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ORIGINAL RESEARCH ARTICLE

Atrial Translocation of *Porphyromonas gingivalis* Exacerbates Atrial Fibrosis and Atrial Fibrillation

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BACKGROUND: Recent studies have indicated an association between periodontitis and atrial fibrillation (AF), although the underlying mechanisms remain unclear. *Porphyromonas gingivalis (P ginigivalis)* is a causative agent of periodontal disease and is highly pathogenic. This study focused on *P gingivalis* and aimed to investigate the relationship among periodontitis, atrial translocation of *P gingivalis*, and atrial fibrosis and AF.

METHODS: An experiment was conducted using *P gingivalis*–infected C57BL/6J mice, in which *P gingivalis* was inoculated into the pulp of the molars. Immunohistochemistry was used to visualize the localization of *P gingivalis*, and loop-mediated isothermal amplification was employed to detect *P gingivalis* DNA in the left atrium. AF inducibility was examined by intracardiac stimulation. Moreover, left atrial appendage specimens were obtained from 68 patients with AF. A periodontal examination was conducted before the surgery, and the periodontal epithelial surface area and periodontal inflamed surface area, which are quantitative indices used to determine the clinical severity of periodontitis, were measured. The bacterial number of *P gingivalis* in human atrial tissue was analyzed via quantitative polymerase chain reaction. Atrial fibrosis was assessed using Azan-Mallory staining.

RESULTS: The translocation path of *P gingivalis* from the dental granuloma to the left atrium via the circulatory system was demonstrated by immunohistochemistry and loop-mediated isothermal amplification in *P gingivalis*–infected mice, which showed a higher degree of atrial fibrosis (21.9% versus 16.3%; P=0.0003) and a higher AF inducibility (30.0% versus 5.0%; P=0.04) than the control mice. Upregulation of galectin-3 and transforming growth factor-beta 1 in the left atrium was observed in *P gingivalis*–infected mice. Moreover, immunohistochemistry revealed that *P gingivalis* was also present in human atrial tissue. The number of *P gingivalis* in the human atrial tissue was positively correlated with periodontal epithelial surface area (ρ=0.35; P=0.004), periodontal inflamed surface area (ρ=0.52, P<0.0001), and the degree of atrial fibrosis (ρ=0.38; P=0.002).

CONCLUSIONS: *P gingivalis* translocation to the left atrium correlates with the clinical severity of periodontitis, which may exacerbate atrial fibrosis and AF. Atrial translocation of *P gingivalis* is a potential pathway explaining the causal relationship between periodontitis and AF.

Key Words: atrial fibrillation ■ bacterial translocation ■ periodontitis ■ *Porphyromonas gingivalis*

Although the relationship between periodontitis and atrial fibrillation (AF) has attracted attention,[1] the underlying mechanism has yet to be clarified. Inflammation plays a key role in the pathogenesis of atrial fibrosis and AF as well as in their interaction.[2] One possible mechanism linking periodontitis and AF may be the systemic inflammation elicited by periodontitis. We previously reported a positive correlation between the

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Clinical Perspective

What Is New?

- *Porphyromonas gingivalis (P gingivalis)* translocation to the left atrium correlates with the clinical severity of periodontitis.
- Atrial translocation of *P gingivalis* exacerbates atrial fibrosis and results in higher atrial fibrillation inducibility.
- Activation of the galectin-3/transforming growth factor-beta 1 pathway is a potential mechanism linking atrial translocation of *P gingivalis* to the exacerbation of atrial fibrosis.

What Are the Clinical Implications?

- Periodontal inflamed surface area may represent a clinical indicator of *P gingivalis* translocation from periodontitis lesions to the left atrium.
- Periodontal treatment, which can significantly reduce the periodontal inflamed surface area, may prevent atrial translocation of *P gingivalis* from the inflamed periodontal tissue.
- Not only periodontitis but *P gingivalis* itself may serve as an upstream target for atrial fibrillation treatment.

Nonstandard Abbreviations and Acronyms

AF	atrial fibrillation
LAA	left atrial appendage
PCR	polymerase chain reaction
PESA	periodontal epithelial surface area
PISA	periodontal inflamed surface area

severity of periodontal inflammation and the degree of atrial fibrosis, suggesting that periodontitis worsens the substrate of AF.[3] Moreover, severe periodontal inflammation is associated with the activation of systemic inflammation and a higher recurrent rate of AF after radiofrequency catheter ablation.[4] These clinical findings are supported by a few experimental studies in animals.[5,6]

Another mechanism could involve the hematogenous dissemination of periodontal pathogens from inflamed periodontal tissue to the atrial tissue of the heart. Several studies have succeeded in detecting DNA of some periodontal pathogens in the atrial and ventricular myocardium, heart valves, and atherosclerotic plaques,[7–9] although there is still no consensus on their pathogenicity to cardiovascular disease, including AF.[10] Among various periodontal pathogens, *Porphyromonas gingivalis (P gingivalis)* is considered the most influential and tightly associated with periodontitis,[11] and its pathogenic role in the oral-systemic disease connection has been evidenced.[12] Regarding AF, limited evidence suggests a positive association between *P gingivalis* and AF using serum immunoglobulin G antibody titer measurement.[13–15] In our previous study, an elevated anti–*P gingivalis* antibody titer was associated with a higher AF recurrence rate after radiofrequency catheter ablation.[15] Therefore, we focused on the pathogenic role of *P gingivalis* in the association of periodontitis with AF.

To elucidate this issue, we established a *P gingivalis* odontogenic infection mouse model that mimics *P gingivalis* hematogenous dissemination via periodontitis foci[16] and investigated the translocation of *P gingivalis* from the periodontitis foci to the left atrium and its involvement in the exacerbation of atrial fibrosis and AF. Furthermore, to validate the findings from the mouse model in humans, we conducted a clinical study using surgically resected left atrial appendages (LAAs). This study aimed to investigate the relationship between clinical periodontitis status and atrial translocation of *P gingivalis*, as well as their impact on atrial fibrosis in patients with AF.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animal Experiments

Animal experiments were conducted in strict accordance with recommendations of the *Guide for the Care and Use of Laboratory Animals* of the Hiroshima University animal research committee and the *Guidelines on Euthanasia* of the American Veterinary Medical Association. This experimental design was approved by the Hiroshima University animal experiment ethics committee (approval No. A21-129). Male C57BL/6J mice 13 weeks of age were purchased from Charles River Japan Inc (Yokohama, Japan). The mice were randomly divided into 2 groups: one with *P gingivalis* odontogenic infection and the other without infection. Each group was further divided into 2 subgroups: one was reared for 12 weeks, and the other for 18 weeks (Figure S1). The W83 strain of *P gingivalis*, classified as fimA type IV and known for its aggressiveness, was purchased from Nissui Pharmaceutical Co Ltd (Tokyo, Japan). The mouse models of *P gingivalis* infection were created at 13 weeks of age, as reported previously,[16] and are overviewed in Figure 1A and detailed in the Supplemental Methods section. All mice were housed in a specific pathogen-free facility with 12-hour light/dark cycles and had ad libitum access to water and food. Health monitoring was conducted daily. Up to 5 mice were kept in one cage. After 12 or 18 weeks of rearing, intracardiac stimulation was performed to investigate AF inducibility. Then, the left atrial and ventricular tissues were obtained for histological analyses. Randomly selected mice were euthanized without intracardiac stimulation, and their blood and left atrial tissues were subjected to serum biomarker measurements and tissue gene expression analyses in addition to histological evaluations. Moreover, in mice with *P gingivalis* infection, the jaw lesion was histologically analyzed to confirm the successful establishment of the model.

