Oral Microbiology Immunology 2008: 23: 170–172
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ORAL MICROBIOLOGY
AND IMMUNOLOGY

Short communication

Distribution of *Porphyromonas gingivalis fimA* genotypes in cardiovascular specimens from Japanese patients

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Nakano K, Inaba H, Nomura R, Nemoto H, Takeuchi H, Yoshioka H, Toda K, Taniguchi K, Amano A, Ooshima T. Distribution of Porphyromonas gingivalis fimA *genotypes in cardiovascular specimens from Japanese patients.*
Oral Microbiol Immunol 2008: 23: 170–172. © 2008 The Authors. Journal compilation © 2008 Blackwell Munksgaard.

Introduction: *Porphyromonas gingivalis*, a major periodontal pathogen, is gaining increasing attention for its possible association with cardiovascular diseases. Its fimbriae are classified into six genotypes (types I–V and Ib) based on the diversity of the *fimA* genes encoding the fimbrial subunits. In this study, *fimA* genotypic distribution was analyzed in *P. gingivalis*-infected cardiovascular specimens.
Methods: A total of 112 heart valves and 80 atheromatous plaque specimens were collected from patients undergoing cardiovascular surgery, as well as 56 dental plaque specimens. Bacterial DNA was extracted from each, and polymerase chain reaction analysis was carried out with a *P. gingivalis*-specific set of primers. *P. gingivalis*-positive specimens were further analyzed to discriminate the *fimA* genotype using polymerase chain reaction with *fimA* type-specific primer sets.
Results: *P. gingivalis* was detected in 10.4% of the cardiovascular specimens and 50.0% of the dental plaque samples. In the latter, type II was most frequently detected (35.7%), followed by types I (28.6%) and IV (21.4%), while types IV and II were detected with considerable frequencies of 45.0% and 30.0%, respectively, in the cardiovascular specimens. In contrast, the occurrence of type I was limited (5.0%) in the cardiovascular specimens.
Conclusion: These results suggest that specific *fimA* genotypic clones, which are reportedly associated with periodontitis, are also frequently harbored in cardiovascular specimens, indicating the possible involvement of type II and IV clones in the initiation and progression of cardiovascular diseases.

Key words: cardiovascular specimens; *fimA* genotype; polymerase chain reaction; *Porphyromonas gingivalis*

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Accepted for publication June 23, 2007

Porphyromonas gingivalis fimbriae are classified into six genotypes (types I–V, and Ib) based on the different nucleotide sequences of the *fimA* genes encoding the fimbrial subunits (4). Previously, we reported that a majority of *P. gingivalis* isolates from periodontitis patients were found to carry type II *fimA*, followed by type IV (2). In contrast, periodontally healthy subjects carried type I organisms. A significant association of *P. gingivalis* clones carrying type II *fimA* with periodontitis has also been reported by other studies conducted in other countries with different cohorts (13, 22). Based on analyses of Japanese populations, the odds ratios regarding the relationship between periodontitis and *fimA* were shown to be 77.80 for type II, 7.64 for type IV, and 6.51 for type Ib, while those for types III, V, and I were 2.51, 1.05, and 0.198, respectively (15). Thus, a strong relationship between periodontitis and *P. gingivalis* with types II, IV, and Ib *fimA* genes has been suggested.

Recently, the correlation between periodontitis and cardiovascular disease has

received attention, with several reports of *P. gingivalis* being detected in atheromatous plaque specimens (5, 8, 12). We previously analyzed the presence of bacterial DNA in cardiovascular specimens collected during surgery, and found that the detection frequency of *P. gingivalis* in 35 heart valve and 27 atheromatous plaque specimens was 11.4% and 7.4%, respectively (17). However, we were unable to analyze the prevalence of *fimA* genotypes in that small number of *P. gingivalis*-positive cardiovascular specimens; we therefore attempted to collect as many specimens as possible over a period of more than 2 1/2 years. The purpose of the present study was not only to confirm the *P. gingivalis* detection rate using a greater number of cardiovascular specimens, but also to analyze the distribution of *fimA* genotypes in the *P. gingivalis*-positive specimens.

