と[28]であるが，自検例では片アレルに2つのexonの欠失が存在した．このような大きな欠失の場合片アレルは正常のため，通常のPCRを用いたシークエンスでは正常アレルのみが増幅され変異の存在が検出できないうえに，1万塩基対を超える解析は通常困難である．大きなDNAの解析に適したLA-PCR法およびMLPA法を用いることで，exon単位での大きな欠失が明らかとなった．これまでの報告でもこのような大きな欠失を確認した報告は2010年に海外の1例のみであり，非常に稀と考えられる．国内例でも臨床像はnMFSと思われるにもかかわらず，遺伝子変異が検出されなかった報告[29]もあり，LA-PCR法やMLPA法での再検討が望まれる．

Table 1 Comparison of nMFS characteristics between foreign and Japanese cases (quote from reference 12, 17, 21 and 30-36)

	Foreign cases		Domestic cases	
General				
Sporadic occurrence	100%	(22/22)	100%	(12/12)
Diagnosis within first week of life	83%	(15/18)	83%	(10/12)
Death within first year of life	82%	(18/22)	75%	(9/12)
Skeletal				
Arachnodactyly	95%	(21/22)	100%	(12/12)
Joint hypermorbility	81%	(13/16)	67%	(2/3)
Contractures	91%	(20/22)	89%	(8/9)
Scoliosis	22%	(4/18)	50%	(1/2)
Pectus deformity	55%	(11/20)	60%	(3/5)
Joint dislocation	36%	(5/14)	66%	(2/3)
Pes planus	100%	(7/7)	100%	(2/2)
Facial appearance				
Distinctive face	100%	(12/12)	100%	(9/9)
Dolichocephaly	57%	(8/14)		
Crumpled ears	55%	(6/11)	100%	(5/5)
Loose skin, cutis laxa	94%	(15/16)	100%	(1/1)
Senile appearance	91%	(10/11)	100%	(8/8)
Down-slanting palpebral fissures	100%	(9/9)	100%	(2/2)
Optic				
Ectopia lentis	32%	(6/19)	33%	(2/6)
Cardiac				
Mitral insufficiency	95%	(19/20)	91%	(11/12)
Tricuspid insufficiency	100%	(11/11)	50%	(6/12)
Aortic insufficiency			42%	(5/12)
Pulmonary insufficiency			25%	(3/12)
Aortic root dilatation	100%	(20/20)	100%	(12/12)
Aortic dissection	0%	(0/5)	8%	(1/12)
Cardiomegaly	100%	(11/11)	78%	(7/9)
Congestive heart failure	100%	(11/11)	78%	(7/9)
Heart murmur	100%	(12/12)	83%	(5/6)
Respiratory				
Pulmonary emphysema	57%	(4/7)	67%	(8/12)

Table 2 Difference in neonatal Marfan syndrome, Loeys-Dietz syndrome, congenital contractural arachynodactyly (Beals syndrome) and classical Marfan syndrome

	Gene	Cardiovascular involement	Inheritance	Prognosis
Neonatal Marfan syndrome	*FBN1*	severe from neonate	sporadic	poor
Loeys-Dietz syndrome	*TGFBR1, TGFBR2*	progressive	AD	variable
Congenital contractural arachnodactyly	*FBN2*	rare	AD	good
classical Marfan syndrome	*FBN1*	not severe in childhood	AD (75%)	good

AD: autosomal dominant

Table 3-1 12 domestic neonatal Marfan syndrome cases

No	Sex	Case/Year	Age at initial diagnosis	Prognosis		Genetic analysis	Family history	Others
1	M	Hibi (1985)	0d	7m	dead	–	–	sudden death after surgery for cataract
2	F	Oshima (1988)	0d	5d	dead	–	–	
3	M	Morimoto (1993)*	9d	4m	dead	–	–	
4	M	Morimoto (1993)**	4m	6m	alive	–	–	
5	M	Kishiro (1994)	1d	4m	dead	–	–	
6	F	Oota (1994)	0d	5m	dead	–	–	glaucoma
7	F	Oota (1994)*	9d	4m	dead	–	–	
8	F	Oota (1994)	4m	3y	alive	–	–	scoliosis
9	M	Oota (1994)**	4m	10m	dead	–	–	
10	F	Iwatani (1998)	0d	1y6m	alive	(N.D.)	–	
11	F	Matsumoto (2001)	1d	3m	dead	Ex26 point mutation	–	
12	F	Koriyama (2002)	0d	15d	dead	no mutation	–	
13	F	Shinohara (2009)	2d?	2m	alive	no mutation	–	surgery for intestinal atresia
14	M	Present Case	0d	27d	dead	Ex27-28 large deletion	–	
	Male 4, Female 8			mean 11.1month			0/12	

*, ** same cases, M: male, F: female, N.D.: not described

Table 3-2 Cardiopulmonary characteristics of 12 neonatal Marfan syndrome cases in Japan

No.	Cardiac involvement						Emphysema	Cause of death	Therapy
	AoD	AR	MR	PR	TR	others			
1	+		slight				+, (autopsy)	sudden death	
2	+		+			Aortic dissection	N.D.	N.D.	
3*	+		moderate		slight		+, bilateral	Cor pulmonale	
4**	+		slight				+, bilateral	(alive)	
5	+		severe		severe		N.D.	Congestive heart failure	
6	+		+		+		+, bilateral	Respiratory distress	
7*	+		+		+		+, bilateral	Cor pulmonale	
8	+		severe			VSD (peri, small)	+, bilateral	(alive)	MVP
9**	+		slight				+, bilateral	Respiratory distress	
10	+	slight	severe				N.D.	(alive)	MVR
11	+	+	+	+	+		+, (autopsy)	N.D.	ACEI
12	+	severe	slight	+	severe		N.D.	Congestive heart failure	ACEI, IMV
13	+	+	+?				N.D.	(alive)	ACEI
14	+	severe	severe	severe	severe		+, bilateral	Respiratory distress	IMV
	12/12	5/12	11/12	3/12	6/12		8/12		MVP: 1, MVR: 1 ACEI: 3, IMV: 2

*, ** same cases
AoD: aortic root dilatation, AR: aortic regurgitation, MR: mitral regurgitation, PR: pulmonary regurgitation, TR: tricuspid regurgitation, VSD: ventricular septal defect, N.D.: not described, MVP: mitral valvuloplasty, MVR: mitral valve replacement, ACEI: angiotensin converting enzyme inhibitor, IMV: intermittent mandatory ventilation

本邦のnMFS症例のまとめ(Table 3-1. 3-2)[3, 12, 21, 30-36]

本邦では重複しているものを除き12例の報告があり9例が死亡している．いずれも家族性は認められていない．平均寿命は11.1カ月(生後5日～3歳)で，海外報告より短く，多くは1歳に到達しない．心血管病変ではARは少なく，大動脈弁輪の拡大とMRがほぼ全例で確認され，特にMRは重篤との記載が散見される．多くの症例が生後10日以内に診断されており，特徴的な外表奇形やエコー所見などから本疾患を類推することは難しくないと思われる．

循環器治療は3例でACE阻害剤の投与，2例で僧帽弁への手術介入が行われている．ACE阻害剤の投与例では2例が3カ月以内に死亡しており，生存期間の延長には至らないことが示唆される．ARがわずか5例(42%)に留まることも関連する可能性がある．僧帽弁への手術介入後の2症例の経過は，報告のあった短期の観察では良好である．僧帽弁置換の一例では生後6カ月，体重3.1 kgで手術が実施され，僧帽弁輪が32 mmに拡大していたことから自己弁を温存のうえでCarboMedics社23Mを使用し，術後1年の経過は良好であった．僧帽弁形成術を実施した一例では，詳細な手術時の記載はなかったが，1歳時に僧帽弁形成後3歳までの生存が報告されている．いずれも本来早期に死亡する本疾患患児が1年以上生存しており，MRへの早期介入が予後改善をもたらす可能性が示唆される．