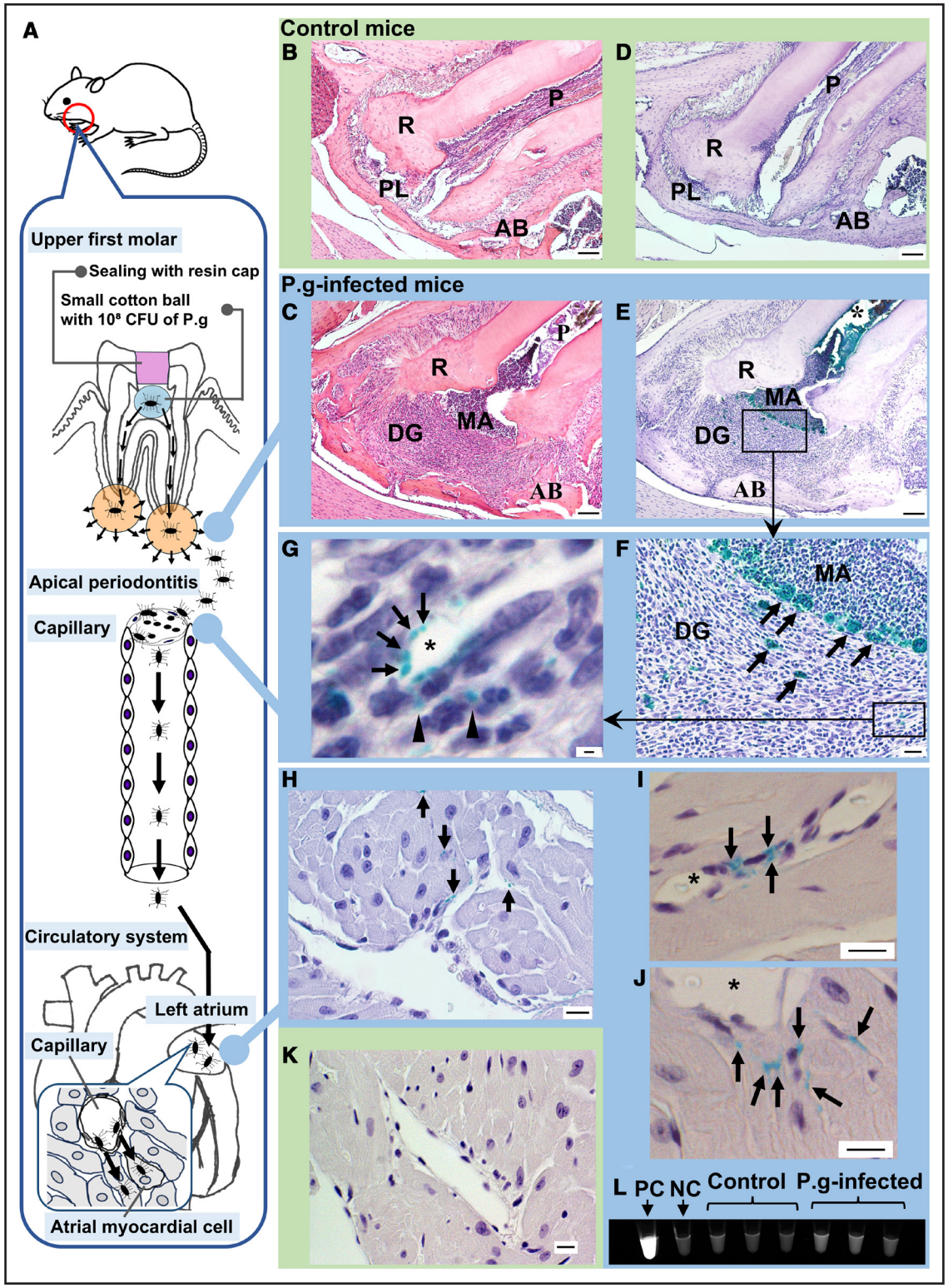


Figure 1. Odontogenic infection and atrial translocation of *Porphyromonas gingivalis (P gingivalis)* in the mouse model.
A, Overview of the mouse model. In this model, a small cotton ball containing 10^8 colony-forming units of *P gingivalis* is inoculated into the bilateral first molars and encapsulated with a resin cap. The dental granuloma serves as the sole gateway for *P gingivalis* to translocate to the circulatory system and reach the left atrium. The following histological images depict the path of *P gingivalis* encapsulated in the molar as it translocates from the dental granuloma to the left atrium. **B** and **C**, Hematoxylin-eosin staining of the root apex area in the jaw (control mouse [**B**]; *P gingivalis*–infected mouse [**C**]). Magnification ×40; scale bar=100 µm. **D** through **G**, Immunohistochemistry of *P gingivalis* in the jaw. **D**, Control mouse: *P gingivalis* is absent in the pulp and root apex areas. Magnification ×40; scale bar=100 µm. **E**, *P gingivalis*–infected mouse: an asterisk marks necrotic pulpal tissue containing abundant *P gingivalis*. Magnification ×40; scale bar=100 µm. **F**, Arrows highlight *P gingivalis*–positive macrophages. Note the linear arrangement of macrophages between the microabscess and dental granuloma. (*Continued*)

Figure 1 Continued. Magnification ×200; scale bar=20 μm. **G**, An asterisk marks the capillary, arrows indicate *P gingivalis* on endothelial surfaces, and arrowheads denote *P gingivalis*–positive neutrophils. Magnification ×1000; scale bar=1 μm. **H** through **J**, Immunohistochemistry of left atrial tissues from *P gingivalis*–infected mice. Atrial translocation of *P gingivalis* was observed in atrial myocardial cells (**H**), on the surface of capillary endothelial cells (**I**), and around capillaries (**J**). Arrows indicate *P gingivalis*, and asterisks mark capillaries. **H**, Magnification ×400; scale bar=10 μm. **I** and **J**, Magnification ×1000; scale bar=10 μm. **K**, Immunohistochemistry of *P gingivalis* of the atrial tissues in a control mouse. Magnification ×400; scale bar=10 μm. **L**, Loop-mediated isothermal amplification for *P gingivalis* DNA from the left atrium. AB indicates alveolar bone; CFU, colony-forming unit; DG, dental granuloma; MA, microabscess; NC, negative control; P, pulp; PC, positive control; P.g, *Porphyromonas gingivais*; PL, periodontal ligament; and R, root.

Quantification of the Degree of Cardiac Fibrosis

The mouse heart samples were fixed in sagittal sections, displaying all 4 chambers, and then subjected to hematoxylin-eosin and Azan-Mallory staining. Seven microscopic fields with ×200 magnification, focusing on the left atrium and left ventricle, were randomly selected and imaged. The areas of fibrosis and intercellular space expansion (which is considered the preliminary stage of fibrosis,)[17] were quantified using the BZ-X800 Analyzer software (Keyence, Osaka, Japan) (Figure S2). Measurements were averaged from 2 images of the left atrium and 5 images of the left ventricle, respectively.

Gram Staining

The methodology of the Gram staining is described in the Supplemental Methods.

Immunohistochemistry of *P gingivalis*

Immunohistochemistry of *P gingivalis* was performed on jaw and heart specimens using an anti–*P gingivalis* polyclonal antibody (1:500), provided by Kazuyuki Ishihara (Tokyo Dental University, Tokyo, Japan), as described in the Supplemental Methods. In addition, to clarify the staining specificity, the specimen was stained without the primary anti–*P gingivalis* antibody (Figure S3).

Detection of *P gingivalis* in the Blood and Left Atrium

In addition to immunohistochemistry, the translocation of *P gingivalis* into the circulatory system and the left atrium in mice was confirmed using loop-mediated isothermal amplification, which targets the 16S rRNA gene of the W81 strain of *P gingivalis*. This method is briefly described in the Supplemental Methods, as detailed in a previous report.[18]

Measurement of Serum Cytokines and Biomarkers

The blood samples were collected from the inferior vena cava before euthanasia. Serum inflammatory cytokines and biomarkers were quantified as described in the Supplemental Methods.

Cell Culture and Infection Protocol

Cell experiments were conducted using HL-1 cardiomyocytes (Sigma-Aldrich, St. Louis, MO) as described in the Supplemental Methods.

Gene Expression Analysis

Total RNA was extracted and purified from the left atrium, and quantitative real-time polymerase chain reaction (PCR) was performed. The reaction product was quantified using glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the reference gene. Detailed methodology is provided in the Supplemental Methods, with applied primers listed in Table S1.

Echocardiography

Transthoracic echocardiography was performed as described in the Supplemental Methods.

Intracardiac Stimulation

Intracardiac stimulation was conducted to investigate AF inducibility. Briefly, mice were anesthetized with isoflurane inhalation (FUJIFILM Wako Pure Chemical Corp, Osaka, Japan). A midline incision was made on the neck to expose the right internal jugular vein. An ultraminiature electrophysiology catheter (Millar Inc, Houston, TX) was inserted into the right atrium via the right internal jugular vein. The catheter was fixed at the point where the intracardiac ECG demonstrated 1:1 atrial and ventricular wave potentials. Atrial extrasystoles were counted for 180 s before the initial stimulation. Overdrive pacing at a cycle length of 40 ms (25 Hz) was applied for 30 s and repeated 3 times for each mouse with intervals of 180 s between applications. AF was defined as the occurrence of rapid, fragmented atrial electrograms with irregular R-R intervals lasting at least 3 s following at least one overdrive pacing. AF duration was measured from the last pacing stimulation to the beginning of the first P wave after returning to sinus rhythm. The PowerLab system and the LabChart software (ADInstruments, Inc, Colorado Springs, CO) were used for the study.

Clinical Study

LAA samples were obtained from 68 patients with AF who underwent LAA excision during elective cardiac surgery. All patients underwent a periodontal examination before the surgery in which periodontal epithelial surface area (PESA) and periodontal inflamed surface area (PISA) were measured to quantify their clinical periodontitis status. The study flowchart is depicted in Figure S4, and the methodology for collecting LAA samples and acquiring baseline data is detailed in the Supplemental Methods. Additionally, the primary cardiac diseases and surgical procedures are presented in Tables S2 and S3, respectively. The clinical study was conducted in accordance with the ethical principles of the Declaration of Helsinki with approval from the Hiroshima University Ethics Committee, and written informed consent was obtained from all patients.

