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Functional polymorphisms of the *FPR1* gene and aggressive periodontitis in Japanese

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Abstract

Aggressive periodontitis (AgP), a severe and early onset type of periodontitis, is thought to be subject to significant genetic background effects. Formyl peptide receptor 1 (FPR1) is a gene strongly implicated in AgP. To determine whether variations in this gene are associated with AgP, we performed an association study with 49 AgP patients and 373 controls using 30 variations identified by sequencing the 21.1-kb gene region. Five polymorphisms (−12915C > T, −10056T > C, −8430A > G, 301G > C, and 546C > A) showed significant association with AgP. Polymorphonuclear neutrophils from subjects carrying the −12915T allele expressed significantly lower levels of *FPR1* transcripts than those homozygous for the −12915C allele. Furthermore, the −12915T allele decreased activity of transcriptional regulation in a luciferase assay. Haplotype association analysis with three SNPs (−12915C > T, 301G > C, and 546C > A) revealed that one haplotype (−12915T–301G–546C) was significantly represented in AgP patients ($p = 0.000020$). Thus, altered FPR1 function might confer increased risk to AgP.

Keywords: *FPR1*; Aggressive periodontitis; Single nucleotide polymorphisms (SNPs); Real-time PCR; Luciferase assay; Haplotype association analysis

Periodontitis is an infectious disease caused by periodontopathic bacteria, including *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* [1]. Aggressive periodontitis (AgP) is a severe type of periodontitis that causes rapid alveolar bone destruction and possible familial aggregation, affecting individuals without any systemic disorders [2]. Disease progression of AgP is so severe and rapid compared with chronic periodontitis that, if not treated, patients' periodontal ligaments and teeth are lost early in life.

Periodontitis is considered a multifactorial disease influenced by both environmental factors (e.g. smoking, stress, and lifestyle) and genetic factors. Some inflammation-related genes such as interleukin-1 (IL-1) [3], tumor necrosis factor-α (TNF-α) [4], Fc-γ receptors (Fc-γR) [5], interleukin-10 (IL-10) [6], and prostaglandin D2 synthase (PTGDS) [7] have been investigated as candidate genes for AgP susceptibility.

In mammalian immune systems, polymorphonuclear neutrophils (PMNs) have an important role in killing bacteria and fungi [8]. In gingival sulcus, PMNs are essential as the first line of defense against periodontopathic bacteria

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[9]. Various functional defects in PMNs isolated from AgP patients have been reported and are believed to be related to the pathogenesis of AgP [10–13].

Formyl peptide receptor 1 (FPR1) is a chemotactic G-protein coupled receptor that is expressed on the surface of PMNs and other phagocytes [14]. The binding of agonists to FPR1 was shown to be related to various leukocyte functions [15]. FPR1 of PMNs in AgP patients has been reported to be defective [16] and a role for *FPR1* SNPs in AgP progression has been suggested [17–20], but these results have not yet been fully confirmed. The purpose of this study was to investigate the possible role of the *FPR1* gene in AgP through an association study in Japanese in which exhaustively identified SNPs in the gene, and haplotype analysis, are employed.

Materials and methods

Subjects. All 49 patients were diagnosed with AgP at Tokyo Medical and Dental University according to the criteria of the 1999 International Classification of Periodontal Diseases [2]. The patients' information is summarized in Table 1. The 373 ethnically matched controls were volunteers from Keio University and Tokyo Medical and Dental University. This study was approved by the ethics committees of RIKEN, Tokyo Medical and Dental University and Keio University, and all patients and controls provided written informed consent for blood sampling and DNA analysis. Genomic DNA was extracted from peripheral leukocytes by standard protocols.

Polymerase chain reactions. The *FPR1* reference sequence was obtained from the National Center for Biotechnology Information (NCBI, USA), accession number NT_011109.15. We targeted the approximately 21.1-kb genomic region containing all exons and introns, as well as 10 kb of the 5'flanking region and 5 kb of the 3'flanking region of *FPR1*. Repetitive sequences were masked with the RepeatMasker program (http://repeatmasker.genome.washington.edu/) and excluded from the screening. We synthesized 13 primer pairs for PCR (Supplementary Table 1). Each PCR amplification was performed in 10 μl containing 1.25 mM dNTPs, 1.25 mM Mg^{2+}, 10 pmol of each primer, 0.25 U of Ex *Taq* DNA polymerase (Takara, Japan), and 10 ng of genomic DNA. All PCRs were performed in GeneAmp 9700 thermal cyclers (Applied Biosystems, Foster City CA, USA). PCR thermocycling parameters used for discovery and genotyping of SNPs were an initial 5 min denaturation step at 94 °C, followed by 37 cycles of 30 s each at 94, 60, and 72 °C, and a final 7 min extension step at 72 °C.