Cardiovascular specimens were collected in the Department of Cardiovascular Surgery at Osaka Rosai Hospital, Sakai, Osaka, Japan, from December 2004 to May 2007. Those collected from December 2004 to March 2006 have also been used in other studies (17–19) and were included in the present analyses. This study was carried out after the protocol had been approved by the Ethics Committee of Osaka Rosai Hospital. The specimens consisted of 112 heart valves extirpated under the diagnosis of infective endocarditis (IE group; 14 specimens) or non-infective endocarditis (Non-IE group; 98 specimens), and 80 atheromatous plaque specimens from patients diagnosed with a thoracic or abdominal aortic aneurysm (Aneurysm group). In addition, dental plaque specimens were taken from some of the same patients during visits to the Department of Dentistry and Oral Surgery before the cardiovascular operations, with six, 27, and 23 specimens collected from subjects in the IE, Non-IE, and Aneurysm groups, respectively. Whole DNA fractions were extracted from the heart valve, atheromatous plaque, and dental plaque specimens using a method described previously (17).

First, we confirmed the appropriate extraction of DNA by polymerase chain reaction (PCR) using a ubiquitous primer set (5′-AGA GTT TGA TCM TGG CTC AG-3′ and 5′-CTG CTG CSY CCC GTA G-3′) (6). Then, *P. gingivalis* was detected using a PCR method with a *P. gingivalis*-specific primer set (5′-TGT AGA TGA CTG ATG GTG AAA ACC-3′ and 5′-ACG TCA TCC CCA CCT TCC TC-3′), as described previously (21). *P. gingivalis*-positive specimens were analyzed further to differentiate their *fimA* genotypes using a PCR method with specific primer sets for each *fimA* type (type I: 5′-CTG TGT GTT TAT GGC AAA CTT C-3′ and 5′-AAC CCC GCT CCC TGT ATT CCG A-3′; type II: 5′-ACA ACT ATA CTT ATG ACA ATG G-3′ and 5′-AAC CCC GCT CCC TGT ATT CCG A-3′; type III: 5′-ATT ACA CCT ACA CAG GTG AGG C-3′ and 5′-AAC CCC GCT CCC TGT ATT CCG A-3′; type IV: 5′-CTA TTC AGG TGC TAT TAC CCA A-3′ and 5′-AAC CCC GCT CCC TGT ATT CCG A-3′; type V: 5′-AAC AAC AGT CTC CTT GAC AGT G-3′ and 5′-TAT TGG GGG TCG AAC GTT ACT GTC-3′; and type Ib: 5′-CAG CAG AGC CAA AAA CAA TCG-3′ and 5′-TGT CAG ATA ATT AGC GTC TGC-3′), as previously reported (3, 14, 15). Genomic DNA samples extracted from *P. gingivalis* strains ATCC33277 (type I), OMZ314 (II), 6/26 (III), HG564 (IV), HNA99 (V), and HG1691 (Ib) were used as positive controls. Statistical analysis was performed using Fisher's exact probability test.

Table 1 summarizes the prevalence of *P. gingivalis* and its *fimA* genotypes in the dental plaque and cardiovascular specimens. *P. gingivalis* was detected in 10.4% of the cardiovascular specimens and 50.0% of the dental plaque samples. In the latter, type II was the most frequently detected (35.7%), followed by types I (28.6%) and IV (21.4%). Furthermore, types IV (45.0%) and II (30.0%) were frequently detected in the cardiovascular specimens, while the occurrence of type I was limited (5.0%). There were no statistically significant differences in the detection rates for each *fimA* genotype because of the low total number of *P. gingivalis*-positive specimens. However, the detection rate of the type IV genotype among all cardiovascular specimens, including the heart valve and atheromatous plaque specimens, was significantly higher than any other genotypes, except type II. As for type II, there was only one genotype (type V) with a statistically significant difference.

Studies have shown that clonal variations of fimbriae are related to bacterial virulence, which influences the development and deterioration of periodontitis (reviewed in refs 1, 4). The *fimA* types II, IV, and Ib clones are considered virulent, whereas, types I, III, and V have been shown to be either avirulent or less virulent. Thus, the *P. gingivalis*-positive specimens were divided into two groups; those with type I, III, or V fimbriae (less virulent), and those with type II, IV, or Ib (virulent). Statistical analysis showed that the rate of distribution for the virulent group in *P. gingivalis*-positive heart valve and aneurysm specimens was significantly higher than that for the less virulent group ($P < 0.01$ and $P < 0.05$, respectively) (Table 2). Taken together, these results suggest that specific *fimA* genotypic clones associated with periodontitis are also frequently harbored in cardiovascular specimens, indicating the possible involvement of types II and IV in the initiation and progression of cardiovascular diseases. These findings are the first known report of a predominant occurrence of specific *fimA* genotypes of *P. gingivalis* in cardiovascular specimens.