Quantification of Clinical Periodontitis Status

Before the surgery, trained dentists conducted complete periodontal examinations and analyzed the results. The number of remaining teeth was noted through visual inspection. The

gingival margin, probing depth, and bleeding on probing in all periodontal pockets were measured. The PESA and PISA were calculated using spreadsheets on the basis of probing depth and bleeding on probing.[19] PESA represents the total epithelial area within periodontal pockets, regardless of the presence of inflammation, whereas PISA represents the total epithelial area with bleeding, indicating the degree of clinical periodontal inflammation.[19]

Quantification of Atrial Fibrosis in Humans

The 2 formalin-fixed and paraffin-embedded LAA sections were deparaffinized and subjected to Azan-Mallory staining. The degree of atrial fibrosis was determined by quantifying the average from 4 microscopic photographs, as reported previously.[3]

Quantification of the Bacterial Number of *P gingivalis* in Human Atrial Tissue

DNA was extracted from LAA specimens, and bacterial cell numbers were calculated using quantitative PCR, as described in a previous report.[20] In addition to *P gingivalis*, bacterial counts of *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* were also determined. The detailed methodology is provided in the Supplemental Methods, and applied primers are listed in Table S4.

Immunohistochemistry and Gene Expression Analysis in Humans

Immunohistochemistry of *P gingivalis* and gene expression analysis were conducted on human LAA specimens using methods similar to those used in mice. The primers used in humans are listed in Table S5.

Statistical Analysis

Data were tested for normality using the Anderson-Darling test. Continuous variables are presented as mean (±SD) or as median (interquartile range), and categorical variables are presented as proportions. In the animal and cell experiments, the degrees of fibrosis and intercellular space expansion, biomarker levels, gene expression levels, and the prevalence of extrasystoles were compared using Student *t* test or Wilcoxon rank-sum test, as appropriate. AF inducibility was compared between the 2 groups (*P gingivalis*–infected versus control) using the χ^2 test. In the clinical study, correlations between continuous variables (PESA, PISA, the degree of fibrosis, and the number of *P gingivalis*) were assessed using the Spearman rank correlation coefficient. Locally weighted scatterplot smoothing was used to visualize trends in the data. The associations between clinical factors and the number of *P gingivalis* in relation to the degree of fibrosis were assessed using univariable linear regression analyses, and variables with $P<0.10$ in the univariable analysis were included in the multivariable models. The standardized partial regression coefficient was presented. In addition, patients were divided into 4 groups on the basis of the quartile of the number of *P gingivalis*, and gene expression was compared using Kruskal-Wallis test. The JMP software version 15.0 (SAS Institute, Cary, NC) was used to perform all statistical analyses, and $P<0.05$ indicated statistical significance.

RESULTS

Animal Experiments

Atrial Translocation of P gingivalis in the Mouse Model

Figure 1 shows representative histological photographs of the jaw and the left atrium in this mouse model, which trace the path of *P gingivalis* encapsulated in the molar as it translocates from the dental granuloma to the left atrium via the circulatory system. In the jaws of the control mice, the pulp is viable, and a homogeneous width of the periodontal ligament is observed between the tooth root and the alveolar bone (Figure 1B). In contrast, in the jaws of the *P gingivalis*–infected mice, the pulp was completely necrotic, and numerous neutrophils had accumulated (forming a microabscess) at the root apex area. Inflammatory granulation tissue, associated with alveolar bone destruction (dental granuloma), proliferated and surrounded the microabscess (Figure 1C). Figure 1D through 1K shows the immunohistochemistry of *P gingivalis*. Although *P gingivalis* was not identified in the control mice (Figure 1D), numerous *P gingivalis* were observed in necrotic pulpal tissue of the *P gingivalis*–infected mice (Figure 1E). In the microabscess and dental granuloma at the root apex area of the *P gingivalis*–infected mice, neutrophils and macrophages phagocytosing *P gingivalis* were observed. Many *P gingivalis*–positive macrophages lineally aligned at the interface between the microabscess and dental granuloma (Figure 1E and 1F). The detailed observation showed microvascular invasion of *P gingivalis* in the dental granuloma (Figure 1G). *P gingivalis* was also detected in the atrial myocardial cells, indicating its atrial translocation (Figure 1H). Moreover, *P gingivalis* was observed on capillary endothelial cells (Figure 1I) and around the capillaries in the left atrium (Figure 1J), indicating its path of translocation to the left atrium. Conversely, *P gingivalis* was not detected in the left atrium of control mice (Figure 1K). Gram staining showed a similar distribution of gram-negative bacteria, likely *P gingivalis*, in the jaw and left atrium of *P gingivalis*–infected mice (Figure S5). Additionally, the loop-mediated isothermal amplification detected *P gingivalis* DNA in the left atrium (Figure 1L), as well as in the blood (Figure S6).

P gingivalis, Atrial Fibrosis, and AF Inducibility in the Mouse Model

Representative microscopic images indicate that increased collagen fibers (colored blue) were observed in the expanded intercellular spaces between atrial myocardial cells in *P gingivalis*–infected mice (Figure 2A). Additionally, *P gingivalis*–infected mice exhibited a significantly higher degree of fibrosis and intercellular space in the left atrium compared to control mice 12 and 18 weeks after *P gingivalis* infection (Figure 2B through 2E). The degree of fibrosis and intercellular space in the

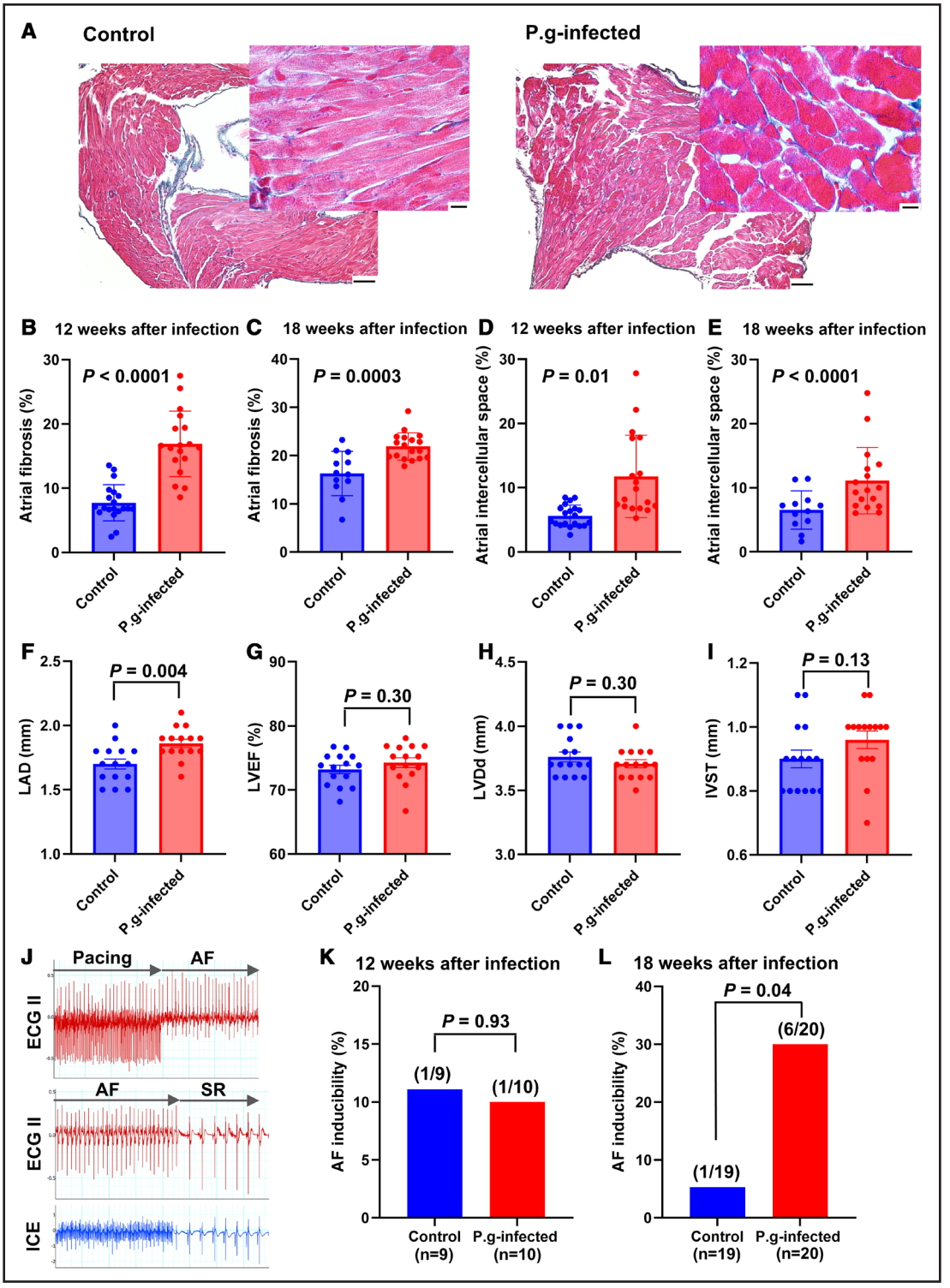


Figure 2. *Porphyromonas gingivalis (P gingivalis)* infection, atrial fibrosis, and atrial fibrillation inducibility.
A, Representative Azan-Mallory-stained images of left atrial tissue from the control group (**left**) and *P gingivalis*–infected group (**right**) 18 weeks after infection. Low magnification ×100; scale bar=50 μm; high magnification ×400; scale bar=10 μm. **B** through **E**, Quantitative comparison of left atrial fibrosis and intercellular space between the control and *P gingivalis*–infected groups. **F** through **I**, Echocardiographic parameter comparisons between the control and *P gingivalis*–infected groups 12 weeks after infection. **J**, Representative ECG traces showing atrial fibrillation induction (**top**) and termination (**bottom**) after atrial overdrive pacing (cycle length, 40 ms; 3 repetitions with 180-s intervals). **K** and **L**, Atrial fibrillation inducibility comparisons between the control and *P gingivalis*–infected groups 12 weeks (**K**) and 18 weeks (**L**) after infection. Error bars represent SE. AF indicates atrial fibrillation; ECG II, body surface II ECG; ICE, intracardiac ECG; IVST, interventricular septum thickness; LAD, left atrial dimension; LVDd, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; P.g, *Porphyromonas gingivalis*; and SR, sinus rhythm.