SNP identification. Genomic DNAs of 24 AgP patients and 24 healthy controls were amplified by each primer set. After the PCR products were purified and diluted, they were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit v3.1 (Applied Biosystems) according to the manufacturer's instructions. For each amplicon, one of the PCR primers was used for direct sequencing, conducted on an ABI 3700 automated sequencer (Applied Biosystems). The GAP4 program contained in the Staden Package (http://www.mrc-lmb.cam.ac.uk/pubseq/) was used to identify the presence of nucleotide polymorphisms.

Linkage disequilibrium (LD) analysis. Pairwise LD analysis of 25 variations discovered during screening was performed using the SNPAlyze V3.2. program (Dynacom, Mobara, Japan). Tightly linked ($r^2 \geqslant 0.7$) SNPs were grouped, and representative SNPs from each group were analyzed in the case–control study.

Genotyping of SNPs. For the remaining 25 AgP patients and 349 controls, genotyping was conducted either by direct sequencing or PCR-RFLP. For fpr1-01, fpr1-02, fpr1-03, fpr1-04, fpr1-05, and fpr1-07, PCR-RFLP was used. In PCR-RFLP, mismatched primers designed to generate restriction enzyme recognition sequences that included SNPs within them were used for one of each PCR primer pair. After PCR amplification, products were digested with the appropriate restriction enzyme according to the manufacturer's instructions. Primers and restriction enzymes used in the PCR-RFLP analysis are listed in Supplementary Table 2. Separation was performed on 4% agarose gels in TBE buffer, and the gels were stained with ethidium bromide to visualize the fragments. For the remaining SNPs, genotyping was performed by direct sequencing because these SNPs were located very close to each other and thus could be genotyped simultaneously with one sequencing run.

Quantification of FPR1 gene expression using real-time RT-PCR. We prepared PMNs using Polymorphprep (Axis-Shield PoC AS, Oslo, Norway) from 22 healthy volunteers. Total RNA from the resting PMNs was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized using SuperScriptIII reverse transcriptase (Invitrogen) from 1 μg total RNA. mRNA levels of both *FPR1* and *β-actin* genes were quantified using TaqMan probes (Assay ID Hs00181830_m1 for *FPR1* gene and catalogue no. 4310881E for *β-actin* gene, Applied Biosystems) on a Stratagene Mx3000 P (Stratagene, La Jolla, CA, USA) in accordance with the manufacturer's instructions. The relative expression levels of *FPR1* mRNA were analyzed using the comparative Ct method, employing *β-actin* as the reference gene in each sample.

Luciferase assay. Twenty-nine oligonucleotide bases surrounding the SNPs were generated and cloned into pGL3-basic vector (Promega, Madison, WI, USA) in the 5'–3' orientation to test the transcriptional activity of fpr1-01, fpr1-04, and fpr1-05. We then transfected subconfluent human embryonic kidney 293 (HEK293) cells (1×10^6) cultured in 6-well plates with 2 μg of each construct and 25 ng of phRG-TK Renilla luciferase vector (Promega) as an internal control. Tranfection was conducted using Amaxa Nucleofector (Amaxa Biosystems, Cologne, Germany) according to the manufacturer's instructions. After 24 h, the cells were lysed and firefly and *Renilla* luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega). Each experiment was repeated three times.

Table 1
Clinical parameters of AgP patients

Trait	
Number	49
Sex ratio (F/M)	23/26
Age (year of first diagnosis)	33.4 ± 6.0
Number of present teeth	26.7 ± 3.6
Mean of PPD (mm)	3.90 ± 1.08
Ratio of teeth PPD > 4 mm	0.79 ± 0.21

Values are means ± SD. PPD, probing pocket depth.

Results

Variations identified in FPR1

Twenty-five variations were identified by screening 24 AgP and 24 control subjects, and five more were identified in the subsequent genotyping step by direct sequencing. Of these 30 variations, 11 were within the coding region, eight within the 5'flanking region and 11 within the 3'flanking region (Fig. 1). Pertinent information relating to these SNPs is summarized in Table 2.

Pairwise LD in FPR1

LD of each SNP pair was evaluated using the r^2 and $|D'|$ values. Four high LD groups ($r^2 \geqslant 0.7$) were identified; the LD structure of the SNPs identified in this study is shown in Fig. 2.