Clinical records of periodontal conditions were not available for the subjects in this study. In our previous study, which also studied cardiovascular patients who did not receive periodontal examinations (17), the detection rates of *P. gingivalis* in dental plaque and cardiovascular speci-

Table 1. Prevalence of *Porphyromonas gingivalis fimA* genotypes in dental plaque and cardiovascular specimens

	Dental plaque specimens			Cardiovascular specimens		
	Heart valve operation ($n = 33$)	Aneurysm operation ($n = 23$)	Total ($n = 56$)	Heart valve ($n = 112$)	Aneurysm ($n = 80$)	Total ($n = 192$)
P. gingivalis (all *fimA* types)[a]	17 (51.5%)	11 (47.8%)	28 (50.0%)	12 (10.7%)	8 (10.0%)	20 (10.4%)
fimA genotype[b]						
I	5 (29.4%)	3 (27.2%)	8 (28.6%)	1 (8.3%)	0 (0%)	1 (5.0%)[e]
Ib	0 (0%)	2 (18.2%)	2 (7.1%)[d]	1 (8.3%)	1 (12.5%)	2 (10.0%)[e]
II	6 (35.3%)	4 (36.4%)	10 (35.7%)	3 (25.0%)	3 (37.5%)	6 (30.0%)
III	1 (5.9%)	0 (0%)	1 (3.6%)[c,d]	1 (8.3%)	1 (12.5%)	2 (10.0%)[e]
IV	4 (23.5%)	2 (18.2%)	6 (21.4%)	6 (50.0%)	3 (37.5%)	9 (45.0%)
V	1 (5.9%)	0 (0%)	1 (3.6%)[c,d]	0 (0%)	0 (0%)	0 (0%)[d,e]

[a]The percentages are expressed in comparison to all of the specimens.
[b]The percentages are expressed in comparison to all of the *P. gingivalis*-positive specimens. Significant differences ($P < 0.05$) were found in comparisons among types I[c], II[d], and IV[e] in the dental plaque and cardiovascular specimens.

Table 2. Comparison of the prevalence of two groups of *fimA* genotypes in *Porphyromonas gingivalis*-positive specimens

	Dental plaque specimens			Cardiovascular specimens		
	Heart valve operation (n = 17)	Aneurysm operation (n = 11)	Total (n = 28)	Heart valve (n = 12)	Aneurysm (n = 8)	Total (n = 20)
fimA genotype						
I, III, and V	7 (41.2%)	3 (27.3%)	10 (35.7%)	2 (16.7%)	1 (12.5%)	3 (15.0%)
II, IV, and Ib	10 (58.8%)	8 (72.7%)	18 (64.3%)	10 (83.3%)[b]	7 (87.5%)[a]	17 (85.0%)[c]

Significant differences were found in comparisons between the groups ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$).

mens were similar to those in the present study. In addition, *P. gingivalis* was detected in oral samples from 91.4% of subjects with and 36.8% of those without periodontitis (4). In another study, a majority of periodontitis patients (66%) were found to carry type II clones (2). Based on these prevalence rates for *P. gingivalis* type II, half of the present subjects may have suffered from periodontitis. However, a future epidemiological study employing a larger cohort of cardiovascular patients with periodontitis is necessary to elucidate the relationships between cardiovascular diseases and specific *fimA* genotypes of *P. gingivalis*.

It has also been reported that *P. gingivalis* possesses properties that contribute to the acceleration of atherogenic plaque progression in an apolipoprotein E-deficient murine model (11), while atherosclerosis was shown to be accelerated following infection with invasive *P. gingivalis* in the same model (7). Also, invasive *P. gingivalis* and fimbriae were reported to stimulate endothelial cell activation, which is regarded as an important step in the development of atherosclerosis (20). We previously showed that type II fimbriae mediate the bacterial invasion of human epithelial cells more efficiently than other types of fimbriae (9), while a type II fimbria-deficient mutant strain underwent a dramatic loss of its invasive ability (16). In addition, a mutant strain with the *fimA* type I gene replaced with the type II gene showed enhanced invasive ability, whereas replacement of type II with type I weakened that property (10). Nevertheless, although the significant invasive ability of type II fimbriae seems to be related to the etiology of *P. gingivalis*-related cardiovascular diseases, further analyses of *P. gingivalis* strains in atheromatous plaque specimens should be conducted to elucidate other potential factors.

Acknowledgments

This study was supported by the 21st Century Center of Excellence program entitled 'Origination of Frontier BioDentistry' at Osaka University Graduate School of Dentistry supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant-in-Aid for Young Scientists (A), 18689050, from the Ministry of Education, Culture, Sports, Science and Technology.

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