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Neutrophil Formylpeptide Receptor Single Nucleotide Polymorphism 348T>C in Aggressive Periodontitis

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Abstract

Background—Neutrophil formylpeptide receptors (FPRs) play an important role in bacterial recognition and chemotaxis. Defective *FPR1* expression and impaired polymorphonuclear leukocyte (PMN) chemotaxis toward bacterial formylpeptides are associated with aggressive periodontitis (AP). The objective of this study was to determine whether single nucleotide polymorphisms (SNPs) in *FPR1* are associated with AP.

Methods—Genomic DNA was isolated from blood samples obtained from African-Americans (30 AP cases, 33 healthy controls) and Turks (75 AP cases, 63 healthy controls). A highly polymorphic fragment of the *FPR1* gene was amplified by polymerase chain reaction (PCR) and sequenced for analysis of SNPs.

Results—Five previously identified SNPs were detected in African Americans, while six were detected in Turkish subjects. The frequency of synonymous SNP c.348T>C was significantly higher in African American AP cases than in controls ($P = 0.029$). The 348T allele was significantly associated with AP ($P = 0.010$). Seven of the AP subjects, but none of the controls, were homozygous for 348T. *FPR1* haplotypes 348T.568A, 348T.576T and 348T.568A.576T were significantly increased in African American AP cases ($P < 0.02$). The Turkish population did not exhibit significant differences in *FPR1* SNP frequencies between AP cases and controls.

Conclusions—*FPR1* SNP c.348T>C is associated with AP in African Americans. Individuals who are homozygous for 348T may have an increased risk of developing this disorder.

Keywords

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

Aggressive periodontitis (AP) is a rapidly progressing periodontal disease with a tendency toward familial aggregation, occurring in either localized or generalized forms.^{1,2} Aside from periodontal manifestations, a frequent finding in patients with AP is abnormal polymorphonuclear leukocyte (PMN) chemotactic function.^{3–5} PMNs serve as the primary host defense against bacterial infections via a complex process that involves adherence to endothelial cells, transmigration into tissues and chemotaxis toward the invading microorganisms. Once they have migrated to an infection site, PMNs phagocytose bacteria and attempt to kill them with reactive oxygen metabolites and microbicidal proteins.^{6,7}

Formylpeptide receptors (FPRs, approximately 55,000 per cell) are found on the surface of PMNs and other phagocytes.⁸ Like other G-protein-coupled receptors, the FPR contains seven transmembrane domains separated by extracellular or intracellular loops. Formyl-Met-Leu-Phe (fMLF) and other formylpeptides are produced by bacteria and occur at high concentrations at infection sites. fMLF binding to these receptors triggers a cascade of intracellular signals that coordinate cytoskeletal reorganization, formation of pseudopodia and migration toward a chemotactic gradient.⁹ PMNs from patients with AP exhibit reduced fMLF binding and less than 20% to 50% of the chemotactic activity toward fMLF observed in healthy control subjects.³⁻⁵ Other PMN abnormalities have also been reported in AP subjects, including decreased phagocytosis,¹⁰ increased superoxide production¹¹ and signal transduction abnormalities¹¹ (reduced expression of diacylglycerol kinase and increased intracellular diacylglycerol levels relative to PMNs from healthy controls).

Single nucleotide polymorphisms (SNPs) are more common in *FPR1* than in most genes.¹² Initial analysis of polymorphisms in the *FPR1* open reading frame (ORF) by direct sequencing of cloned alleles from random North American blood donors revealed five common non-synonymous SNPs (c.32C>T, c.301G>C, c.568A>T, c.576T>C>G and c.1037C>A) as well as two synonymous SNPs (c.348T>C and c.546C>A).¹² Recent reports have suggested that AP is associated with some of these *FPR1* ORF SNPs. A case-control analysis of *FPR1* polymorphisms in African American, Turkish and Brazilian subjects reported that c.568A>T and c.576T>C>G are significantly associated with AP in African Americans.¹³ A more recent report found no differences in the frequencies of SNPs c.301G>C, c.546C>A and c.568A>T in African American and Caucasian AP cases and controls.¹⁴ In Japanese subjects, the SNPs c.301G>C and c.546C>A are reportedly associated with AP.¹⁵ The frequencies of *FPR1* SNPs differ among ethnic groups,¹²⁻¹⁵ and previous reports are in some respects contradictory. Therefore, we screened relatively homogenous populations of African American and Turkish AP cases and healthy controls to determine whether SNPs in the *FPR1* ORF are associated with AP.