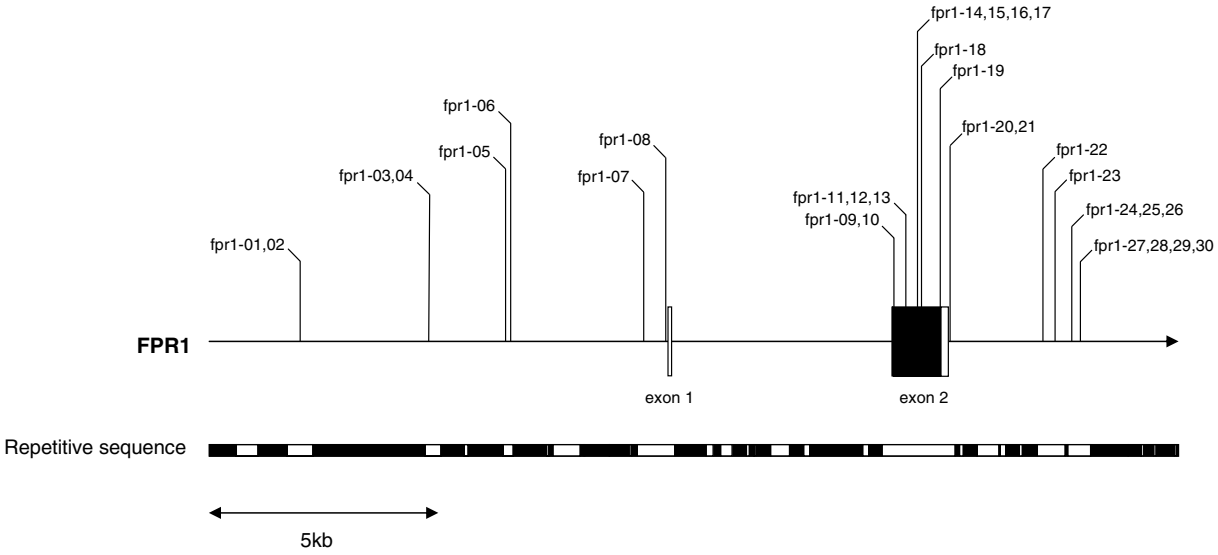

Fig. 1. Variations identified in the *FPR1* region. Open and filled boxes represent untranslated and coding regions, respectively. Repetitive sequences are indicated by black filled zones in the bar under the diagram of the gene structure.

Case–control association analysis

Of these 30 SNPs, 26, including representative SNPs selected from each high LD group (fpr1-09 and fpr1-27), were genotyped. The allele and genotype distribution of these SNPs among the AgP patients and controls were compared; the results are summarized in Table 2. All SNPs with a minor allele frequency larger than 0.01 were in agreement with Hardy–Weinberg equilibrium expectations ($p > 0.01$). The SNPs with a minor allele frequency larger than 0.10 were tested by the χ^2 test, and those lower than 0.10 were tested by Fisher's exact test. Three SNPs (fpr1-01, frr1-04, and fpr1-14) were significantly associated with AgP in the allele frequency ($p < 0.05$; Table 2). In addition, two SNPs (fpr1-05 and fpr1-12) were associated with AgP in the association analysis of the genotype frequency in a recessive model.

Expression level of FPR1 transcripts and reporter gene analysis

We compared the expression level of *FPR1* transcripts in PMNs from healthy volunteers with different genotypes of fpr1-01 ($-12915C > T$). Real-time PCR analysis showed that *FPR1* mRNA expression was significantly lower in subjects carrying a T allele (C/T genotype; $n = 10$, T/T genotype; $n = 3$) than in those carrying no T allele (C/C genotype; $n = 9$) (Mann–Whitney *U*-test, $p = 0.022$ (C/C vs. C/T), $p = 0.0091$ (C/C vs. T/T), $p = 0.0043$ (C/C vs. C/T and T/T); Fig. 3).

The luciferase assay indicated some enhancer-like activity in the DNA fragment containing the fpr1-01 C allele, which was significantly reduced by the C to T substitution (Student's *t*-test, $p < 0.05$; Fig. 4). No enhancer or silencer activity was observed due to either fpr1-04 or fpr1-05 (data not shown). These results strongly suggest that the difference in expression levels of *FPR1* transcripts might be due to altered enhancer activity corresponding to fpr1-01 alleles.