肺病変は12例中8例に認め，その全例が肺気腫である．太田の報告のように生後数カ月で顕在化してくる例や，剖検時に指摘される例もある．肺病理の評価が記載されていたものでは全例で肺胞の破壊・拡大を認めており，国内例においてはcMFSで報告されているようなLAの拡大による圧迫，側臥による圧迫等肺外要因での閉塞性肺過膨張や，海外のnMFSの報告にあるような気管軟化症の報告はない．自検例は例外的に早期の肺気腫を来しており，Shinawi[14]の指摘のように陽圧換気による気道損傷の影響が否定できない．人工換気を施行した2例はいずれも1カ月未満の早期死亡を来しており，人工換気をせざるを得ない呼吸不全の存在は，予後不良因子の可能性が示唆される．

遺伝子診断は4例で施行されているが，遺伝子変異を検出できたのはわずかに2例である．*FBN1*遺伝子がMarfan症候群の原因と同定された1991年以前の報告も多く，鑑別されるべき類縁疾患との区別やGhent基準に沿った診断はなされておらず，これらが本当に*FBN1*遺伝子の異常によるnMFSであるかは，はっきりしない．

終わりに

新生児マルファン症候群と遺伝子の変異について概説するとともに，国内報告12症例をまとめた．国内報告例でも主要症状・呼吸循環器系合併症など臨床像は海外報告と似通っており，多くの例で治療が奏功せず死亡しているが，僧帽弁閉鎖不全への手術介入を行った症例は比較的良好であった．遺伝子診断施行例は少ないが，今後はGhent基準を考慮し，分子生物学的手法を用いた診断の普及が望まれる．自検例のように大きなexonの欠失を認める例もあり，遺伝子異常が認められない症例でも大きな遺伝子変異についての解析が期待される．

なお本稿の内容の一部については2010年7月9日に行われた第46回日本小児循環器学会総会・学術集会において発表を行った．

謝辞

本稿を作成するにあたり，本症例の遺伝子診断を行っていただいた国立循環器病研究センター研究所分子生物学部，森崎隆幸先生，小野晶子先生，また当院産婦人科，菱川賢治先生に深謝致します．

【参考文献】

1) 堀井　正，黒木良和，新川昭夫，ほか：新先天奇形症候群アトラスMarfan症候群．東京，南江堂 1998, pp254-255
2) 賀藤　均：小児期におけるマルファン症候群の診断・管理．呼吸と循環 2009; 57(11): 1133-1139
3) 菱川賢治，大仲　恵，浮田真吾，ほか：新生児マルファン症候群の1例．滋賀県産科婦人科雑誌 2010(投稿中)
4) 森崎裕子，森崎隆幸：大動脈疾患：マルファン症候群ほか．ゲノム医学 2008; 8(1): 73-78
5) 森崎裕子，森崎隆幸：マルファン症候群の病因遺伝子に関する最近の知見．進歩する心臓研究 2007; Vol. XXVII(1): 12-21
6) 森崎隆幸，森崎裕子：マルファン症候群・類縁疾患に対する遺伝子診断とTGF-βの意義．呼吸と循環 2009; 57(11): 1141-1146
7) Godfrey M, Raghunath M, Cisler J, et al: Abnormal morphology of fibrillin microfibrils in fibroblast cultures from patients with neonatal Marfan syndrome. Am J Pathol 1995; 146(6): 1414-1421
8) Superti-Furga A, Raghunath M, Willems PJ: Deficiencies of

fibrillin and decorin in fibroblast cultures of a patient with neonatal Marfan syndrome. J Med Genet 1992; 29: 875-878
9) Raghunath M, Superti-Furga A, Godfrey M, et al: Decreased extracellular deposition of fibrillin and decorin in neonatal Marfan syndrome fibroblasts. Hum Genet 1993; 90: 511-515
10) Kochilas L, Gundogan F, Atalay M, et al: A novel mutation of the fibrillin-1 gene in a newborn with severe Marfan syndrome. J Perinat 2008; 28: 303-305
11) Bresters D, Nikkels PGJ, Meijboom EJM, et al: Clinical, pathological and molecular genetic findings in a case of neonatal Marfan syndrome. Acta Paediatr 1999; 88: 98-101
12) 大島孝一：新生児 Marfan 症候群 臨床と剖検．福岡大紀要 1988; 15(3): 442-443
13) Day DL, Burke BA: Pulmonary emphysema in a neonate with Marfan syndrome. Pediatr Radiol 1986; 16: 518-521
14) Shinawi M, Boileau C, Brik R, et al: Splicing mutation in the fibrillin-1 gene associated with neonatal Marfan syndrome and severe pulmonary emphysema with tracheobronchomalacia. Pediatr Pulmonol 2005; 39: 374-378
15) Chemke J, Nisani R, Feigl A, et al: Homozygosity for autosomal dominant Marfan syndrome. J Med Genet 1984; 21: 173-177
16) Tekin M, Cengiz FB, Ayberkin E, et al: Familial Neonatal Marfan Syndrome Due to Parental Mosaicism of a Missence Mutation in the *FBN1* Gene. Am J Med Genet 2007; 143A: 875-880
17) Faivre L, Masurel-Paulet A, Collod-Béroud G, et al: Clinical and molecular study of 320 children with Marfan syndrome and related type I fibrillinopathies in a series of 1009 probands with pathogenic *FBN1* mutations. Pediatrics 2009; 123(1): 391-397
18) Booms P, Cisler J, Mathews KR, et al: Novel exon skipping mutation the fibrillin-1 gene: two 'hot spots' for the neonatal Marfan syndrome. Clin Genet 1999; 55: 110-117
19) Morse RP, Rockenmacher S, Pyeritz RE, et al: Diagnosis and management of infantile Marfan syndrome. Pediatrics 1990; 86(6): 888-895
20) Geva T, Stephen PS, Maria SD, et al: Two-dimensional and Doppler echocardiographic and pathologic characteristics of the infantile Marfan syndrome. Am J Cardiol 1990; 65: 1230-1237
21) 稲代雅彦，島崎信次郎，秋本かつみ，ほか：重篤な弁膜症により乳児期早期に心不全で死亡した新生児 Marfan 症候群の1例．小児科診療 1994; 121(10): 1843-1847
22) Hennekam RC: Severe infantile Marfan syndrome versus neonatal Marfan Syndrome. Am J Med Genet 2005; 139A: 1
23) Loeys BL, Dietz HC, Braverman AC, et al: The revised Ghent nosology for the Marfan syndrome. J Med Genet 2010; 47: 476-485
24) Loeys BL, Chen J, Neptune ER, et al: A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutation in *TGFBR1* or *TGFBR2*. Nat Genet 2005; 37: 275-281
25) Yetman AT, Beroukhim RS, Ivy DD, et al: Importance of the Clinical Recognition of Loeys-Dietz Syndrome in the Neonatal Period. Pediatrics 2007; 119: e1199-1202
26) Faivre L, Collod-Beroud G, Loeys BL, et al: Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and *FBN1* mutations: an international study. Am J Hum Genet 2007; 81: 454-466
27) Tiecke F, Katzke S, Booms P, et al: Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and genotype-phenotype correlations in *FBN1* exons 21-40. Eur J Hum Genet 2001; 9: 13-21
28) Comeglio P, Johnson P, Arno G, et al: The importance of mutation detection in Marfan syndrome and Marfan-related disorders: report of 193 *FBN1* mutations. Hum Mut 2007; 28: 928
29) 中林玄一，足立雄一，橋本邦夫，ほか：肺気腫の進行を阻止できなかった新生児マルファン症候群の1例．日小呼誌 2003; (6): 66
30) 森本雄次，米田吉宏，秋田裕司，ほか：新生児マルファン症候群の2例－マルファン症候群および先天性拘縮性クモ状指症との臨床的比較検討－．小児科臨床 1993; 46: 2054-2060
31) 太田 明，吉川正強，森本雄次：新生児 Marfan 症候群の肺気腫．日新生児会誌 1997; 33(1): 150-154
32) 日比成美，大塚拓治，山本 稔，ほか：先天性多関節拘縮症を合併した新生児マルファン症候群の1例．小児科診療 1985; 48(5): 46-47
33) 松本居子，今井未央，川真田光，ほか：新生児マルファン症候群の1例．小児内科 2001; 33(5): 737-740
34) 郡山 健，村上洋介，江原英治，ほか：Infantile Marfan syndrome の新生児の1例．日未熟児新生児会誌 2002; 14(2): 97-104
35) 篠原貴子，多賀直行，岡田 修，ほか：新生児マルファン症候群を伴った小腸閉鎖症の麻酔経験．日小児麻酔会誌 2009; 15:130-132
36) 岩谷文夫，星野俊一，猪狩次雄，ほか：Superior Septal Approach により僧帽弁置換術を行った新生児マルファン症候群の1例．日小外会誌 1998; 34(6): 122-126