left ventricle was also higher in the *P gingivalis*–infected mice than in control mice (Figure S7). Echocardiographic analysis revealed a significantly larger left atrial dimension in the *P gingivalis*–infected mice compared to the control mice (Figure 2F). In contrast, ventricular parameters were not different between the 2 groups (Figure 2G through 2I). Atrial overdrive pacing with a cycle length of 40 ms was applied to examine AF inducibility (Figure 2J), and detailed information, including procedural time, baseline heart rate, and AF inducibility and duration in each mouse, is described in Table S6. Although the AF inducibility was not different between the 2 groups 12 weeks after *P gingivalis* infection (Figure 2K), *P gingivalis*–infected mice showed a significantly higher AF inducibility than the controls 18 weeks after *P gingivalis* infection (Figure 2L). The prevalence of atrial extrasystoles was not different between the 2 groups (Figure S8).

Figure 3 shows the inflammatory cytokine and biomarker levels in the serum and the mRNA expression levels of fibrosis-related molecules in the left atrial tissue. The serum GAL3 (galectin 3) level was significantly higher (Figure 3A), and TGF-b1 (transforming growth factor beta 1) level tended to be higher (Figure 3B) in *P gingivalis*–infected mice than in control mice. No significant difference was observed in serum levels of interleukin (IL)-6, IL10, and tumor necrosis factor-alpha between the 2 groups (Figure 3C through 3E). Furthermore, levels of high-sensitive C-reactive protein, N-terminal pro-B-type natriuretic peptide, and cardiac troponin I did not differ between the 2 groups (Figure 3F through 3H). In contrast, mRNA expression of *Gal3*, *Tgfb1*, and *Il6* in the left atrium were significantly higher in the *P gingivalis*–infected mice than in the controls (Figure 3I through 3N).

Cell Experiments

In HL-1 cells with *P gingivalis* infection, the mRNA expression of *TGFB1* was significantly upregulated 1 day after infection (Figure S9).

Clinical Study

Periodontitis Status and Clinical and Histological Characteristics

Baseline clinical characteristics are listed in Table S7. Table S8 compares the relationships between the PESA and PISA scores and specific clinical factors. PISA was associated with higher hemoglobin A1C levels and neutrophil/lymphocyte ratio, whereas PESA was associated with a higher neutrophil/lymphocyte ratio. In addition, patients with high PISA tended to have a higher prevalence of LAA thrombus, hypertension, and diabetes mellitus as well as elevated levels of N-terminal pro-B-type natriuretic peptide. However, these differences were not statistically significant. Figure 4 shows that PISA was positively correlated with the degree of atrial fibrosis, whereas PESA was not.

Clinical Periodontitis Status and Atrial Translocation of P gingivalis

P gingivalis was also detected in human atrial tissue by quantitative PCR, and the number of *P gingivalis* was positively correlated with PESA and PISA. The PISA demonstrated a stronger correlation with the number of *P gingivalis* than the PESA (Figure 5A and 5B). In contrast, the numbers of *F nucleatum* and *A actinomycetemcomitans* in the human atrial tissue were below the detection sensitivity. Furthermore, *P gingivalis* was detected in the human atrial tissue using immunohistochemistry. Representative immunohistochemistry images are shown in Figures 5C, 5D, and S10. As shown in Figure 5C, *P gingivalis* was found in and around the capillary. As shown in Figure 5D, *P gingivalis* was detected inside the atrial myocardium.

Atrial Translocation of P gingivalis and Atrial Fibrosis in Humans

The number of *P gingivalis* in the atrial tissue was found to be positively correlated with the degree of atrial fibrosis (Figure 5E). In the multivariable linear regression analysis, the positive association between the number of *P gingivalis* and the degree of atrial fibrosis was significant, regardless of other clinical confounders (Table). The case with the highest PISA, in which a higher number of *P gingivalis* was detected in the atrial tissue, was presented as a representative case, illustrated by its immunohistochemistry photograph (Figure 5F). The patients were divided into 4 groups (Q1–Q4) on the basis of the quartile of the number of *P gingivalis*. No significant differences were observed in the mRNA expression levels of fibrosis-related molecules in human atrial tissue among the quartile groups (Figure 5G through 5K).

DISCUSSION

This animal model, which mimics hematogenous dissemination of *P gingivalis* via periodontitis foci, revealed that *P gingivalis* translocates from the periodontitis foci to the atrial tissue via the circulatory system, which exacerbates the progression of atrial fibrosis and increases AF inducibility. In addition, upregulation of IL6, Gal3, and TGFb1 was observed in the atrial tissue, related to *P gingivalis* infection. Atrial translocation of *P gingivalis* and its association with atrial fibrosis was also confirmed in human atrial tissue. Moreover, the amount of *P gingivalis* translocating to the left atrium was observed to correlate with the clinical severity of periodontitis, as indicated by PESA and PISA.

To the best of our knowledge, this study is the first to reveal a pathogenic impact of *P gingivalis* on atrial fibrosis and AF, which may represent a potential pathway