MATERIALS AND METHODS

Subject recruitment and DNA isolation

Human subjects were enrolled under protocols approved by the Institutional Review Boards of The Ohio State University and Hacettepe University. African American and Turkish Subjects were recruited between July 2001 and November 2007. The criteria for diagnosis of AP were consistent with the 1999 International Workshop for Classification of Periodontal Diseases and Conditions. The AP group included individuals diagnosed with either localized aggressive periodontitis (LAP) or generalized aggressive periodontitis (GAP) who were otherwise healthy. Cases with attachment loss not involving more than two permanent teeth other than the first molars and incisors, were diagnosed with LAP.¹ Specifically, subjects with ≥ 4 mm attachment loss involving at least two permanent first molars and incisors, including at least one first molar, were recruited.² Cases involving at least three teeth other than the first molars and incisors, with an attachment loss of ≥ 4 mm were diagnosed with GAP.^{1,2}

After obtaining informed consent from each subject, a sample of peripheral blood was drawn. Genomic DNA was isolated from blood with an established laboratory method¹⁶ or by using a DNA isolation kit.[§]

[§]QIAamp DNA Blood Mini Kit, Qiagen, Valencia, CA, USA.

Polymerase Chain Reaction (PCR) and sequencing

To detect SNPs c.301G>C, c.306T>C, c.348T>C, c.546C>A, c.568A>T and c.576T>C>G, primers were designed to amplify a 439 bp fragment of the *FPRI* gene containing these SNPs. The forward and reverse primers were ²²⁰TTCACCTCCACTTTGCCATT²³⁹ and ⁶⁵⁸TGACAGCAACGATGGACATG,⁶³⁹ respectively. PCR was carried out for 35 cycles in a mixture of 45 µl PCR supermix^{||}, 1 µl of each of the two primers, 1.5 µl of the DNA sample (18-30 pg) and 1.5 µl of water. Prior to initiating PCR, the reaction mixture was heated at 95°C for one minute to dissociate antibody from the Taq polymerase. The parameters for one cycle of the PCR reaction include denaturation at 95°C for one minute, annealing at 60°C for one minute and extension at 72°C for one minute. All samples yielded PCR products, which were processed with a purification kit[¶] and sequenced with an automated DNA analyzer.[#]

Data Analysis

Allele frequencies in both population groups for each of the polymorphisms identified were calculated separately in patients and controls and analyzed by the Fisher Exact test for all SNPs except c.576T>C>G (which was analyzed by the Chi-Square test). Allele association of SNP c.348T>C in African Americans was analyzed in AP cases and controls from our study as well as in sequence data obtained from 55 randomly-selected African American subjects by the International HapMap project.¹⁷ The HapMap data are freely accessible at https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=5030879). The statistical significance of the observed allele associations was assessed using a web-based program (<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>). Hardy-Weinberg equilibrium analysis and haplotype frequency analysis were conducted with commercially available software.^{**} Secondary mRNA structures associated with the two predominant haplotypes in the African American population were predicted using the mfold web server (<http://mfold.bioinfo.rpi.edu/cgi-bin/rna-form1-2.3.cgi>).¹⁸

RESULTS

The African American subject population included a case group of 30 subjects (mean age 24 years, 60% female) and a healthy control group of 33 subjects (mean age 27.3 years, 63.6% female). The Turkish subject population included a case group of 75 subjects (mean age 27.8 years, 64.9% female) and a control group of 63 subjects (mean age 37.2 years, 68.3% female). The mean ($\pm$ SEM) pocket probing depth and clinical attachment loss in the African American case group was 3.70 ± 0.20 mm and 2.98 ± 0.27 mm, respectively. In the Turkish case group, these values were 3.67 ± 0.11 mm and 4.21 ± 0.14 mm, respectively. None of the subjects in the control groups exhibited radiographic evidence of bone loss. Clinical attachment loss in the control groups was only observed at isolated sites and was predominantly related to gingival recession.