Original Article

Elevated Antibody Titers to *Porphyromonas gingivalis* as a Possible Predictor of Ischemic Vascular Disease: Results from the Tokamachi-Nakasato Cohort Study

Koichi Tabeta[1], Naohito Tanabe[2], Daisuke Yonezawa[3], Hirotaka Miyashita[1,3], Tomoki Maekawa[1,3], Naoki Takahashi[1,3], Takafumi Okui[1,3], Takako Nakajima[1,4] and Kazuhisa Yamazaki[1,3]

Koichi Tabeta and Naohito Tanabe equally contributed to this work.

[1]Center for Transdisciplinary Research, Niigata University, Niigata, Japan
[2]Division of Health Promotion, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[3]Laboratory of Periodontology and Immunology, Department of Oral Health and Welfare, Faculty of Dentistry, Niigata University, Niigata, Japan
[4]General Dentistry and Clinical Education Unit, Niigata University Medical and Dental Hospital, Niigata, Japan

Aim: Limited epidemiological studies have investigated the relationship between ischemic vascular disease and periodontitis in non-Western populations. We investigated this relationship in a Japanese cohort by measuring serum titers of antibodies to periodontopathic bacteria.
Methods: As part of the Tokamachi-Nakasato cohort study, we followed up 7021 participants regarding cardiovascular events over 5 years, and observed 99 ischemic vascular events: 66 cerebral infarctions and 33 cases of ischemic heart disease (IHD). For a nested case-control study, we selected 495 sex- and age-matched control subjects. Conditional logistic regression analysis was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) of ischemic vascular events associated with antibody titers to *Porphyromonas gingivalis* FDC381 and SU63. Multivariable models were adjusted for traditional cardiovascular risk factors using propensity scores.
Results: The highest tertile category of antibody titers to *P. gingivalis* FDC381 in men was significantly associated with an increased risk of cerebral infarction in only the crude model. The 2nd and 3rd tertile categories of antibody titers to *P. gingivalis* SU63 were significantly associated with an increased risk of cerebral infarction in men (multivariable ORs (95% CIs) were 7.12 (1.51-33.5) and 9.03 (1.97-41.5), respectively). The association was not appreciably modified when we further adjusted for serum high-sensitivity C-reactive protein levels. Antibody titers to *P. gingivalis* were not dose-dependently associated with the risk of IHD.
Conclusion: High serum antibody titers to *P. gingivalis* SU63 could be a predictor of cerebral infarction in Japanese men independent of traditional risk factors and inflammation.

J Atheroscler Thromb, 2011; 18:808-817.

Key words; Serum antibody, Cerebral infarction, Ischemic heart disease, *Porphyromonas gingivalis*, Periodontitis

Address for correspondence: Kazuhisa Yamazaki, Periodontology and Immunology, Department of Oral Health and Welfare, Faculty of Dentistry, Niigata University, 5274 Gakkocho 2-ban-cho, Niigata 951-8514, Japan
E-mail: kaz@dent.niigata-u.ac.jp
Received: August 29, 2010
Accepted for publication: April 5, 2011

Introduction

Several studies have suggested chronic periodontitis as a possible risk factor for ischemic vascular disease, such as cerebral infarction and ischemic heart disease (IHD)[1-4]. Some studies have suggested an as-

sociation between elevated antibody titers against common pathogens, such as *Chlamydia pneumoniae* and *Helicobacter pylori*, and sustained chronic infections or risk of mortality from coronary heart disease[5-8]. Hence, a bacterial burden in gingival tissues that is sufficient to induce systemic inflammation may modulate atherogenesis and inflammation of vascular walls[9] in periodontitis patients[10]. Indeed, several studies have confirmed the relationship between periodontal tissue destruction in periodontitis patients and serum C-reactive protein (CRP) levels[11, 12].

Porphyromonas gingivalis, a periodontopathic Gram-negative bacterium, has been extensively analyzed with regard to its role in the pathogenesis of atherogenesis[13-17]. A high frequency of antibody positivity for *P. gingivalis* SU63 was observed in patients with IHD, suggesting an association between the presence of a particular highly virulent strain and IHD[18]. Previous reports have demonstrated an association between antibody titers to *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* and the incidence of stroke[4] and IHD[19, 20] in a Western population. A recent nested case-control study in a larger cohort demonstrated the influence of systemic exposure to *P. gingivalis* on the incidence of stroke[21]. No studies to date have investigated the association between ischemic vascular disease and periodontitis in an Asian population using a nested case-control model. Differing genetic backgrounds and lifestyles among populations may affect the incidence of ischemic vascular disease in patients. The aim of our research was to investigate the association between serum antibody levels to *P. gingivalis* strains FDC381 and SU63 and ischemic vascular disease in a Japanese population using a nested case-control study.

Materials and Methods

Study subjects and outcome

We designed a nested case-control study as a part of the Tokamachi-Nakasato cohort study[22]. The study areas were Tokamachi City and its neighboring village, Nakasato, Niigata Prefecture, Japan. According to the census in 2000, the total population of the area was 49,424, including 29,323 people between the ages of 40 and 89 years. The Tokamachi-Nakasato cohort included 7,753 inhabitants of the study area, aged 40-89 years, who agreed to participate in the cohort study and whose baseline data and serum samples were collected in 1998. They were followed up from 1998 to 2003 regarding the incidence of cardiovascular events.

Baseline data and serum samples were collected during the official population-based health checkup program from June to September 1998, and written informed consent for participation in the cohort study was obtained from the participants. They were interviewed by public health nurses to collect baseline information on cardiovascular disease history, diabetes mellitus, hypertension treatment status, smoking status, and alcohol consumption. To evaluate their diabetic status, fasting or postprandial plasma glucose levels were measured and subjects with a fasting level ≥ 7.0 mmol/L (126 mg/dL), postprandial level ≥ 11.1 mmol/L (200 mg/dL), and those who declared themselves diabetic were considered diabetic. Blood pressure was measured using a manual sphygmomanometer. After measuring total and high-density lipoprotein (HDL) cholesterol levels, serum samples were stored at −80℃ until antibody titers against *P. gingivalis* were measured.