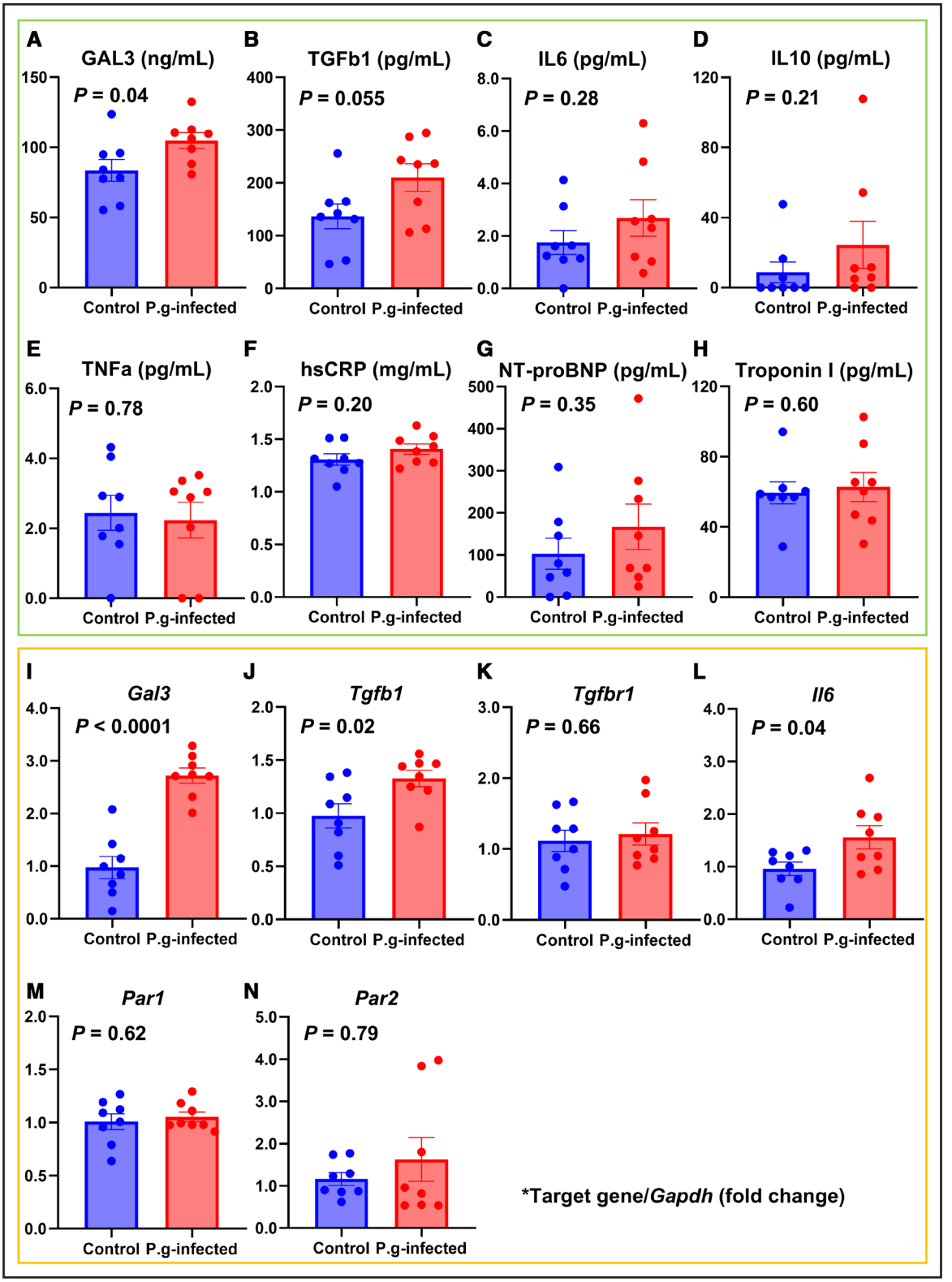


Figure 3. Serum inflammatory cytokine levels and gene expression in the left atrium.
Serum inflammatory cytokine and biomarker levels (**A** through **H**) and mRNA expression in the left atrium (**I** through **N**) were compared between the control and *Porphyromonas gingivalis (P gingivalis)*–infected groups euthanized without atrial stimulation 18 weeks after infection. Data were analyzed using the Student *t* test or Wilcoxon rank sum test, as appropriate. Gene expression levels were normalized to *GAPDH* as the reference gene. Error bars represent SE. GAL3/*Gal3* indicates galectin-3; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; hsCRP, high-sensitive C-reactive protein; IL6/*Il6*, interleukin-6; IL10, interleukin-10; NT-proBNP, N-terminal pro-brain natriuretic peptide; *Par1*, protease-activated receptor 1; *Par2*, protease-activated receptor 2; P.g, *Porphyromonas gingivalis*; TGFb1/*Tgfb1*, transforming growth factor-beta 1; *Tgfbr1*, transforming growth factor beta receptor 1; and TNFa, tumor necrosis factor-alpha.

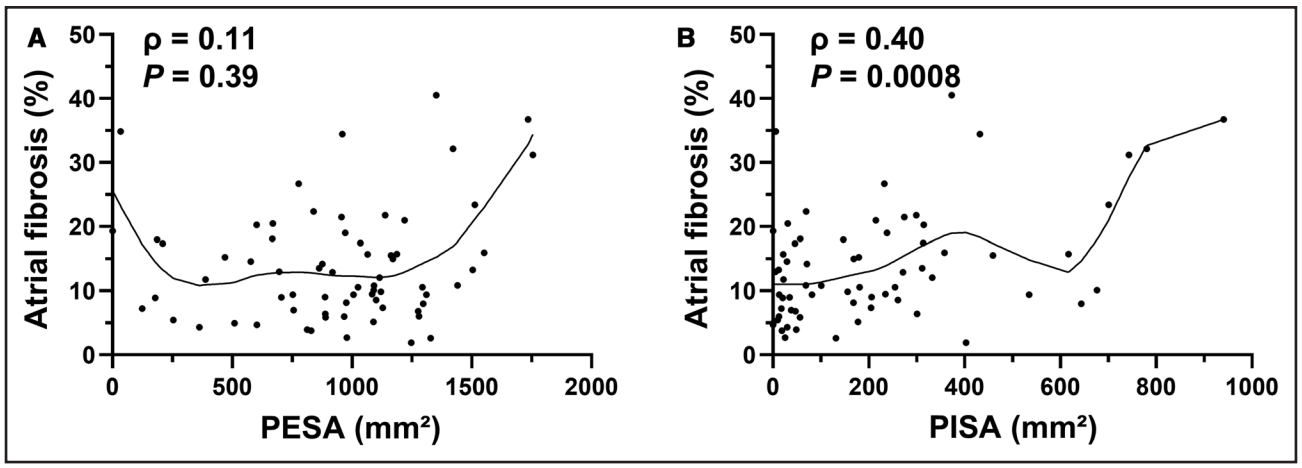


Figure 4. Correlations of periodontal epithelial surface area and periodontal inflamed surface area with the degree of atrial fibrosis.

Locally weighted scatterplot smoothing curves illustrate the relationships of periodontal epithelial surface area and periodontal inflamed surface area with the degree of atrial fibrosis. Spearman rank correlation coefficient quantifies these correlations. PESA indicates periodontal epithelial surface area; and PISA, periodontal inflamed surface area.

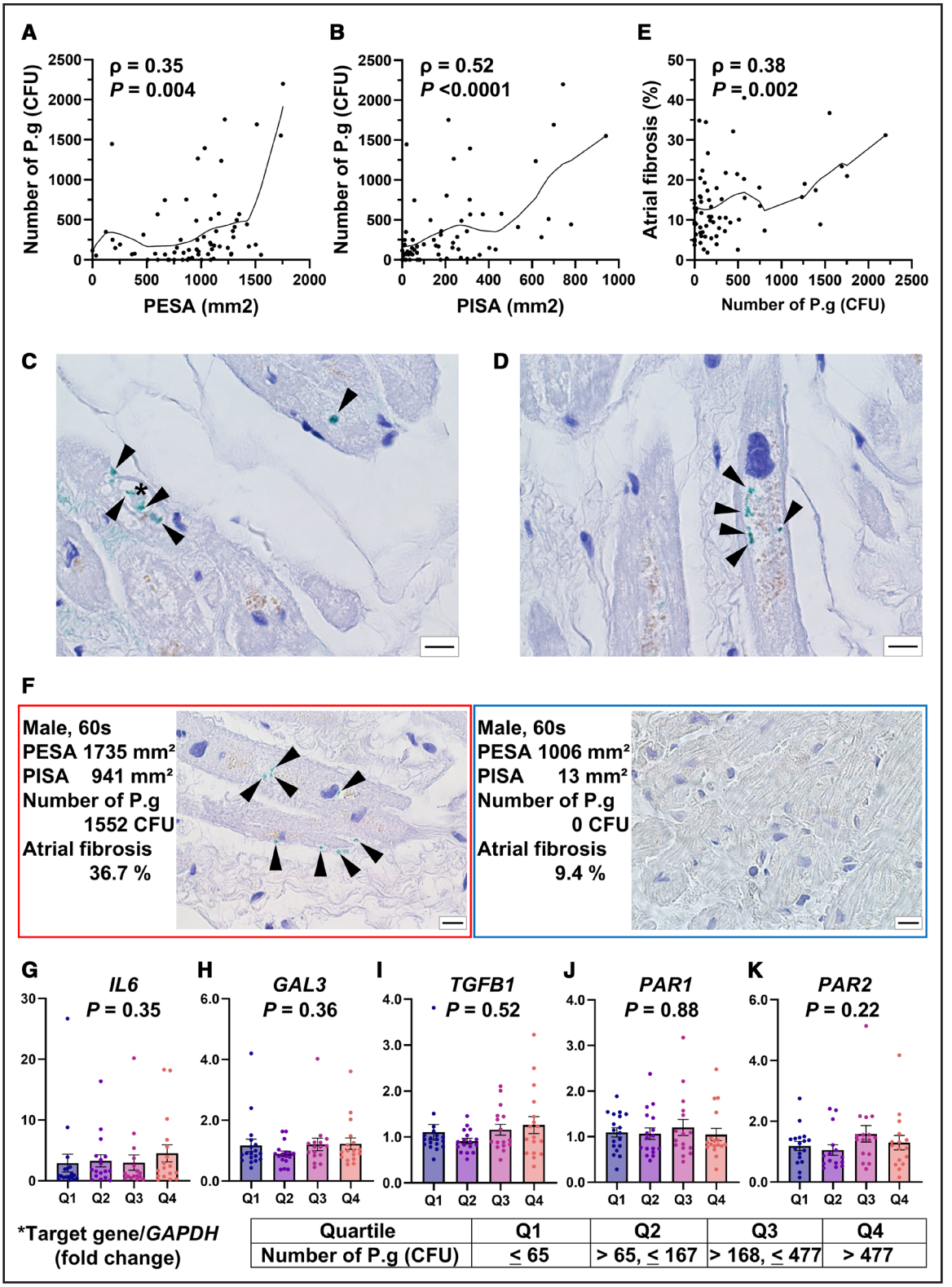
explaining the causal relationship between periodontitis and AF.

Periodontal Inflammation, Atrial Fibrosis, and AF

Atrial fibrosis is a common manifestation of atrial myopathy and is closely linked to atrial arrhythmogenesis.[21] In the process of atrial myopathy, inflammation plays a key role in the progression of atrial fibrosis and the occurrence of AF through several pathways, including the activation of IL1b, IL6, IL8, tumor necrosis factor-alpha, and monocyte chemoattractant protein 1.[22] Periodontitis, in turn, is a chronic inflammation in the periodontal tissue. From the inflamed periodontal tissue, inflammatory mediators spread hematogenously and impact some systemic diseases (eg, diabetes mellitus, atherosclerosis, and rheumatoid arthritis),[23] and patients with periodontitis show elevated levels of some inflammatory cytokines in the saliva and serum,[24] which may influence the progression of atrial fibrosis and AF occurrence. Previous epidemiological studies have indicated a positive association between periodontitis and AF.[25–27] A recent meta-analysis revealed that periodontitis increases the risk of AF (or atrial flutter) by 1.3-fold.[1] Although other conventional risk factors, such as habitual alcohol consumption,[28] may confound the association between periodontitis and AF, and the causal relationship remains unclear, our previous studies have reported a positive correlation between the degree of clinical periodontal inflammation, quantified by PISA, and the extent of atrial fibrosis.[3] Moreover, another study from our group revealed that high PISA levels, associated with elevated circulating inflammatory cytokines such as C-reactive protein, IL1b, IL6, and tumor necrosis factor-alpha, predicted the recurrence of AF after radiofrequency catheter ablation.[4] Supporting these clinical findings, previous animal experiments using a periodontitis canine model with ligature injury observed the development of experimental AF and demonstrated mild inflammation in the atria.[5] Therefore, atrial inflammation induced by systemic inflammation attributable to periodontitis is one plausible mechanism linking periodontitis and AF.