Five previously identified *FPRI* SNPs (c.301G>C, c.348T>C, c.546C>A, c.568A>T and c.576T>C>G) were detected in the African American and Turkish populations. In addition, a previously identified SNP c.306T>C was detected in the Turkish population. In both control groups, all SNPs were in Hardy-Weinberg equilibrium. There was a significant difference in the allele frequency of c.348T>C between the African American AP cases and healthy controls ($P = 0.029$, odds ratio = 2.5, 95% confidence interval = 1.1-5.6, Table 1). In the African American population, seven of the case subjects and none of the control subjects were

^{||}PCR SuperMix, Invitrogen, Carlsbad, CA, USA.

[¶]QIAquick PCR Purification Kit, Qiagen, Valencia, CA, USA.

[#]3730 automated DNA analyzer, Applied Biosystems, Foster City, CA, USA.

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

homozygous for 348T (table 2). There was a significant relationship between 348T and case/control status ($P = 0.010$, Table 2). Among African Americans randomly sampled by the HapMap project, three of the fifty-five subjects were homozygous for 348T. There was a significant association of 348T with AP case status when these HapMap subjects were used as controls ($P = 0.02$, table 2). This association was more significant when the case group was controlled by pooling our controls with the HapMap subjects ($P = 0.002$, table 2). The association of 348T homozygotes with AP case status was highly significant compared to 348C homozygotes when cases were compared to our study controls ($P = 0.004$), to HapMap subjects ($P = 0.006$), or to our controls pooled with HapMap subjects ($P = 0.0005$) (table 3). In all three comparisons, the resulting odds ratios were similar whether the allele association of the 348T/T genotype was compared to that of 348C/C alone, or to a pool of 348C/C and 348T/C genotypes. The odds ratios were considerably lower when 348T/C was compared to 348C/C (table 3). In contrast, no significant differences between cases and controls were observed with respect to frequencies of the other *FPRI* SNPs. Moreover, no significant differences between cases and controls were observed with regard to frequencies of the six *FPRI* SNPs detected in Turkish subjects (Table 1).

Haplotypes with a predicted frequency of at least 0.1 were analyzed in the African American population. SNPs c.568A>T and c.576T>C>G, which were previously reported to be significantly associated with AP in African Americans,¹³ were included in the analysis. Twelve haplotypes were predicted in the case and control groups when SNPs c.348T>C, c.568A>T and c.576T>C>G were analyzed (Table 4). The haplotypes 348T.568A, 348T.576T and 348T.568A.576T were predicted to occur more frequently in AP cases compared to controls ($P = 0.0036$, 0.017 and 0.017 respectively), while 348C.568A, 348C.576T and 348C.568A.576T were significantly increased in controls compared to cases ($P = 0.041$, 0.014 and 0.014 respectively, Table 4). The mRNA associated with haplotype 348T.568A exhibited higher free energy (lower stability) and a different secondary structure than that predicted for 348C.568A (Fig. 1).

DISCUSSION

The results of the present study suggest that the 348T variant is associated with an increased risk of developing AP in African Americans. Zhang et al. (2003)¹³ previously reported a significant association between c.348T>C and AP in Brazilian subjects, but did not observe this relationship in African Americans. The significance of the 348T allele association with AP in African Americans was further validated by comparison of our study's AP cases with randomly-selected African American subjects sampled by the HapMap project. The 348T/T allele exhibited a strong association with AP case status when our study controls were pooled with HapMap subjects and compared to our AP case group ($P = 0.0007$, table 3). Since the prevalence of AP in African Americans is approximately 2%,^{19,20} this could explain why a small number of 348T homozygotes were present in the HapMap subject group. The allele frequencies of SNPs observed in our African American control group were reasonably similar to the report of Zhang et al. (2003).¹³ In disagreement with their findings, SNPs c.568A>T and c.576T>C>G were not significantly associated with AP cases in our African American study population. Four haplotypes (568A.576T, 568A.576C, 568A.576G and 568T.576C) reportedly have a statistically significant association with AP in African Americans. We did not find similar haplotype associations in our African American population, but haplotypes 348T.568A, 348T.576T and 348T.568A.576T were significantly increased in AP cases, whereas haplotypes 348C.568A, 348C.576T and 348C.568A.576T were significantly increased in controls. Disparities between our findings and the previous study¹³ could be due to differences in selection criteria of the study population. We recruited a relatively homogenous population of African Americans whose ancestral origins are in West Africa or the Bantu territory. Our findings are in agreement with a recent report,¹⁴ which found no

differences in the frequencies of SNPs c.301G>C, c.546C>A and c.568A>T in African American AP cases and controls.