From the 7753 participants in the entire Tokamachi-Nakasato cohort, 61 subjects aged 85 years or older were excluded because there were not enough subjects to obtain sex-matched and age-matched control subjects for some incident cases of ischemic vascular events. From the remaining 7692 participants, 7021 were included in the baseline cohort for the present nested case-control study because they had no history of IHD and cerebral infarction at baseline.

Diagnosis of cerebral infarction and IHD (acute coronary syndrome and angiographically proven stable angina pectoris) was determined by medical chart review. First, we checked information on incident cardiovascular events in a health checkup survey in 2003. From participants who declared that they had experienced cardiovascular events and from nonparticipants, potential candidates for medical chart review were identified by reviewing either death certificates or the answers to a mailed 2004 questionnaire survey. A single physician reviewed all medical charts. Diagnosis of cerebral infarction was based on typical clinical features and characteristic changes on computed tomography and/or magnetic resonance imaging brain scans. Diagnosis of acute coronary syndrome was based on chest pain, cardiac enzyme levels, electrocardiograms, and coronary angiography. Subjects with stable angina were also included as IHD cases on detection of relevant coronary stenosis by angiography. Although there were many more cases of clinically diagnosed angina pectoris, we selected only the cases described above because angiographically defined coronary stenosis warranted that the disease was caused by atherosclerosis. Our procedures allowed us to identify the presence or absence of cardiovascular events during the follow-up period for 92% of the entire cohort.

During a follow-up period of 35,131 person-

Table 1. Baseline characteristics of cases and control subjects

	Case (n=99)	Control (n=495)
Sex, number of men (%)	58 (58.6)	290 (58.6)
Age (years)	69.7±7.7	69.7±7.6
Smoking status		
Never	48 (48.5)	270 (54.5)
Former	10 (10.1)	104 (21.0)
Current	41 (41.4)	121 (24.4)
Diabetes mellitus	8 (8.1)	35 (7.1)
Treated hypertension	38 (38.4)	112 (22.6)
Systolic blood pressure (mmHg)	138.6±17.7	134.3±19.4
Diastolic blood pressure (mmHg)	77.3±11.8	75.4±11.6
Hypertension*	59 (59.6)	210 (42.4)
Serum total cholesterol (mg/dL)	206.9±35.1	199.2±35.8
Serum HDL-cholesterol (mg/dL)	49.7±9.2	54.3±13.6
hs-CRP ($\log_{10}$, ng/mL)	2.95±0.44	2.74±0.49
Mean $\log_{10}$ antibody titers to *P. gingivalis*		
FDC381	4.18±0.67	4.04±0.66
SU 63	3.95±0.35	3.89±0.44

Baseline data of the study subjects were collected in 1998 and the subjects were followed up until the end of 2003
hs-CRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein
Data are the mean ± standard deviation or number (%)
*Treated or systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure of ≥ 90 mmHg

years for the baseline cohort, 99 subjects were confirmed to have experienced a first-ever event of cerebral infarction (66 events), acute coronary syndrome (23 events), or angiographically proven stable angina pectoris (10 events). None of these ischemic vascular events were observed in the other 6922 subjects during the follow-up period. Five sex- and age-matched control subjects were selected for each case from these non-diseased subjects. A total of 495 control subjects were subsequently enrolled in our study. Baseline characteristics of cases and control subjects are summarized in **Table 1.**

The study protocol was reviewed and approved by the ethics committee of Niigata University School of Medicine.

Antibody Titers to *P. gingivalis* and CRP in serum

Antibody titers to *P. gingivalis* lysates were measured by enzyme-linked immunosorbent assay (ELISA) and evaluated according to a method described previously, with some modifications[23]. For the ELISA assay, *P. gingivalis* lysates for FDC381 and SU63 strains (Institute of Immunology Co., Ltd., Tokyo, Japan) were used as antigens. The lysates (0.05 μg) were used to coat 96-well ELISA plates (100 μL/well; 0.5 μg/mL in 0.05 M bicarbonate buffer, pH 9.6) and were incubated overnight at 4℃. The plates were then washed with 0.05% Tween-saline, and 100 μL test samples diluted with 0.05% Tween-saline containing 4% goat serum (1:400 to 1:12,800) were added and incubated for 1 h at room temperature. After washing 3 times with 0.05% Tween-saline, horseradish peroxidase-conjugated goat anti-human IgG (Sigma, Ronkonkoma, NY) was added and the samples were incubated for 1 h at room temperature. The reaction of peroxidase was accomplished by the addition of 75 μL/well of 0.04% H_2O_2 and ortho-phenylenediamine (OPD, 1 mg/mL) in sodium citrate buffer. Color development was stopped by the addition of 4 M sulphuric acid, and absorbance was read at a wavelength of 490 nm. Pooled serum for control subjects was prepared from systemically and periodontally healthy subjects with considerably low antibody responses to *P. gingivalis*, and was used to titrate the antibody levels into ELISA units (EU). Serum reactivity of 3200-fold diluted control serum was determined to be 100 EU. High sensitivity CRP (hs-CRP) was also measured from pooled serum with a nephelometric assay.

Statistical analysis

For antibody titers to *P. gingivalis*, descriptive statistics are presented as the mean and standard deviation of $\log_{10}$ values. Hs-CRP was also analyzed using $\log_{10}$ values because the distribution was close to log-normal. Statistical differences in baseline characteristics between cases and control subjects were analyzed by crude conditional logistic regression analysis. Odds ratios (OR) and 95% confidence intervals (CI) of continuous variables were calculated for every +1 SD of all control subjects.

Antibody titers to *P. gingivalis* were classified into 3 categories based on the tertiles of all study subjects. Differences in the tertile category distributions between cases and control subjects were analyzed by the Mann-Whitney *U*-test. Conditional logistic regression analysis was used to estimate the crude and multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) to develop ischemic vascular events, associated with tertile categories of antibody titers to *P. gingivalis*, treating the lowest tertile category as a reference. Multivariable-adjusted ORs were adjusted for the effects of confounders, such as the current smoking status at baseline (yes/no, only for men), treated hypertension (present/absent), diabetes mellitus (present/absent), systolic blood pressure (continuous), diastolic blood pressure (continuous), serum total cholesterol (continuous), and serum HDL-cholesterol (continuous) using propensity scores. Smoking status was not adjusted for women because of the very low prevalence of current female smokers. A multiple propensity score method was used in this study to adjust for the effects of these confounders[24], because the number of cases was too small to apply standard multivariable models; event numbers of less than 5 per independent variable can lead to major problems in logistic regression analysis[25]. Multinomial logistic regression analysis was performed using sex-specific data from all matched control subjects ($n=495$) to calculate parameter estimates for calculating propensity scores. Previously described confounders were entered as independent variables in a multinomial logistic regression model, wherein a tertile category variable of antibody titers to *P. gingivalis* was entered as the dependent variable. Using the parameter estimates obtained for the confounders, we estimated the probability of each study subject being categorized into each tertile category of antibody titers to *P. gingivalis*. The probabilities for the middle and highest tertile categories were used as propensity scores to be entered as covariates in a multivariable-adjusted conditional logistic regression model instead of confounders themselves. Hs-CRP level was further adjusted in order to assess the role of inflammation in the association between antibody titers to *P. gingivalis* and the risk of ischemic vascular events by including a covariate ($\log_{10}$ values of hs-CRP level) in every multivariable model.

All statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). Because no statistical procedure of conditional logistic regression analysis is available in SPSS, we utilized the COXREG procedure to construct conditional logistic regression models. For this purpose, a dummy time variable was created so that cases had a positive value smaller than that for control subjects, e.g., a value of "1" was given for cases whereas a value of "2" was given for control subjects. This dummy time variable was moved into the "Time" slot as a dependent variable. We also created a variable that denotes each set of cases and matched control subjects, and then moved it into the "Strata" slot. A status variable for which cases were given a value of "1" and control subjects were given "0" was moved into the "Status" slot. A tertile category variable of antibody titers and the covariates were entered as independent variables. A P value of < 0.05 was considered significant.