Dose *P gingivalis* Translocate to the Atrium?

Bacterial translocation may be another underlying mechanism explaining the relationship between periodontitis and AF, and we focused on *P gingivalis*, which is one of the most pathogenic periodontal bacteria. Our previous study showed an association between elevated serum anti–*P gingivalis* antibodies and a higher recurrence rate of AF after radiofrequency catheter ablation,[15] and some studies have reported a positive association between anti–*P gingivalis* titers and the prevalence of AF among the general population[13] and patients with ischemic stroke.[14] In addition, *P gingivalis* has been detected in some organs, including the brain, liver, and placenta, suggesting its pathogenic role in some systemic diseases beyond AF.[12] Although the atrial translocation of *P gingivalis* has not been well elucidated, this study provides evidence in animal models and humans. Interestingly, this study found that *P gingivalis* was detected in human atrial tissue, and its presence correlated with the clinical severity of periodontitis. Furthermore, atrial translocation of *P gingivalis* was also identified in a mouse model that is unique in mimicking the hematogenous dissemination of *P gingivalis* from periodontitis foci. In this model, *P gingivalis* was applied to the pulp chamber, and it led to the development of chronic apical periodontitis (dental granuloma). Immunolocalization of *P gingivalis* was observed not only in macrophages and neutrophils but also in capillary endothelial cells within the dental granuloma. These



Quartile	Q1	Q2	Q3	Q4
Number of P.g (CFU)	≤ 65	> 65, ≤ 167	> 168, ≤ 477	> 477

Figure 5. Clinical periodontitis status, atrial translocation of *Porphyromonas gingivalis (P gingivalis),* and atrial fibrosis in humans.

A and **B**, Locally weighted scatterplot smoothing curves illustrate the relationships of periodontal epithelial surface area and periodontal inflamed surface area with the number of *P gingivalis* in human atrial tissue. Spearman rank correlation coefficient quantifies these correlations. **C** and **D**, Representative immunohistochemistry images of *P gingivalis* in human atrial tissue. *P gingivalis* was observed within and around capillaries (**C**) and inside atrial myocardial cells (**D**). *P gingivalis* appears green (arrowheads), and the capillary is marked with an asterisk. Magnification ×400; scale bar=10 μm. **E**, A locally weighted scatterplot smoothing curve illustrates the relationship between the number of *P gingivalis* in human atrial tissue and the degree of atrial fibrosis. Spearman rank correlation coefficient quantifies the correlation. **F**, Representative cases with high (*Continued*)

Figure 5 Continued. periodontal inflamed surface area (**left**) and low periodontal inflamed surface area (**right**). *P gingivalis* is indicated by arrowheads. Magnification ×400; scale bar=10 μm. **G** through **K**, mRNA expression levels of inflammation- and fibrosis-related genes in the left atrium. Quartile groups Q1 to Q4 were stratified by the number of *P gingivalis* in atrial tissue. Gene expression was normalized to *GAPDH* as the reference gene and analyzed using the Kruskal-Wallis test. Error bars represent SE. AF indicates atrial fibrillation; CFU, colony-forming unit; *GAL3*, galectin-3; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *IL6*, interlukin-6; *PAR1*, protease-activated receptor 1; *PAR2*, protease-activated receptor 2; PESA, periodontal epithelial surface area; P.g, *Porphyromonas gingivalis*; PISA, periodontal inflamed surface area; and *TGFB1*, transforming growth factor-beta 1.

findings indicate that a dental granuloma is a possible reservoir of *P gingivalis*, which can continuously release *P gingivalis* into the circulatory system. Moreover, *P gingivalis* was encapsulated in the molar lesions by resin caps. Therefore, the dental granuloma served as the only gateway for the translocation of *P gingivalis* to the circulatory system and the left atrium. The results from the animal and clinical studies indicate that *P gingivalis* translocates to the left atrium and that inflamed periodontal tissue is the plausible gateway for its invasion.

Several studies have shown that *P gingivalis* can invade host cells and survive intracellularly by avoiding autophagosomal degradation in myeloid dendritic cells,[29] gingival fibroblasts, and gingival epithelial cells.[30] In a recent report, the invasion of *P gingivalis* into cardiomyocytes was observed in vitro, and intracytoplasmic *P gingivalis* inhibited the formation of autophagolysosomes to survive within the autophagosome.[31] Additionally, in our immunohistochemistry photographs, *P gingivalis* was observed inside the atrial myocardial cells. This suggests that *P gingivalis*, which escapes autophagosome-related bacterial killing, survives in the atrial myocardial cells in vivo and negatively affects atrial remodeling, leading to the onset and perpetuation of AF. Further studies are needed to clarify the effects of *P gingivalis* translocation on atrial myocardial cells and atrial fibroblasts in the future.

P gingivalis, Atrial Fibrosis, and AF

This animal experiment revealed that *P gingivalis*–infected mice showed a higher degree of atrial fibrosis and intercellular space expansion than the control mice. An association between *P gingivalis* infection and upregulation of IL6, Gal3, and TGFb1 was observed in the left atrium. Atrial fibrosis and its preliminary intercellular space expansion are known to form substrates for AF, clinically characterized by atrial voltage reduction, slow conduction zones, and fractionated electrograms.[17] Indeed, *P gingivalis*–infected mice showed increased AF inducibility. Furthermore, the clinical study demonstrated a correlation between atrial translocation of *P gingivalis* and atrial fibrosis in patients with AF. Serum biomarkers and echocardiographic parameters showed no evidence of inflammation, heart failure, or myocarditis in this animal model. Although the involvement of mild systemic inflammation cannot be completely ruled out, the local atrial response to the translocated *P gingivalis* is considered the most important factor in our animal model.

IL6 plays an important role in fibrotic disorders in various organs, as it promotes the transformation of fibroblasts into myofibroblasts through the Janus kinase/signal transducer and activator of transcription 3 signaling pathway.[32] It is also widely accepted that Gal3 plays a key role in the development of fibrotic lesions in the heart,[33] lungs,[34] kidneys,[35] and liver.[36] In the left atrium, Gal3 is known to retain TGFb1 receptors on the cell membrane and amplify the TGFb1/Smad pathway in atrial myocardial cells and myofibroblasts. This activation exacerbates atrial fibrosis and perpetuates AF.[37] In this regard, Nagasaki et al.[38] reported previously that *P gingivalis*, when orally infected, translocates to the fatty liver tissue in the same *P gingivalis* infection mouse model with high-fat diet–induced fatty liver, exacerbating liver fibrosis and inflammation. They revealed that *P gingivalis* induces Gal3 production from hepatocytes and hepatic stellate cells (liver myofibroblasts) and amplifies the TGFb1/Smad pathway, leading to liver fibrosis in an autocrine and paracrine manner.[38] Taken together with the current results, a similar mechanism is possibly responsible for the aggravation of atrial fibrosis induced by *P gingivalis*.

Previous findings suggest that enzymatic activation of protease-activated receptor (PAR) 1/PAR2 may also be involved in the progression of fibrosis through crosstalk with TGFb1 signaling.[39] Interestingly, gingipain, a trypsin-like enzyme secreted by living *P gingivalis*, activates PAR1/PAR2 in gingival fibroblasts and induces TGFb1 production.[40] Upregulation of TGFb1 through enzymatic activation of PAR1/PAR2 is a critical mechanism in liver fibrosis. Nagasaki et al[38] showed that *P gingivalis* infection promotes TGFb1 production from hepatocytes and hepatic stellate cells through gingipain, resulting in hepatic stellate cell activation. It has also been reported that TGFb1 signaling, activated via PAR1/PAR2, promotes cardiac fibrosis,[41] and we have shown upregulation of TGFb1 expression in HL-1 myocardial cells induced with *P gingivalis* infection. Therefore, gingipain, which is secreted from *P gingivalis* translocated to the left atrium, potentially acts on atrial myocardial cells and myofibroblasts, including TGFb1 production via PAR1/PAR2 activation, which ultimately leads to fibrosis.