In our Turkish case population, frequencies of all SNPs were similar to those previously reported.¹³ The allele frequencies of SNP observed in our Turkish control group were also very similar with the exception of c.306T>C, which was more prevalent in our Turkish population group than previously reported.¹³ In contrast to previous work, c.306T>C was not associated with AP in our Turkish population.

The African American and Turkish populations recruited for the present study exhibited some differences with respect to frequencies of *FPR1* SNPs. The SNP c.306T>C was detected only in Turkish subjects, while the 348T variant was much less common in Turks than in African Americans. These findings are consistent with previous work.¹³ A recent study of Japanese AP cases and controls detected eleven polymorphisms in the entire *FPR1* ORF, including the five SNPs detected in all of our subjects as well as six other SNPs (c.32C>T, c.117C>T, c.289C>A, c.553A>G, c.634G>A and c.1037C>A).¹⁵ In Japanese subjects, SNPs c.301G>C and c.546C>A exhibited a significant association with AP, but c.568A>T, c.576T>C>G or c.348T>C were not significantly associated. In the populations screened to date, it is clear that the frequency of *FPR1* SNPs can vary widely between races.

While it is unclear how a synonymous SNP like c.348T>C could influence FPR expression, the free energy of the predicted secondary structure of mRNA associated with haplotype 348T.568A was higher than that of 348C.568A (Fig. 1). This was associated with changes in mRNA structure. The significance of this change has not been evaluated, but higher free energy and associated changes in structure could potentially lower mRNA stability and decrease translational efficiency. It is also possible that 348T could be linked to one or more polymorphisms in the FPR promoter region that could influence FPR gene expression. In a recent study in Japanese subjects, a 21.1 kb region including the *FPR1* ORF, *FPR1* promoter region, 5' and 3' flanking areas were screened for polymorphisms. Thirty SNPs were reported, eight of which occurred in the 5' region. Three of these SNPs (-12915C>T, -10056T>C, -8430A>G) were significantly associated with AP.¹⁵ The -12915 T/T genotype was associated with a marked reduction in *FPR1* mRNA levels of AP patients compared to those expressing the -12915 C/C genotype.¹⁵ A decrease in mRNA levels would be expected to result in reduced receptor expression which would impair PMN chemotaxis toward bacteria, making individuals more susceptible to development of AP. Linkages involving synonymous and non-synonymous coding region polymorphisms have been observed in genes of other receptors.^{21,22} Studies suggest that approximately 50% of genes may have an association with one or more regulatory SNPs (outside coding regions) and many of them occur in the promoters. Promoter SNPs may alter stability, curvature or flexibility of the promoter and this could affect transcription.²³

A significant number of African Americans are affected by aggressive periodontitis.^{19,20,24} Our study suggests that individuals who are homozygous for 348T in the *FPR1* gene could have an increased risk for developing AP. If untreated, AP patients are at a greater risk of premature tooth loss. Early diagnosis and intervention could mitigate the consequences of this disease and help reduce treatment costs for these patients.