Results

Comparison of baseline characteristics

In crude analyses, current smoking in men and hypertension in both sexes showed significantly positive associations with cerebral infarction. Higher antibody titers to *P. gingivalis* SU63 was significantly associated with an elevated risk of cerebral infarction in men (**Table 2**). Traditional risk factors, except current smoking and hs-CRP as well as antibody titers to *P. gingivalis*, were not associated with the risk of IHD (**Table 3**).

Serum antibody titers to *P. gingivalis* FDC 381 and SU63

Tertile distributions of titers of antibodies to *P. gingivalis* FDC381 and SU63 are compared in **Table 4**. Regarding cerebral infarctions, men tended to have higher antibody titers to *P. gingivalis* FDC381 and SU63 than control subjects ($P=0.036$ and 0.001, respectively). In women, there were no significant differences in antibody-titer distribution between cases of cerebral infarction and control subjects and between IHD cases and control subjects.

Relative odds for ischemic vascular events associated with antibody titers to *P. gingivalis* FDC381 are listed in **Table 5**. In conditional logistic regression analyses for men, the 3rd tertile categories of antibody

Table 2. Comparison of risk factors between cases of cerebral infarction and sex- and age-matched control subjects with crude odds ratios

	Case	Control	OR	95% CI	*P*
Men	(*n* = 34)	(*n* = 170)			
Age (years)	67.9 ± 7.7	67.9 ± 7.6			
Smoking status					
Never	4 (11.8)	39 (22.9)	1.00	(reference)	
Former	6 (17.6)	56 (32.9)	1.01	(0.27-3.73)	0.993
Current	24 (70.6)	75 (44.1)	3.12	(1.02-9.49)	0.045
Diabetes mellitus	2 (5.9)	16 (9.4)	0.60	(0.13-2.74)	0.512
Treated hypertension	10 (29.4)	32 (18.8)	1.89	(0.79-4.53)	0.154
Systolic blood pressure (mmHg)*	137.2 ± 16.8	129.3 ± 17.0	1.32	(0.89-1.96)	0.166
Diastolic blood pressure (mmHg)*	79.9 ± 10.2	74.5 ± 11.4	1.71	(1.13-2.61)	0.012
Hypertension†	21 (61.8)	60 (35.3)	3.03	(1.39-6.58)	0.005
Serum total cholesterol (mg/dL)*	178.0 ± 36.6	186.4 ± 33.3	0.76	(0.51-1.14)	0.182
Serum HDL-cholesterol (mg/dL)*	52.6 ± 12.1	52.5 ± 14.4	1.01	(0.70-1.44)	0.973
hs-CRP ($\log_{10}$, ng/mL)*	2.97 ± 0.60	2.67 ± 0.53	1.59	(1.15-2.20)	0.006
Mean $\log_{10}$ antibody titers to *P. gingivalis*					
FDC381*	4.22 ± 0.43	4.02 ± 0.59	1.48	(0.99-2.22)	0.054
SU 63*	4.09 ± 0.32	3.89 ± 0.45	1.62	(1.10-2.39)	0.015
Women	(*n* = 32)	(*n* = 160)			
Age	71.9 ± 7.2	71.9 ± 7.1			
Smoking status				Not examined	
Never	31 (96.9)	154 (96.3)			
Former	1 (3.1)	3 (1.9)			
Current	0 (0.0)	3 (1.9)			
Diabetes mellitus	0 (0.0)	5 (3.1)		Not examined	
Treated hypertension	15 (46.9)	40 (25.0)	2.52	(1.17-5.43)	0.018
Systolic blood pressure (mmHg)*	136.3 ± 21.3	131.3 ± 16.8	1.32	(0.89-1.96)	0.166
Diastolic blood pressure (mmHg)*	75.9 ± 14.7	72.6 ± 10.1	1.38	(0.92-2.07)	0.120
Hypertension†	20 (62.5)	69 (43.1)	2.21	(1.01-4.85)	0.048
Serum total cholesterol (mg/dL)*	213.3 ± 33.2	208.4 ± 32.6	1.17	(0.78-1.75)	0.445
Serum HDL-cholesterol (mg/dL)*	53.3 ± 18.1	54.5 ± 13.9	0.92	(0.64-1.33)	0.664
hs-CRP ($\log_{10}$, ng/mL)*	2.74 ± 0.53	2.70 ± 0.54	1.08	(0.77-1.53)	0.644
Mean $\log_{10}$ antibody titers to *P. gingivalis*					
FDC381*	4.06 ± 0.70	4.00 ± 0.69	1.07	(0.77-1.51)	0.679
SU 63*	3.97 ± 0.55	3.84 ± 0.44	1.31	(0.90-1.90)	0.153

Data are the mean ± standard deviation or number (%) as otherwise noted
OR, odds ratio; CI, confidence interval
*OR of continuous variables were calculated for + 1SD of total control subjects, which are described in Table 1
†Treated or systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure of ≥ 90 mmHg

titers were associated with an elevated risk of cerebral infarction in the crude model (OR 2.88; 95% CI, 1.06-7.85); however, this association was not statistically significant in the multivariable model (multivariable OR for the 3rd vs. 1st category 2.64; 95% CI, 0.94-7.41). For women, no significant association was observed between antibody titers to *P. gingivalis* FDC381 and the risk of cerebral infarction.

Significantly elevated risk of IHD was observed in the 2nd tertile category antibody titers to *P. gingivalis* FDC381 (multivariable OR 2.90; 95% CI, 1.04-13.1); however, no dose-dependent association was observed between antibody titers to *P. gingivalis* FDC381 and the risk of IHD. The risk of IHD was not substantially changed when analyzed only for men: multivariable ORs (95% CIs) in the 2nd and 3rd tertile categories were 3.98 (1.21-13.1) and 1.78 (0.47-6.63), respectively (not shown in tables).

Table 3. Comparison of risk factors between cases of ischemic heart disease and sex-matched and age-matched control subjects with crude odds ratios

	Case	Control	OR	95% CI	*P*
Men and women	(n = 33)	(n = 165)			
Sex, number of men (%)	24 (72.7)	120 (72.7)			
Age (years)	69.7 ± 7.7	69.7 ± 7.6			
Smoking status					
Never	13 (39.4)	77 (46.7)	1.00	(reference)	
Former	3 (9.1)	45 (27.3)	0.43	(0.10-1.84)	0.254
Current	17 (51.5)	43 (26.1)	3.15	(1.09-9.11)	0.034
Diabetes mellitus	6 (18.2)	14 (8.5)	2.55	(0.85-7.63)	0.094
Treated hypertension	13 (39.4)	40 (24.2)	2.05	(0.93-4.51)	0.076
Systolic blood pressure (mmHg)*	138.6 ± 17.7	134.3 ± 19.4	1.24	(0.86-1.79)	0.250
Diastolic blood pressure (mmHg)*	77.3 ± 11.8	75.4 ± 11.6	1.18	(0.82-1.71)	0.375
Hypertension†	18 (54.5)	81 (49.1)	1.23	(0.59-2.56)	0.578
Serum total cholesterol (mg/dL)*	206.9 ± 35.1	199.2 ± 35.8	1.26	(0.86-1.85)	0.243
Serum HDL-cholesterol (mg/dL)*	49.7 ± 9.2	54.3 ± 13.6	0.66	(0.42-1.03)	0.065
hs-CRP ($\log_{10}$, ng/mL)*	2.95 ± 0.44	2.74 ± 0.49	1.46	(1.02-2.08)	0.038
Mean $\log_{10}$ antibody titers to *P. gingivalis*					
FDC381*	4.18 ± 0.67	4.04 ± 0.66	1.23	(0.86-1.76)	0.260
SU 63*	3.95 ± 0.35	3.89 ± 0.44	1.15	(0.78-1.70)	0.478