Our animal experiment revealed that *P gingivalis* infection induces ventricular fibrosis with a pattern resembling

Table. Association of Clinical Factors and *Porphyromonas gingivalis* Infection With Atrial Fibrosis

	Univariable		Multivariable model 1		Multivariable model 2	
Variables	β	*P* value	β	*P* value	β	*P* value
Age, y	0.216	0.08	0.081	0.46	0.087	0.43
Sex, male (yes or no)	0.112	0.36	…	…	…	…
Body mass index, kg/m^2	−0.023	0.85	…	…	…	…
AF duration, months	0.441	0.0002	0.268	0.01	0.301	0.005
AF type, nonparoxysmal (present or absent)	0.378	0.002	0.188	0.07	0.229	0.03
Current or former smoking (yes or no)	−0.185	0.13	…	…	…	…
Habitual drinking (yes or no)	0.253	0.04	0.148	0.14	0.110	0.27
Hypertension (present or absent)	0.156	0.20	…	…	…	…
Diabetes mellitus (present or absent)	0.045	0.72	…	…	…	…
$CHADS_2$ score	0.294	0.01	0.117	0.29	0.104	0.35
Hemoglobin A1c level, %	0.174	0.16	…	…	…	…
LDL cholesterol level, mg/dL	−0.067	0.60	…	…	…	…
Triglyceride level, mg/dL	−0.041	0.75	…	…	…	…
NT-proBNP level, pg/dL	0.133	0.28	…	…	…	…
PESA, mm^2	0.144	0.24	…	…	…	…
PISA, mm^2	0.468	<0.0001	0.362	0.0005	…	…
Number of *P gingivalis*, CFU	0.412	0.0005	…	…	0.345	0.0009

Univariable and multivariable linear regression analyses were used for the degree of atrial fibrosis (n=68). Variables with *P*<0.10 in the univariable analysis were included in the multivariable models. Periodontal inflamed surface area and the number of *Porphyromonas gingivalis* (*P gingivalis)* were applied separately to multivariate models 1 and 2, respectively.

AF indicates atrial fibrillation; β, standardized partial regression coefficient; CFU, colony-forming unit; $CHADS_2$, congestive heart failure, hypertension, age, diabetes, previous stroke/transient ischemic attack; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PESA, periodontal epithelial surface area; and PISA, periodontal inflamed surface area.

that observed in the left atrium. However, the effect is notably more pronounced in the left atrium. This study primarily focused on the left atrium and AF, leaving the relationship between *P gingivalis* and ventricular remodeling, as well as its clinical significance, to be elucidated in future investigations.

Clinical Implications and Perspectives

First, this study indicates the importance of periodontal care in preventing progression of atrial fibrosis and AF. We have recently demonstrated that nonsurgical periodontal treatment improves the PISA and may contribute to successful rhythm control in patients with AF.[4] This study showed that atrial translocation of *P gingivalis* is observed in correlation with the severity of PISA. Thus, periodontal treatment not only suppresses the inflammation in the periodontal tissues but also possibly inhibits the atrial translocation of *P gingivalis* by blocking the gateway for its invasion. Accumulating evidence suggests that periodontitis should be recognized as a modifiable risk factor for AF and that periodontal care should be considered as an upstream treatment for AF in addition to addressing conventional risk factors.[42] Furthermore, targeting *P gingivalis* or its byproducts, such as gingipain, could lead to the development of novel therapeutic strategies for AF.

Study Limitations

In the animal study, the electrophysiological impact of *P gingivalis* on the left atrium was described by AF inducibility following atrial pacing. However, further electrophysiological studies, such as atrial optical mapping and atrial action potential measurements, could not be conducted in this study and will be the subject of future investigations. Several limitations should also be noted regarding the clinical study. First, LAA samples were collected from patients with underlying cardiac disease that necessitated cardiac surgery. However, obtaining LAA samples from healthy people is ethically impossible. This may be the reason behind our inability to reproduce the results of our gene expression analyses, which were obtained in the animal experiment, using the human LAA samples. Additionally, we did not evaluate atrial tissues of patients who were in sinus rhythm. Second, this was a cross-sectional study with a relatively small sample size. Particularly, the small number of patients with high-range PISA values (>500 mm^2) should be considered when interpreting the correlation between PISA and other factors. Third, although we calculated the extent of atrial fibrosis using an average of measurements from 4 different views of the LAA, we may have missed areas of disease. Additionally, the number of *P gingivalis* was calculated from a single section of the LAA, which

was randomly resected. The bacterial localization in the LAA or the entire left atrium was not examined. Fourth, referral and selection bias may have existed, especially in patients referred for a thoracoscopic standalone LAA excision.

Conclusions

In patients with severe periodontitis, a higher number of *P gingivalis* translocates to the left atrium, which may exacerbate atrial fibrosis and result in a worse AF outcome. This suggests that periodontal treatment, which can block the gateway for bacterial translocation, is important for managing AF in addition to modifying conventional risk factors. Moreover, *P gingivalis* itself may be a potential novel therapeutic target for AF.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Supplemental Methods
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REFERENCES

1. Leelaviwat N, Kewcharoen J, Poomprakobsri K, Trongtorsak A, Del Rio-Pertuz G, Abdelnabi M, Benjanuwattra J, Navaravong L. Periodontal disease and risk of atrial fibrillation or atrial flutter: a systematic review and meta-analysis. *J Arrhythm*. 2023;39:992–996. doi: 10.1002/joa3.12921
2. Guo Y, Lip GY, Apostolakis S. Inflammation in atrial fibrillation. *J Am Coll Cardiol*. 2012;60:2263–2270. doi: 10.1016/j.jacc.2012.04.063
3. Miyauchi S, Nishi H, Ouhara K, Tokuyama T, Okubo Y, Okamura S, Miyamoto S, Oguri N, Uotani Y, Takasaki T, et al. Relationship between periodontitis and atrial fibrosis in atrial fibrillation: histological evaluation of left atrial appendages. *JACC Clin Electrophysiol*. 2023;9:43–53. doi: 10.1016/j.jacep.2022.08.018
4. Miyauchi S, Ouhara K, Shintani T, Tokuyama T, Okubo Y, Okamura S, Miyamoto S, Oguri N, Uotani Y, Takemura T, et al. Periodontal treatment during the blanking period improves the outcome of atrial fibrillation ablation. *J Am Heart Assoc*. 2024;13:e033740. doi: 10.1161/JAHA.123.033740
5. Yu G, Yu Y, Li YN, Shu R. Effect of periodontitis on susceptibility to atrial fibrillation in an animal model. *J Electrocardiol*. 2010;43:359–366. doi: 10.1016/j.jelectrocard.2009.12.002
6. Xiang J, Cao J, Shen J, Wang X, Liang J, Li X, Zhang L, Tang B. Bioinformatics analysis reveals the potential common genes and immune characteristics between atrial fibrillation and periodontitis. *J Periodontal Res*. 2024;59:104–118. doi: 10.1111/jre.13192
7. Nakano K, Nemoto H, Nomura R, Inaba H, Yoshioka H, Taniguchi K, Amano A, Ooshima T. Detection of oral bacteria in cardiovascular specimens. *Oral Microbiol Immunol*. 2009;24:64–68. doi: 10.1111/j.1399-302X.2008.00479.x
8. Raffaelli L, Santangelo R, Falchetti P, Galluccio F, Luciani N, Anselmi A, Nowzari H, Verdugo F, Fadda G, D'Addona A. Examination of periodontal pathogens in stenotic valve specimens and in whole blood samples in patients affected by aortic valve stenosis and chronic periodontitis. *Int J Immunopathol Pharmacol*. 2010;23:561–566. doi: 10.1177/039463201002300218
9. Atarbashi-Moghadam F, Havaei SR, Havaei SA, Hosseini NS, Behdadmehr G, Atarbashi-Moghadam S. Periopathogens in atherosclerotic plaques of patients with both cardiovascular disease and chronic periodontitis. *ARYA Atheroscler*. 2018;14:53–57. doi: 10.22122/arya.v14i2.1504
10. Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, Taubert KA, Newburger JW, Gornik HL, Gewitz MH, et al; American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, Council on Epidemiology and Prevention, Council on Peripheral Vascular Disease, and Council on Clinical Cardiology. Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association?: a scientific statement from the American Heart Association. *Circulation*. 2012;125:2520–2544. doi: 10.1161/CIR.0b013e31825719f3
11. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol*. 1998;25:134–144. doi: 10.1111/j.1600-051x.1998.tb02419.x
12. Bregaint S, Boyer E, Fong SB, Meuric V, Bonnaure-Mallet M, Jolivet-Gougeon A. *Porphyromonas gingivalis* outside the oral cavity. *Odontology*. 2022;110:1–19. doi: 10.1007/s10266-021-00647-8
13. Hoshino T, Kaneko N, Yoshihara A, Iwasaki M, Suwama K, Ito Y, Tanaka J, Narita I, Ogawa H. Associations between plasma antibody levels against *Porphyromonas gingivalis* and atrial fibrillation among community-dwelling older individuals in Japan: a cross-sectional study. *Oral Health Prev Dent*. 2023;21:339–346. doi: 10.3290/j.ohpd.b4528813
14. Nezu T, Hosomi N, Aoki S, Nishi H, Nakamori M, Shiga Y, Imamura E, Shintani T, Kawaguchi H, Maruyama H. Assessment of serum IgG titers to various periodontal pathogens associated with atrial fibrillation in acute stroke patients. *J Stroke Cerebrovasc Dis*. 2022;31:106301. doi: 10.1016/j.jstrokecerebrovasdis.2021.106301
15. Miyauchi S, Tokuyama T, Shintani T, Nishi H, Hamamoto Y, Ouhara K, Furusho H, Miyauchi M, Komatsuzawa H, Nakano Y. Periodontitis and the outcome of atrial fibrillation ablation: *Porphyromonas gingivalis* is related to atrial fibrillation recurrence. *J Cardiovasc Electrophysiol*. 2021;32:1240–1250. doi: 10.1111/jce.14952
16. Furusho H, Miyauchi M, Hyogo H, Inubushi T, Ao M, Ouhara K, Hisatune J, Kurihara H, Sugai M, Hayes CN, et al. Dental infection of *Porphyromonas gingivalis* exacerbates high fat diet-induced steatohepatitis in mice. *J Gastroenterol*. 2013;48:1259–1270. doi: 10.1007/s00535-012-0738-1
17. Takahashi Y, Yamaguchi T, Otsubo T, Nakashima K, Shinzato K, Osako R, Shichida S, Kawano Y, Fukui A, Kawaguchi A, et al. Histological validation of atrial structural remodelling in patients with atrial fibrillation. *Eur Heart J*. 2023;44:3339–3353. doi: 10.1093/eurheartj/ehad396
18. Kitagawa M, Ouhara K, Oka H, Sakamoto S, Yamane Y, Kashiwagi A, Kanamoto R, Miyauchi M, Nagamine K. Selective and easy detection of the *Porphyromonas gingivalis* fimA type II and IV genes by loop-mediated isothermal amplification. *J Microbiol Methods*. 2021;185:106228. doi: 10.1016/j.mimet.2021.106228
19. Nesse W, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, Vissink A. Periodontal inflamed surface area: quantifying inflammatory burden. *J Clin Periodontol*. 2008;35:668–673. doi: 10.1111/j.1600-051X.2008.01249.x
20. Yoshida A, Suzuki N, Nakano Y, Oho T, Kawada M, Koga T. Development of a 5′ fluorogenic nuclease-based real-time PCR assay for