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REFERENCES

1. Lang N, Bartold PM, Cullinan M, et al. Consensus report: aggressive periodontitis. *Ann Periodontol* 1999;4:53.
2. Tonetti MS, Mombelli A. Early-onset periodontitis. *Ann Periodontol* 1999;4:39–52. [PubMed: 10863374]
3. Van Dyke TE, Levine MJ, Tabak LL, Genco RJ. Reduced chemotactic peptide binding in juvenile periodontitis: A model for neutrophil function. *Biochem Biophys Res Commun* 1981;100:1278–1284. [PubMed: 6268085]
4. Sigusch B, Eick S, Pfister W, Klinger G, Glockmann E. Altered chemotactic behavior of crevicular PMNs in different forms of periodontitis. *J Clin Periodontol* 2001;28:162–167. [PubMed: 11168741]
5. Perez HD, Kelly E, Elfman F, Armitage G, Winkler J. Defective polymorphonuclear leukocyte formyl peptide receptors in juvenile periodontitis. *J Clin Invest* 1991;87:971–976. [PubMed: 1999504]
6. Miyasaki KT. The neutrophil-mechanisms of controlling periodontal bacteria. *J Periodontol* 1991;62:761–774. [PubMed: 1765939]
7. Smith JA. Neutrophils, host defense and inflammation: a double-edged sword. *J Leukoc Biol* 1994;56:672–686. [PubMed: 7996043]
8. Pike MC, Snyderman R. Leukocyte chemoattractant receptors. *Methods Enzymol* 1988;162:236–245. [PubMed: 3226308]
9. Katanaev VL. Signal transduction in neutrophil chemotaxis. *Biochemistry (Mosc)* 2001;66:351–368. [PubMed: 11403641]
10. Sigusch B, Klinger G, Holtz H, Suss J. In vitro phagocytosis by crevicular phagocytes in various forms of periodontitis. *J Periodontol* 1992;63:496–501. [PubMed: 1625149]
11. Gronert K, Kantarci A, Levy BD, et al. A molecular defect in intracellular lipid signaling in human neutrophils in localized aggressive periodontal tissue damage. *J Immunol* 2004;172:1856–1861. [PubMed: 14734770]
12. Sahagun-Ruiz A, Colla JS, Juhn J, Gao JL, Murphy PM, McDermott DH. Contrasting evolution of the human leukocyte N-formylpeptide receptor subtypes FPR and FPRL1R. *Genes Immun* 2001;2:335–342. [PubMed: 11607790]
13. Zhang Y, Syed R, Uygur C, et al. Evaluation of human leukocyte N-formylpeptide receptor (FPR1) SNPs in aggressive periodontitis patients. *Genes Immun* 2003;4:22–29. [PubMed: 12595898]
14. Nibali L, Parkar M, Brett P, Knight J, Tonetti MS, Griffiths GS. NADPH oxidase (CYBA) and FcγR polymorphisms as risk factors for aggressive periodontitis: a case-control association study. *J Clin Periodontol* 2006;33:529–539. [PubMed: 16899095]
15. Gunji T, Onouchi Y, Nagasawa T, et al. Functional polymorphisms of the FPR1 gene and aggressive periodontitis in Japanese. *Biochem Biophys Res Commun* 2007;364:7–13. [PubMed: 17927965]
16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215. [PubMed: 3344216]
17. The International HapMap Consortium. The International HapMap Project. *Nature* 2003;426:789–796. [PubMed: 14685227]
18. Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 2003;31:3406–3415. [PubMed: 12824337]
19. Melvin WL, Sandifer JB, Gray JL. The prevalence and sex ratio of juvenile periodontitis in a young racially mixed population. *J Periodontol* 1991;62:330–334. [PubMed: 2072246]
20. Fine DH, Markowitz K, Furgang D, et al. Aggregatibacter actinomycetemcomitans and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. *J Clin Microbiol* 2007;45:3859–3869. [PubMed: 17942658]
21. Drysdale CM, McGraw DW, Stack CB, et al. Complex promoter and coding region β2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *PNAS* 2000;97:10483–10488. [PubMed: 10984540]

22. Mummidi S, Banshad M, Ahuja SS, et al. Evolution of human and non-human primate CC chemokine receptor 5 gene and mRNA. *J Biol Chem* 2000;275:18946–18961. [PubMed: 10747879]
23. Buckland PR. The importance and identification of regulatory polymorphisms and their mechanisms of action. *Biochim Biophys Acta* 2006;1762:17–28. [PubMed: 16297602]Epub 2005 Oct 28. Review
24. Loe H, Brown LJ. Early onset periodontitis in the United States of America. *J Periodontol* 1991;62:608–616. [PubMed: 1770420]

Figure 1. Predicted mRNA secondary structures derived from predominant haplotypes 348T.568A (a) and 348C.568A (b). The positions of 348T and 348C are indicated by arrows in magnified portions of the two structures. The 348T variant forms a different predicted structure than the 348C variant and has a higher free energy of folding (dG), making it less stable.

Table 1
Allele frequencies of three *FPR1* SNPs in African-American and Turkish case and control groups