Results of sex-specific analyses are not displayed due to small sample size
Data are the mean ± standard deviation or number (%) as otherwise noted
OR, odds ratio; CI, confidence interval
*OR of continuous variables were calculated for + 1SD of total control subjects, which are described in Table 1
†Treated or systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure of ≥ 90 mmHg

For *P. gingivalis* SU63, a significantly elevated risk of cerebral infarction was observed only in men in the 2nd (multivariable OR 7.12; 95% CI, 1.51-33.5) and 3rd (multivariable OR 9.03; 95% CI, 1.97-41.5) tertile categories (**Table 6**). No significant association was observed for IHD. There was also no significant association for IHD when analyzed only for men: multivariable ORs (95% CIs) in the 2nd and 3rd tertile categories were 1.84 (0.63-5.41) and 1.00 (0.30-3.40), respectively (not shown in tables).

hs-CRP did not considerably modify the association between antibody titers to *P. gingivalis* of both strains and the risk of ischemic vascular events.

Discussion

In this study, elevated antibody titers to the periodontopathic bacterium *P. gingivalis* SU63 in men were significantly associated with the risk of cerebral infarction. Because hs-CRP did not considerably modify the association, periodontitis appears to relate to cerebral infarction in Japanese men independent of inflammation. These findings suggest that *P. gingivalis* SU63 has specific biological effects or may induce specific host responses related to cerebral infarction.

Several studies have demonstrated clonal heterogeneity in virulence that induces an immune response among various *P. gingivalis* strains. Among diverse antigenic substances, fimbriae, filamentous components on the cell surface, playing an important role in the colonization and invasion of periodontal tissues, are potentially responsible for differences in immunogenicity. *P. gingivalis* is classified into 6 genotypes based on the genomic diversity of the *fimA* gene, which codes for fimbriae[26], and is detected in 10% of cardiovascular specimens. Among these, Type IV of the fimA genotype is most frequently detected (45.0%), and is possessed by SU63 strain (30.0%)[27]. *P. gingivalis* is also known to activate blood coagulation factors by arginine-specific cysteine proteases[28], which are other potential candidates for differences in immunogenicity related to cerebral infarction.

In a previous case-control study, we observed that the positive rate of antibody to *P. gingivalis* SU63 was increased in IHD patients[18]. In this study, however, antibody titers to *P. gingivalis* SU63 had no significant association with the risk of IHD. Although the risk of IHD was strongly associated with antibody titers to *P. gingivalis* FDC 381 in the 2nd tertile category, no association was observed in the 3rd category.

Table 4. Comparison of tertile distributions of antibody titers to *P. gingivalis* FDC381 and SU63 between case and control groups

	Tertile categories of *P. gingivalis*			Average rank	*p**
	1st	2nd	3rd		
FDC 381, range	392-6187	6,212-13696	13,746-53,041,276		
Cerebral infarction					
Men					
Case (n=34)	6 (17.6)	11 (32.4)	17 (50.0)	120.8	0.036
Control (n=170)	57 (33.5)	56 (32.9)	57 (33.5)	98.8	
Women					
Case (n=32)	13 (40.6)	7 (21.9)	12 (37.5)	96.9	0.960
Control (n=160)	54 (33.8)	59 (36.9)	47 (29.4)	96.4	
Ischemic heart disease					
Men and women					
Case (n=33)	6 (18.2)	15 (45.5)	12 (36.4)	96.9	0.129
Case (n=165)	62 (37.6)	50 (30.3)	53 (32.1)	112.5	
SU63 (range)	325-5,914	5935-10,462	10472-360,948		
Cerebral infarction					
Men					
Case (n=34)	6 (17.6)	11 (32.4)	17 (50.0)	131.0	0.001
Control (n=170)	57 (33.5)	56 (32.9)	57 (33.5)	96.8	
Women					
Case (n=32)	12 (37.5)	5 (15.6)	15 (46.9)	103.2	0.427
Control (n=160)	57 (35.6)	52 (32.5)	51 (31.9)	95.2	
Ischemic heart disease					
Men and women					
Case (n=33)	10 (30.3)	12 (36.4)	11 (33.3)	98.6	0.605
Case (n=165)	56 (33.9)	61 (37.0)	48 (29.1)	103.9	

Data are n (% among cases or control subjects)
*by Mann-Whitney U test

In contrast to the relationship between cerebral infarction and SU63, the risk of IHD was not dose-dependently associated with titers of antibodies to either *P. gingivalis* FDC381 or SU63 strains even when analyzed only for men. Because we were unable to demonstrate a significant association between the risk of IHD and traditional risk factors, except for current smoking and hs-CRP, the number of events would not have been enough to evaluate the risk of IHD; therefore, we cannot reach any conclusion about the antibody titers to *P. gingivalis* and the risk of IHD in this study. A larger cohort or a longer follow-up is needed to investigate the relationship between IHD and antibody titers to *P. gingivalis* in the Japanese population. In addition, extensive studies investigating the influence of differences in *P. gingivalis* strains on pathogenesis are required to elucidate the relationship between periodontitis and ischemic vascular disease.

In the present study, lower antibody titers to *P. gingivalis* FDC381 and SU63 were observed in women than men, and no association was observed between antibody titers and ischemic vascular events in women. Differences in antibody titers to periodontopathic bacteria and risk for stroke by gender have been observed in previous studies[21, 29]. This disparity may be due to differences in bacterial burden or the severity of periodontitis between genders. Epidemiological surveys showed a higher prevalence and greater extent of attachment loss (a clinical characteristic of periodontitis) in males than in females[30]. Clinical records of periodontitis were unavailable for evaluation in this study; however, the lower antibody titers in women in our study population may reflect the extent of severity of periodontitis. Given that the progression and severity of periodontal disease are determined by the host inflammatory response and reflected in antibody titers to periodontal bacteria, high antibody titers in men may be indicative of the severity of periodontitis and

Table 5. Relative odds for ischemic vascular events associated with antibody titers to *P. gingivalis* FDC381

	Tertile categories of *P. gingivalis* FDC381						
	1st (392-6187)	2nd (6212-13,696)			3rd (13,746-53,041,276)		
	OR	OR	(95% CI)	*P*	OR	(96% CI)	*P*
Cerebral infarction							
Men							
Crude	1.00 (reference)	1.88	(0.65-5.46)	0.247	2.88	(1.06-7.85)	0.039
Multivariable	1.00 (reference)	1.66	(0.56-4.96)	0.362	2.64	(0.94-7.41)	0.066
+ hs-CRP (log_{10})	1.00 (reference)	1.91	(0.63-5.79)	0.252	2.62	(0.90-7.58)	0.076
Women							
Crude	1.00 (reference)	0.47	(0.17-1.30)	0.143	1.07	(0.46-2.50)	0.879
Multivariable	1.00 (reference)	0.41	(0.14-1.17)	0.094	1.04	(0.42-2.57)	0.926
+ hs-CRP (log_{10})	1.00 (reference)	0.41	(0.14-1.17)	0.095	1.07	(0.43-2.69)	0.880
Ischemic heart disease							
Men and women							
Crude	1.00 (reference)	2.90	(1.08-7.82)	0.035	2.31	(0.81-6.56)	0.116
Multivariable	1.00 (reference)	2.90	(1.04-8.08)	0.042	2.35	(0.81-6.85)	0.117
+ hs-CRP (log_{10})	1.00 (reference)	3.00	(1.05-8.56)	0.040	2.27	(0.77-6.65)	0.135