Haplotype association analysis

Three SNPs (fpr1-01, fpr1-12, and fpr1-14) were selected for haplotype association analysis. Eight haplotypes were tested for association with AgP by the χ^2 test using the SNPAlyze program. We found overall significance (global $p = 0.0000081$) and Haplotype 1 (fpr1-01T–fpr1-12G–fpr1-14C) was overrepresented in AgP patients ($p = 0.000020$; Table 3).

Discussion

AgP, a very severe type of periodontitis, is diagnosed by three characteristics: rapid bone destruction, possible familial aggregation, and absence of other systemic disorders in the patient [2]. In periodontal tissue, PMNs are the first line of defense against periodontopathic bacteria, and play an important role in AgP pathogenesis [9]. Several reports suggest that a number of functional abnormalities in PMNs are related to AgP pathogenesis [10–13].

Investigation of the role of the *FPR1* gene in the pathogenesis of AgP resulted in the identification of a functional polymorphism, fpr1-01 ($-12915C > T$), associated with AgP. The T allele was overrepresented in the patient compared to the control group ($p = 0.00098$ for allelic frequency). PMNs carrying the fpr1-01T allele expressed significantly lower levels of *FPR1* transcripts than those homozygous for the fpr1-01C allele (fpr1-01C/C genotype) (Fig. 3). Furthermore, the luciferase assay results indicated that the fpr1-01T allele reduced the efficiency of transcription (Fig. 4). Significant association was observed also in genotype frequency comparison ($p = 0.0062$ for the

Table 2
Identified variations and association analysis for allelic frequency and genotype frequency

SNPID[a]	Location	Position[b]	SNPs	dbSNP number	Substitution	AgP	Control	*p* value[d]	Odds ratio[d]	*p* value[e]	*p* value[f]
fpr1-01	5′flanking region	−12915	C > T	rs11666254		56/98 (57.1)	296/746 (39.7)	0.00098	2.03	0.0062	0.013
fpr1-02	5′flanking region	−12902	C > T			0/98 (0.0)	11/746 (1.5)	0.63	0	NS	NS
fpr1-03	5′flanking region	−10107	A > G	rs8111423		4/98 (4.1)	30/746 (4.0)	1.00	1.02	NS	NS
fpr1-04	5′flanking region	−10056	T > C	rs12460836		56/98 (57.1)	297/746 (39.8)	0.0011	2.02	0.0068	0.013
fpr1-05	5′flanking region	−8430	A > G			31/98 (31.6)	313/746 (42.0)	0.051	0.64	NS	0.018
fpr1-06	5′flanking region	−8318	A > G	rs4802862		1/48 (2.1)	2/48 (4.2)				
fpr1-07	5′flanking region	−5426	G > A			18/98 (18.4)	158/746 (21.2)	0.52	0.84	NS	NS
fpr1-08	5′flanking region	−4949	C > T	rs4802859		1/48 (2.1)	2/48 (4.2)				
fpr1-09	Exon2	32	C > T	rs5030878	Thr 11 Ile	3/98 (3.1)	17/746 (2.3)	0.72	1.35	NS	NS
fpr1-10	Exon2	117	C > T		Leu 39 Leu	1/98 (1.0)	6/746 (0.8)	0.58	1.27	NS	NS
fpr1-11	Exon2	289	C > A		Leu 97 Met	9/98 (9.2)	70/746 (9.4)	1.00	0.98	NS	NS
fpr1-12	Exon2	301	G > C	rs2070745	Val 101 Leu	41/98 (41.8)	358/746 (48.0)	0.25	0.78	NS	0.029
fpr1-13	Exon2	306	T > C	rs28930680	Phe 102 Phe	0/98 (0.0)	2/746 (0.3)	1.00	0	NS	NS
fpr1-14	Exon2	546	C > A	rs2070746	Pro 182 Pro	36/98 (36.7)	354/746 (47.5)	0.045	0.64	NS	NS
fpr1-15	Exon2	553	A > G		Asn 185 Asp	2/98 (2.0)	6/746 (0.8)	0.24	2.57	NS	NS
fpr1-16	Exon2	568	A > T	rs5030880	Arg 190 Trp	18/98 (18.4)	106/746 (14.2)	0.27	1.36	NS	NS
fpr1-17	Exon2	576	T > G > C	rs1042229	Asn 192 Lys	40/98 (40.8)[c]	266/746 (35.7)[c]	0.32	1.24	NS	NS
fpr1-18	Exon2	634	G > A		Ala 212 Thr	1/98 (1.0)	0/746 (0.0)	0.12	0	NS	NS
fpr1-19	Exon2	1037	C > A	rs867228	Ala 346 Glu	29/98 (29.6)	202/746 (27.1)	0.60	1.13	NS	NS
fpr1-20	3′flanking region	1249	C > T	rs867229		35/98 (35.7)	280/746 (37.5)	0.73	0.92	NS	NS
fpr1-21	3′flanking region	1258	C > T	rs1868943		4/98 (4.1)	20/746 (2.7)	0.51	1.54	NS	NS
fpr1-22	3′flanking region	3274	G > C			8/48 (16.7)	14/48 (29.2)				
fpr1-23	3′flanking region	3538	T > C	rs8105268		8/48 (16.7)	14/48 (29.2)				
fpr1-24	3′flanking region	3906	C > T			5/98 (5.1)	23/746 (3.1)	0.36	1.69	NS	NS
fpr1-25	3′flanking region	3927	G > A	rs7250851		37/98 (37.8)	241/746 (32.3)	0.28	1.27	NS	NS
fpr1-26	3′flanking region	3943	G > A			0/98 (0.0)	1/746 (0.1)	1.00	0	NS	NS
fpr1-27	3′flanking region	4091	G > A	rs8104640		22/98 (22.4)	185/746 (24.8)	0.61	0.88	NS	NS
fpr1-28	3′flanking region	4128	G > C			3/98 (3.1)	31/746 (4.2)	0.79	0.73	NS	NS
fpr1-29	3′flanking region	4133	G > A	rs8104633		15/98 (15.3)	167/746 (22.4)	0.11	0.63	NS	NS
fpr1-30	3′flanking region	4232	G > A			1/98 (1.0)	10/746 (1.3)	1.00	0.76	NS	NS