SNP		African American AP Cases n (%)	African American Controls n (%)	P - value ¹	Turkish AP Cases n (%)	Turkish Controls n (%)	P - value ¹
c.301G>C	G	32 (53.3)	41 (62.1)	0.37*	111 (77)	92 (73)	0.96*
(non-synonymous)	C	28 (46.7)	25 (37.9)		39 (23)	34 (27)	
c.306T>C	T	60 (100)	66 (100)	1.00*	131 (87)	111 (88)	0.99*
(synonymous)	C	0 (0)	0 (0)		19 (13)	15 (12)	
c.348T>C	T	23 (38.3)	13 (19.7)	0.029*	7 (5)	3 (2)	0.49*
(synonymous)	C	37 (61.7)	53 (80.3)		143 (95)	123 (98)	
c.546C>A	C	45 (75)	46 (69.7)	0.55*	100 (67)	90 (71)	0.47*
(synonymous)	A	15 (25)	20 (30.3)		50 (33)	36 (29)	
c.568A>T	A	55 (91.7)	58 (87.9)	0.57*	135 (90)	115 (91)	0.878*
(non-synonymous)	T	5 (8.3)	8 (12.1)		15 (10)	11 (9)	
c.576T>C>G	T	27 (45)	34 (51.5)	0.47 [†]	90 (58)	73 (56)	0.425 [†]
(non-synonymous)	C	5 (8.3)	8 (12.1)		20 (15)	12 (9)	
	G	28 (46.7)	24 (36.4)		40 (27)	41 (35)	

* Determined by Fisher Exact test

[†] Determined by Chi Square test

Table 2Allele association of *FPR1* SNP 348T>C with AP cases in African-Americans

Genotype	AP n (%)	Controls [*] n (%)	HapMap randomly selected subjects [†] n (%)	Controls pooled with HapMap subjects [‡] n (%)
348 C/C	14 (46.7)	20 (60.6)	40 (72.7)	60 (68.2)
348 T/C	9 (30)	13 (39.4)	12 (21.8)	25 (28.4)
348 T/T	7 (23.3)	0 (0)	3 (5.5)	3 (3.4)

^{*} Relationship between genotype and AP case/control status is significant ($P = 0.010$, Fisher Exact test)

[†] Relationship between genotype and AP case/random sample status is significant ($P = 0.02$, Chi square)

[‡] Relationship between AP cases and pool of controls plus HapMap random samples significant ($P = 0.002$, Chi square)

Table 3Significance of allele associations of *FPRI* SNP 348T>C with AP in African-Americans*

Comparisons	T vs C	T/T vs C/C	T/T vs C/C+T/C	T/C vs C/C
AP cases vs study controls	P = 0.021 OR = 2.53 (1.1-5.6)	P = 0.004 OR = 21.2 (1.1-401.4)	P = 0.003 OR = 21.4 (1.2-392.9)	P = 0.984 OR = 0.98 (0.3-2.9)
AP cases vs HapMap randomly selected subjects	P = 0.001 OR = 3.2 (1.5-6.6)	P = 0.006 OR = 6.7 (1.5-29.3)	P = 0.014 OR = 5.3 (1.2-22.2)	P = 0.153 OR = 2.1 (0.7-6.2)
AP cases vs study controls pooled with HapMap subjects	P = 0.00097 OR = 2.9 (1.5-5.6)	P = 0.0005 OR = 10 (2.3-43.6)	P = 0.0007 OR = 8.6 (2.1-36)	P = 0.373 OR = 1.5 (0.6-4)

OR = odds ratio (95% confidence interval)

* Assessed with Pearson's goodness-of-fit Chi square (degree of freedom = 1) using <https://ihg.gsf.de/cgi-bin/hw/hwa1.pl>

Table 4Haplotypes of *FPR1* SNPs c.348T>C, c.568A>T and c.576T>C>G in African Americans*

Haplotype	Frequency in AP cases	Frequency in controls	P value [†]	Odds Ratio (95% CI)
348C.568A	34	49	0.041	0.4 (0.21-0.96)
348T.568A	22	9	0.0036	3.7 (1.5-8.8)
348C.576G	20	20	0.848	1.1 (0.5-2.4)
348C.576T	14	30	0.014	0.4 (0.2-0.8)
348T.576T	13	4	0.017	4.2 (1.3-14)
348T.576G	8	4	0.227	2.4 (0.7-8.4)
568A.576G	28	24	0.279	1.5 (0.7-3.1)
568A.576T	27	34	0.481	0.8 (0.4-1.5)
348C.568A.576G	19	20	1	1.1 (0.5-2.3)
348C.568A.576T	14	30	0.014	0.4 (0.2-0.8)
348T.568A.576T	13	4	0.017	4.3 (1.3-14)
348T.568A.576G	8	4	0.226	2.4 (0.7-8.4)

* Haplotypes predicted by expectation-maximization algorithm

[†] Determined by Fisher Exact test