OR, odds ratio calculated by conditional logistic regression analysis; CI, confidence interval
Multivariable model adjusted for current smoking status (only for men), treated hypertension, diabetes mellitus, systolic blood pressure, diastolic blood pressure, serum total cholesterol level, and serum HDL-cholesterol level using sex-specific propensity scores

Table 6. Relative odds for ischemic vascular events associated with antibody titers to *P. gingivalis* Su63

	Tertile categories of *P. gingivalis* SU63						
	1st (325-5914)	2nd (5935-10,462)			3rd (10,472-360,948)		
	OR	OR	(95% CI)	*P*	OR	(96% CI)	*P*
Cerebral infarction							
Men							
Crude	1.00 (reference)	7.94	(1.72-36.7)	0.008	9.62	(2.15-43.0)	0.003
Multivariable	1.00 (reference)	7.12	(1.51-33.5)	0.013	9.03	(1.97-41.5)	0.005
+ hs-CRP (log_{10})	1.00 (reference)	8.58	(1.77-41.7)	0.008	9.39	(2.00-44.2)	0.005
Women							
Crude	1.00 (reference)	0.45	(0.15-1.34)	0.152	1.45	(0.64-3.30)	0.379
Multivariable	1.00 (reference)	0.37	(0.12-1.14)	0.084	1.30	(0.54-3.09)	0.560
+ hs-CRP (log_{10})	1.00 (reference)	0.37	(0.12-1.14)	0.083	1.32	(0.55-3.17)	0.541
Ischemic heart disease							
Men and women							
Crude	1.00 (reference)	1.10	(0.44-2.70)	0.843	1.27	(0.51-3.17)	0.611
Multivariable	1.00 (reference)	1.09	(0.43-2.74)	0.856	1.25	(0.49-3.19)	0.633
+ hs-CRP (log_{10})	1.00 (reference)	1.16	(0.45-2.96)	0.761	1.29	(0.51-3.30)	0.594

OR, odds ratio calculated by conditional logistic regression analysis; CI, confidence interval
Multivariable model adjusted for current smoking status (only for men), treated hypertension, diabetes mellitus, systolic blood pressure, diastolic blood pressure, serum total cholesterol level, and serum HDL-cholesterol level using sex-specific propensity scores

may be associated with ischemic vascular disease.

CRP is a candidate protein which may explain the pathogenetic relationship between IHD and periodontitis, because elevated levels of CRP are observed in periodontitis patients[12]. In our data, adjustment for hs-CRP did not considerably modify the association between antibody titers to *P. gingivalis* and the risk of ischemic vascular events; therefore, a high anti-

body titer did not directly reflect high CRP levels in the cases, suggesting that the measurement of antibody levels to *P. gingivalis* may be helpful in risk evaluation of ischemic vascular disease.

Recent meta-analysis using 5 cohort studies indicated that both the prevalence and incidence of IHD are significantly increased in periodontitis patients[31]; however, current knowledge was mainly derived from Western populations and ethnicity must be taken into account for variations. For example, hs-CRP levels, an important risk factor for IHD, are lower in the Japanese population possibly due to both genetic and lifestyle differences[32, 33]; therefore, levels of antibody titers that may reflect systemic inflammatory responses could differ in the Japanese population.

The potential limitations of this study are the small number of cases and the contribution of residual confounders, such as socioeconomic status, which were not controlled for in this analysis. However, the present serologic case-control study nested in the Tokamachi-Nakasato cohort enabled us to verify the relationship between high titers of IgG antibody to *P. gingivalis* and ischemic vascular disease in the Japanese population. In particular, we successfully demonstrated that high antibody titers to *P. gingivalis* SU63 are a strong independent predictor of cerebral infarction in men. In addition to biomarkers such as interleukin-6 and hs-CRP, an elevated antibody titer to SU63 could be a good candidate marker for predicting the future risk of cerebral infarction.

Acknowledgments

The authors declare that they have no conflicts of interest. This study was supported by the Japan Society for the Promotion of Science (20295426 for KT, 15390195 for NT, and 190536 for KY). The Tokamachi-Nakasato cohort study (principal investigator, NT) was also funded by Niigata Prefecture, Japan in fiscal year 1998 and Niigata Association for Comprehensive Health Promotion and Research in fiscal year 2004.

We thank Sunstar Inc. (Osaka, Japan) for providing us with information pertaining to the preparation of bacterial antigens.

References

1) DeStefano F, Anda RF, Kahn HS, Williamson DF, Russell CM: Dental disease and risk of coronary heart disease and mortality. BMJ, 1993; 306: 688-691
2) Grau AJ, Buggle F, Ziegler C, Schwarz W, Meuser J, Tasman AJ, Buhler A, Benesch C, Becher H, Hacke W: Association between acute cerebrovascular ischemia and chronic and recurrent infection. Stroke, 1997; 28: 1724-1729
3) Mattila KJ, Valtonen VV, Nieminen M, Huttunen JK: Dental infection and the risk of new coronary events: Prospective study of patients with documented coronary artery disease. Clin Infect Dis, 1995; 20: 588-592
4) Pussinen PJ, Alfthan G, Rissanen H, Reunanen A, Asikainen S, Knekt P: Antibodies to periodontal pathogens and stroke risk. Stroke, 2004; 35: 2020-2023
5) Sakurai-Komada N, Koike KA, Kaku Y, Hiraki M, Cui R, Sankai T, Kikuchi S, Date C, Tamakoshi A, Iso H: *Chlamydia pneumoniae* infection was associated with risk of mortality from coronary heart disease in Japanese women but not men: The jacc study. J Atheroscler Thromb, 2010; 17: 510-516
6) Satoh H, Saijo Y, Yoshioka E, Tsutsui H: *Helicobacter pylori* infection is a significant risk for modified lipid profile in Japanese male subjects. J Atheroscler Thromb, 2010; 17: 1041-1048
7) Liu R, Yamamoto M, Moroi M, Kubota T, Ono T, Funatsu A, Komatsu H, Tsuji T, Hara H, Nakamura M, Hirai H, Yamaguchi T: *Chlamydia pneumoniae* immunoreactivity in coronary artery plaques of patients with acute coronary syndromes and its relation with serology. Am Heart J, 2005; 150: 681-688
8) Persson GR, Persson RE: Cardiovascular disease and periodontitis: An update on the associations and risk. J Clin Periodontol, 2008; 35: 362-379
9) Ross R: Atherosclerosis--an inflammatory disease. N Engl J Med, 1999; 340: 115-126
10) Tonetti MS: Periodontitis and risk for atherosclerosis: An update on intervention trials. J Clin Periodontol, 2009; 36 Suppl 10: 15-19
11) Nakajima T, Honda T, Domon H, Okui T, Kajita K, Ito H, Takahashi N, Maekawa T, Tabeta K, Yamazaki K: Periodontitis-associated up-regulation of systemic inflammatory mediator level may increase the risk of coronary heart disease. J Periodontal Res, 2010; 45: 116-122
12) Paraskevas S, Huizinga JD, Loos BG: A systematic review and meta-analyses on c-reactive protein in relation to periodontitis. J Clin Periodontol, 2008; 35: 277-290
13) Dorn BR, Dunn WA, Jr., Progulske-Fox A: Invasion of human coronary artery cells by periodontal pathogens. Infect Immun, 1999; 67: 5792-5798
14) Honda T, Oda T, Yoshie H, Yamazaki K: Effects of *Porphyromonas gingivalis* antigens and proinflammatory cytokines on human coronary artery endothelial cells. Oral Microbiol Immunol, 2005; 20: 82-88
15) Tabeta K, Yamazaki K, Hotokezaka H, Yoshie H, Hara K: Elevated humoral immune response to heat shock protein 60 (hsp60) family in periodontitis patients. Clin Exp Immunol, 2000; 120: 285-293
16) Yamazaki K, Ohsawa Y, Itoh H, Ueki K, Tabeta K, Oda T, Nakajima T, Yoshie H, Saito S, Oguma F, Kodama M, Aizawa Y, Seymour GJ: T-cell clonality to *Porphyromonas gingivalis* and human heat shock protein 60s in patients with atherosclerosis and periodontitis. Oral Microbiol Immunol, 2004; 19: 160-167
17) Yamazaki K, Ohsawa Y, Tabeta K, Ito H, Ueki K, Oda T,