Two numbers divided by '/' represent number of minor alleles and total chromosomes genotyped, respectively. The percentages of minor allele are shown in parentheses. NS, not significant ($p > 0.05$).

[a] SNPs represented by italyc characters were not genotyped for association analysis because of high LD with representative SNPs.

[b] 'position' is the distance from the Adenine of first Methionine.

[c] T and C vs. G.

[d] Major allele vs. minor allele.

[e] Dominant model.

[f] Recessive model.

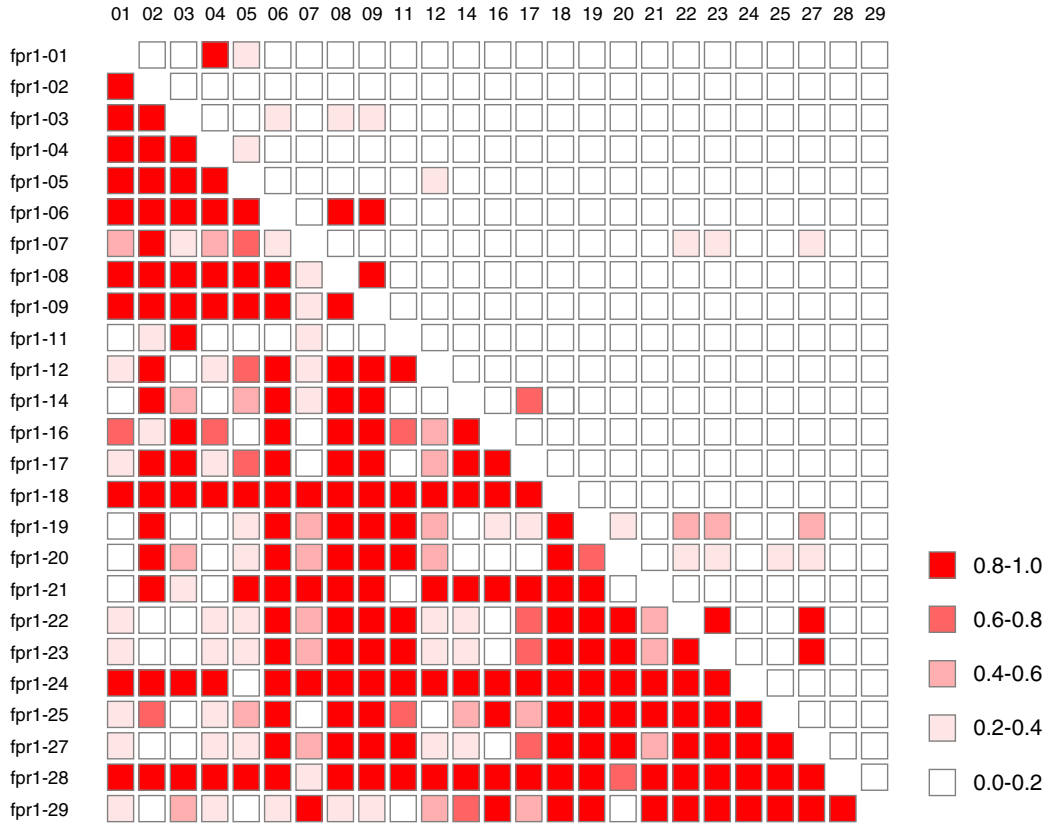

Fig. 2. Pairwise linkage disequilibrium between 25 variations. $|D'|$ value is shown in the lower left, the r^2 value is shown in the upper right.

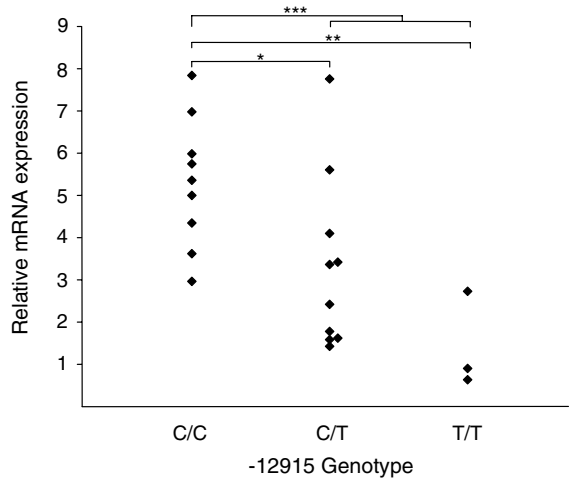

Fig. 3. Decreased *FPR1* mRNA levels in resting polymorphonuclear neutrophils from subjects carrying the −12915T allele (C/T genotype; $n = 10$, T/T genotype; $n = 3$) compared to the—12915C allele homozygous subjects (C/C genotype, $n = 9$). β-Actin was used as an endogenous control. $^{*}p = 0.022$, $^{**}p = 0.0091$, $^{***}p = 0.0043$ by Mann–Whitney *U*-test.

dominant model and $p = 0.013$ for the recessive model). The frequency of the T/T homozygote in AgP patients was significantly higher than in controls (0.33 and 0.18 in AgP patients and controls, respectively). Although the number was small, PMNs from individuals with the T/T genotype ($n = 3$) expressed a significantly lower amount of *FPR1* transcripts than those with the C/C genotype (Fig. 3). Given these results, decreased expression of *FPR1* associated with the fpr1-01T allele might contribute to the pathogenesis of AgP.

FPR1 belongs to the seven-transmembrane domain G-protein coupled receptor (GPCR) family. Gwinn et al. reported that two SNPs in *FPR1*, 329T > C and 378C > G, are related to a localized form of AgP in their African–American patients [17]. These two SNPs change amino acid residues in the third transmembrane domain and the second intracellular loop, respectively. Either change leads to a loss of G protein coupling in FPR1 [18,19]. However, neither of these SNPs was observed in a different African–American AgP group [20]. These SNPs were also not found in our sequence analyses. Instead, we found another non-synonymous SNP significantly associated with AgP ($p = 0.029$ in the recessive model). The SNP fpr1-12 (301G > C) causes an amino acid change in the third transmembrane domain (Val 101 Leu). This SNP might affect the coupling of G-protein to FPR1 in a manner similar to neighboring variations, 329T > C and 378C > G. Alternatively, binding of FPR1 to its ligand might be affected by fpr1-12 because this domain follows the first extracellular loop, and has been reported to play an important role in high-affinity binding to the ligand [21]. Further statistical and biological studies regarding variations in the domain would clarify the role of the domain in FPR1 function.