quantitative detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *J Clin Microbiol*. 2003;41:863–866. doi: 10.1128/JCM.41.2.863-866.2003
21. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, Chugh SS, Corradi D, D'Avila A, Dobrev D, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Heart Rhythm*. 2017;14:e3–e40. doi: 10.1016/j.hrthm.2016.05.028
22. Goette A. Is periodontitis a modifiable risk factor for atrial fibrillation substrate? *JACC Clin Electrophysiol*. 2023;9:54–56. doi: 10.1016/j.jacep.2022.09.013
23. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nat Rev Immunol*. 2021;21:426–440. doi: 10.1038/s41577-020-00488-6
24. D'Aiuto F, Orlandi M, Gunsolley JC. Evidence that periodontal treatment improves biomarkers and CVD outcomes. *J Clin Periodontol*. 2013;40:S85–105. doi: 10.1111/jcpe.12061
25. Chen DY, Lin CH, Chen YM, Chen HH. Risk of atrial fibrillation or flutter associated with periodontitis: a nationwide, population-based, cohort study. *PLoS One*. 2016;11:e0165601. doi: 10.1371/journal.pone.0165601
26. Sen S, Redd K, Trivedi T, Moss K, Alonso A, Soliman EZ, Magnani JW, Chen LY, Gottesman RF, Rosamond W, et al. Periodontal disease, atrial fibrillation and stroke. *Am Heart J*. 2021;235:36–43. doi: 10.1016/j.ahj.2021.01.009
27. Im SI, Heo J, Kim BJ, Cho KI, Kim HS, Heo JH, Hwang JY. Impact of periodontitis as representative of chronic inflammation on long-term clinical outcomes in patients with atrial fibrillation. *Open Heart*. 2018;5:e000708. doi: 10.1136/openhrt-2017-000708
28. Voskoboinik A, Prabhu S, Ling LH, Kalman JM, Kistler PM. Alcohol and atrial fibrillation: a sobering review. *J Am Coll Cardiol*. 2016;68:2567–2576. doi: 10.1016/j.jacc.2016.08.074
29. El-Awady AR, Miles B, Scisci E, Kurago ZB, Palani CD, Arce RM, Waller JL, Genco CA, Slocum C, Manning M, et al. *Porphyromonas gingivalis* evasion of autophagy and intracellular killing by human myeloid dendritic cells involves DC-SIGN-TLR2 crosstalk. *PLoS Pathog*. 2015;10:e1004647. doi: 10.1371/journal.ppat.1004647
30. Liu M, Shao J, Zhao Y, Ma B, Ge S. *Porphyromonas gingivalis* evades immune clearance by regulating lysosome efflux. *J Dent Res*. 2023;102:555–564. doi: 10.1177/00220345221146097
31. Shiheido-Watanabe Y, Maejima Y, Nakagama S, Fan Q, Tamura N, Sasano T. *Porphyromonas gingivalis*, a periodontal pathogen, impairs post-infarcted myocardium by inhibiting autophagosome-lysosome fusion. *Int J Oral Sci*. 2023;15:42. doi: 10.1038/s41368-023-00251-2
32. Li Y, Zhao J, Yin Y, Li K, Zhang C, Zheng Y. The role of IL-6 in fibrotic diseases: molecular and cellular mechanisms. *Int J Biol Sci*. 2022;18:5405–5414. doi: 10.7150/ijbs.75876
33. Asleh R, Enriquez-Sarano M, Jaffe AS, Manemann SM, Weston SA, Jiang R, Roger VL. Galectin-3 levels and outcomes after myocardial infarction: a population-based study. *J Am Coll Cardiol*. 2019;73:2286–2295. doi: 10.1016/j.jacc.2019.02.046
34. Mackinnon AC, Gibbons MA, Farnworth SL, Leffler H, Nilsson UJ, Delaine T, Simpson AJ, Forbes SJ, Hirani N, Gauldie J, et al. Regulation of transforming growth factor-β1-driven lung fibrosis by galectin-3. *Am J Respir Crit Care Med*. 2012;185:537–546. doi: 10.1164/rccm.201106-0965OC
35. Desmedt V, Desmedt S, Delanghe JR, Speeckaert R, Speeckaert MM. Galectin-3 in renal pathology: more than just an innocent bystander. *Am J Nephrol*. 2016;43:305–317. doi: 10.1159/000446376
36. Kram M. Galectin-3 inhibition as a potential therapeutic target in non-alcoholic steatohepatitis liver fibrosis. *World J Hepatol*. 2023;15:201–207. doi: 10.4254/wjh.v15.i2.201
37. Takemoto Y, Ramirez RJ, Yokokawa M, Kaur K, Ponce-Balbuena D, Sinno MC, Willis BC, Ghanbari H, Ennis SR, Guerrero-Serna G, et al. Galectin-3 regulates atrial fibrillation remodeling and predicts catheter ablation outcomes. *JACC Basic Transl Sci*. 2016;1:143–154. doi: 10.1016/j.jacbts.2016.03.003
38. Nagasaki A, Sakamoto S, Chea C, Ishida E, Furusho H, Fujii M, Takata T, Miyauchi M. Odontogenic infection by *Porphyromonas gingivalis* exacerbates fibrosis in NASH via hepatic stellate cell activation. *Sci Rep*. 2020;10:4134. doi: 10.1038/s41598-020-60904-8
39. Ungefroren H, Gieseler F, Kaufmann R, Settmacher U, Lehnert H, Rauch BH. Signaling crosstalk of TGF-β/ALK5 and PAR2/PAR1: a complex regulatory network controlling fibrosis and cancer. *Int J Mol Sci* . 2018;19:1568. doi: 10.3390/ijms19061568
40. Palm E, Demirel I, Bengtsson T, Khalaf H. The role of toll-like and protease-activated receptors in the expression of cytokines by gingival fibroblasts stimulated with the periodontal pathogen *Porphyromonas gingivalis*. *Cytokine*. 2015;76:424–432. doi: 10.1016/j.cyto.2015.08.263
41. Friebel J, Weithauser A, Witkowski M, Rauch BH, Savvatis K, Dörner A, Tabaraie T, Kasner M, Moos V, Bösel D, et al. Protease-activated receptor 2 deficiency mediates cardiac fibrosis and diastolic dysfunction. *Eur Heart J*. 2019;40:3318–3332. doi: 10.1093/eurheartj/ehz117
42. Chung MK, Eckhardt LL, Chen LY, Ahmed HM, Gopinathannair R, Joglar JA, Noseworthy PA, Pack QR, Sanders P, Trulock KM; American Heart Association Electrocardiography and Arrhythmias Committee and Exercise, Cardiac Rehabilitation, and Secondary Prevention Committee of the Council on Clinical Cardiology; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular and Stroke Nursing; and Council on Lifestyle and Cardiometabolic Health. Lifestyle and risk factor modification for reduction of atrial fibrillation: a scientific statement from the American Heart Association. *Circulation*. 2020;141:e750–e772. doi: 10.1161/CIR.0000000000000748
43. Naccarelli GV, Panaccio MP, Cummins G, Tu N. CHADS2 and CHA2DS2-VASc risk factors to predict first cardiovascular hospitalization among atrial fibrillation/atrial flutter patients. *Am J Cardiol*. 2012;109:1526–1533. doi: 10.1016/j.amjcard.2012.01.371
44. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, et al; Chamber Quantification Writing Group. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;18:1440–1463. doi: 10.1016/j.echo.2005.10.005
45. Ohtsuka T, Ninomiya M, Nonaka T, Hisagi M, Ota T, Mizutani T. Thoracoscopic stand-alone left atrial appendectomy for thromboembolism prevention in nonvalvular atrial fibrillation. *J Am Coll Cardiol*. 2013;62:103–107. doi: 10.1016/j.jacc.2013.01.017