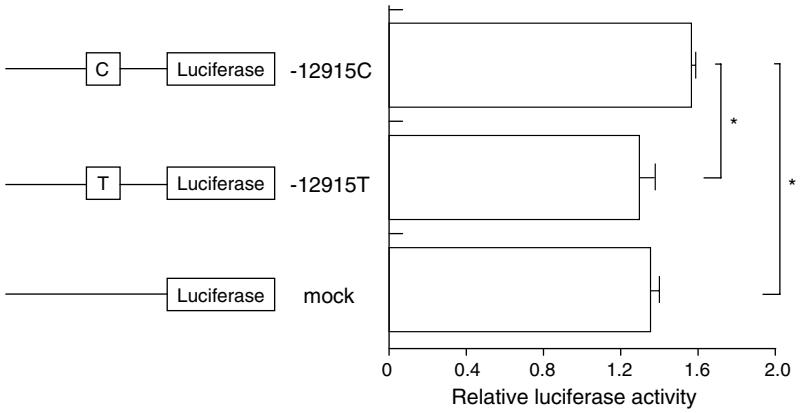

Fig. 4. Effect of −12915C > T polymorphism on transcriptional activity was evaluated by the luciferase assay. The results represent means ± SD. for three independent experiments. $^{*}p < 0.05$ by Student's *t*-test.

Table 3
Association analysis of haplotypes consisted with fpr1-01, fpr1-12, and fpr1-14

	Genotype			Haplotype frequency		χ^2	*p*
	fpr1-01	fpr1-12	fpr1-14	Case	Control		
Haplotype 1	T	G	C	0.36	0.18	18.2	0.000020
Haplotype 2	C	C	A	0.22	0.17	1.7	0.19
Haplotype 3	C	G	C	0.11	0.085	0.75	0.39
Haplotype 4	T	G	A	0.097	0.10	0.033	0.86
Haplotype 5	C	C	C	0.083	0.20	7.4	0.0064
Haplotype 6	T	C	C	0.081	0.069	0.17	0.68
Haplotype 7	T	C	A	0.035	0.048	0.35	0.55
Haplotype 8	C	G	A	0.015	0.16	14.4	0.00015
Global						35.7	0.0000081

FPR1 has three extracellular loops, of which the second has been shown to be important for binding to *N*-formyl peptides (fMLPs) [21]. The SNP fpr1-14 (546C > A), which was associated with AgP in the present study ($p = 0.045$), is located in the second extracellular loop. However, fpr1-14 is synonymous and further investigations are required to evaluate the functional significance of this SNP. In contrast, fpr1-14 is in LD with a neighboring SNP, fpr1-17 (576T > G > C) ($r^2 = 0.72$). The G allele of fpr1-17 alters amino acid translation of the 192nd codon (Asn > Lys) and has been reported to be associated with AgP in African–American subjects [20]. Although we did not observe a significant association between fpr1-17 and AgP (T and C (Asn) vs. G allele (Lys), $p = 0.32$), the result of the haplotype association analysis for two SNPs, fpr1-01 and fpr1-17, showed that a haplotype (fpr1-01T–fpr1-17G) was overrepresented in AgP patients ($p = 0.0030$) (Supplementary Table 3). These results indicate that the fpr1-17G allele also might be associated with AgP in Japanese subjects.

Lack of an association of fpr1-17 with AgP in Japanese might be due to the relatively small sample size, or it is possible that fpr1-14 alone might confer susceptibility to AgP in the Japanese population. An additional study with a larger sample size is needed to clarify this issue.

Haplotype association analysis for three significant SNPs, fpr1-01, fpr1-12, and fpr1-14, identified eight haplotypes and showed highly significant association in global comparisons. The results suggest that a haplotype comprised of susceptibility alleles of each SNP (fpr1-01T – fpr1-12G – fpr1-14C) is dominantly representative in AgP patients ($p = 0.000020$).

In conclusion, an altered *FPR1* gene might be correlated with AgP in the Japanese population. Our data, combined with previous findings may facilitate future investigations of the etiology and pathogenesis of AgP. Growing knowledge about genetic factors that predispose individuals to AgP will hopefully lead to ways to predict and prevent the disease in high-risk individuals.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.09.105.

References

[1] S.S. Socransky, A.D. Haffajee, The bacterial etiology of destructive periodontal disease: current concepts, J. Periodontol. 63 (1992) 322–331.

[2] International Workshop for a Classification of Periodontal Diseases and Conditions, Papers. Oak Brook, Illinois, October 30–November 2, 1999, Ann. Periodontol. 4 (1999) 1–112.

[3] J.M. Parkhill, B.J. Hennig, I.L. Chapple, P.A. Heasman, J.J. Taylor, Association of interleukin-1 gene polymorphisms with early-onset periodontitis, J. Clin. Periodontol. 27 (2000) 682–689.

[4] M. Endo, H. Tai, K. Tabeta, T. Kobayashi, K. Yamazaki, H. Yoshie, Analysis of single nucleotide polymorphisms in the 5′-flanking region of tumor necrosis factor-alpha gene in Japanese patients with early-onset periodontitis, J. Periodontol. 72 (2001) 1554–1559.

[5] K. Yasuda, N. Sugita, T. Kobayashi, K. Yamamoto, H. Yoshie, FcgammaRIIB gene polymorphisms in Japanese periodontitis patients, Genes Immun. 4 (2003) 541–546.

[6] D.F. Kinane, P. Hodge, J. Eskdale, R. Ellis, G. Gallagher, Analysis of genetic polymorphisms at the interleukin-10 and tumour necrosis factor loci in early-onset periodontitis, J. Periodontal. Res. 34 (1999) 379–386.

[7] A. Suzuki, G. Ji, Y. Numabe, M. Muramatsu, K. Gomi, M. Kanazashi, Y. Ogata, E. Shimizu, Y. Shibukawa, A. Ito, T. Ito, A. Sugaya, T. Arai, S. Yamada, S. Deguchi, K. Kamoi, Single nucleotide polymorphisms associated with aggressive periodotitis and severe chronic periodontitis in Japanese, Biochem. Biophys. Res. Commun. 317 (2004) 887–892.

[8] R.R. Sandborg, J.E. Smolen, Early biochemical events in leukocyte activation, Lab. Invest. 59 (1988) 300–320.

[9] S. Offenbacher, Periodontal disease: pathogenesis, Ann. Periodontol. 1 (1996) 821–878.

[10] R.A. Clark, R.C. Page, G. Wilde, Defective neutrophil chemotaxis in juvenile periodontitis, Infect. Immun. 18 (1977) 694–700.

[11] T.E. Van Dyke, M.J. Levine, L.A. Tabak, R.J. Genco, Reduced chemotactic peptide binding in juvenile periodontitis; a model for neutrophil function, Biochem. Biophys. Res. Commun. 100 (1981) 1278–1284.

[12] D. Biasi, L.M. Bambara, A. Carletto, P. Caramaschi, G. Andrioli, G. Urbani, P. Bellavite, Neutrophil migration oxidative metabolism and adhesion in early onset periodontitis, J. Clin. Periodontol. 26 (1999) 563–568.

[13] S. Agarwal, M.A. Reynolds, L.D. Duckett, J.B. Suzuki, Altered free cytosolic calcium changes and neutrophil chemotaxis in patients with juvenile periodontitis, J. Periodontal. Res. 24 (1989) 149–154.

[14] F. Boulay, M. Tardif, L. Brouchon, P. Vignais, Synthesis and use of a novel *N*-formyl peptide derivative to isolate a human *N*-formyl peptide receptor cDNA, Biochem. Biophys. Res. Commun. 168 (1990) 1103–1109.

[15] Y. Le, P.M. Murphy, J.M. Wang, Formyl-peptide receptors revisited, Trends Immunol. 23 (2002) 541–548.

[16] H.D. Perez, E. Kelly, F. Elfman, G. Armitage, J. Winkler, Defective polymorphonuclear leukocyte formyl peptide receptor(s) in juvenile periodontitis, J. Clin. Invest. 87 (1991) 971–976.

[17] M.R. Gwinn, A. Sharma, E. De Nardin, Single nucleotide polymorphisms of the *N*-formyl peptide receptor in localized juvenile periodontitis, J. Periodontol. 70 (1999) 1194–1201.

[18] R. Seifert, K. Wenzel-Seifert, Defective Gi protein coupling in two formyl peptide receptor mutants associated with localized juvenile periodontitis, J. Biol. Chem. 276 (2001) 42043–42049.

[19] B.E. Jones, H.M. Miettinen, A.J. Jesaitis, J.S. Mills, Mutations of F110 and C126 of the formyl peptide receptor interfere with G-protein coupling and chemotaxis, J. Periodontol. 74 (2003) 475–484.

[20] Y. Zhang, R. Syed, C. Uygar, D. Pallos, M.C. Gorry, E. Firatli, J.R. Cortelli, T.E. Van Dyke, P.S. Hart, E. Feingold, T.C. Hart, Evaluation of human leukocyte *N*-formylpeptide receptor (FPR1) SNPs in aggressive periodontitis patients, Genes Immun. 4 (2003) 22–29.

[21] O. Quehenberger, E.R. Prossnitz, S.L. Cavanagh, C.G. Cochrane, R.D. Ye, Multiple domains of the *N*-formyl peptide receptor are required for high-affinity ligand binding. Construction and analysis of chimeric *N*-formyl peptide receptors, J. Biol. Chem. 268 (1993) 18167–18175.