Yoshie H, Seymour GJ: Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. Infect Immun, 2002; 70: 2492-2501
18) Yamazaki K, Honda T, Domon H, Okui T, Kajita K, Amanuma R, Kudoh C, Takashiba S, Kokeguchi S, Nishimura F, Kodama M, Aizawa Y, Oda H: Relationship of periodontal infection to serum antibody levels to periodontopathic bacteria and inflammatory markers in periodontitis patients with coronary heart disease. Clin Exp Immunol, 2007; 149: 445-452
19) Beck JD, Eke P, Heiss G, Madianos P, Couper D, Lin D, Moss K, Elter J, Offenbacher S: Periodontal disease and coronary heart disease: A reappraisal of the exposure. Circulation, 2005; 112: 19-24
20) Pussinen PJ, Nyyssonen K, Alfthan G, Salonen R, Laukkanen JA, Salonen JT: Serum antibody levels to *Actinobacillus actinomycetemcomitans* predict the risk for coronary heart disease. Arterioscler Thromb Vasc Biol, 2005; 25: 833-838
21) Pussinen PJ, Alfthan G, Jousilahti P, Paju S, Tuomilehto J: Systemic exposure to *Porphyromonas gingivalis* predicts incident stroke. Atherosclerosis, 2007; 193: 222-228
22) Tanabe N, Suzuki H, Aizawa Y, Seki N: Consumption of green and roasted teas and the risk of stroke incidence: Results from the Tokamachi-Nakasato cohort study in Japan. Int J Epidemiol, 2008; 37: 1030-1040
23) Murayama Y, Okamura K, Kurihara H, Nomura Y, Kokeguchi S, Kato K: Serum immunoglobulin g antibody to periodontal bacteria. Adv Dent Res, 1988; 2: 339-345
24) Spreeuwenberg MD, Bartak A, Croon MA, Hagenaars JA, Busschbach JJ, Andrea H, Twisk J, Stijnen T: The multiple propensity score as control for bias in the comparison of more than two treatment arms: An introduction from a case study in mental health. Med Care, 2010; 48: 166-174
25) Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR: A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol, 1996; 49: 1373-1379
26) Amano A: Molecular interaction of *Porphyromonas gingivalis* with host cells: Implication for the microbial pathogenesis of periodontal disease. J Periodontol, 2003; 74: 90-96
27) 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
28) Imamura T, Potempa J, Tanase S, Travis J: Activation of blood coagulation factor X by arginine-specific cysteine proteinases (gingipain-rs) from *Porphyromonas gingivalis*. J Biol Chem, 1997; 272: 16062-16067
29) Johansson A, Johansson I, Eriksson M, Ahren AM, Hallmans G, Stegmayr B: Systemic antibodies to the leukotoxin of the oral pathogen *Actinobacillus actinomycetemcomitans* correlate negatively with stroke in women. Cerebrovasc Dis, 2005; 20: 226-232
30) Ojima M, Hanioka T, Tanaka K, Inoshita E, Aoyama H: Relationship between smoking status and periodontal conditions: Findings from national databases in Japan. J Periodontal Res, 2006; 41: 573-579
31) Bahekar AA, Singh S, Saha S, Molnar J, Arora R: The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: A meta-analysis. Am Heart J, 2007; 154: 830-837
32) Arima H, Kubo M, Yonemoto K, Doi Y, Ninomiya T, Tanizaki Y, Hata J, Matsumura K, Iida M, Kiyohara Y: High-sensitivity c-reactive protein and coronary heart disease in a general population of Japanese: the Hisayama study. Arterioscler Thromb Vasc Biol, 2008; 28: 1385-1391
33) Sakkinen P, Abbott RD, Curb JD, Rodriguez BL, Yano K, Tracy RP: C-reactive protein and myocardial infarction. J Clin Epidemiol, 2002; 55: 445-451

Available online at www.sciencedirect.com
SciVerse ScienceDirect
journal homepage: http://www.elsevier.com/locate/aob

Relationship between serum antibody titres to *Porphyromonas gingivalis* and hs-CRP levels as inflammatory markers of periodontitis

Hirotaka Miyashita [a,b,c], *Tomoyuki Honda* [a,b,c], *Tomoki Maekawa* [a,b,c], *Naoki Takahashi* [a,b,c], *Yukari Aoki* [a,b,c], *Takako Nakajima* [a,d], *Koichi Tabeta* [a], *Kazuhisa Yamazaki* [a,b,*]

[a] *Center for Transdisciplinary Research, Niigata University, Niigata 9518514, Japan*
[b] *Laboratory of Periodontology and Immunology, Division of Oral Science for Health Promotion, Niigata University Graduate School of Medical and Dental Sciences, Niigata 9518514, Japan*
[c] *Division of Periodontology, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata 9518514, Japan*
[d] *General Dentistry and Clinical Education Unit, Niigata University Medical and Dental Hospital, Niigata 9518514, Japan*

ARTICLE INFO

Article history:
Accepted 7 November 2011

Keywords:
Periodontitis
CRP
Antibody
Porphyromonas gingivalis

SUMMARY

Objective: The present study was designed to investigate whether titres of antibody to two strains of *Porphyromonas gingivalis*, FDC381 and SU63, are associated with serum high-sensitivity C-reactive protein (hs-CRP) levels in Japanese periodontitis patients.

Design: Forty-nine patients with moderate to advanced periodontitis and 40 periodontally healthy control subjects were included in this study. hs-CRP levels and antibody titres to *P. gingivalis* were measured at baseline and reassessment 3–4 months after periodontal treatment in periodontitis patients as well as at the time of examination in the periodontally healthy subjects. Further, the effect of periodontal therapy, including surgical treatment and use of antibacterials on both markers, was analysed in patients.

Results: hs-CRP levels and antibody titres to *P. gingivalis* were higher in periodontitis patients than in control subjects, and they significantly decreased following periodontal treatment ($p < 0.005$). Also, a significant decrease in hs-CRP levels as a result of periodontal treatment was found in patients with hs-CRP levels $>1\ mg\ l^{-1}$ at baseline ($p < 0.005$). Probing depth, clinical attachment level, and alveolar bone loss in patients were significantly associated with a higher antibody titre to both strains of *P. gingivalis* ($p < 0.05$), but were not related to hs-CRP levels. No relationship was observed between hs-CRP levels and tertiles as defined by titres of antibody to *P. gingivalis* strains FDC381 and SU63.

Conclusions: Our data indicate that hs-CRP levels were independent of antibody titres to *P. gingivalis* in Japanese periodontitis patients.

* *Corresponding author at*: Laboratory of Periodontology and Immunology, Division of Oral Science for Health Promotion, Niigata University Graduate School of Medical and Dental Sciences, Niigata 9518514, Japan. Tel.: +81 25 227 0744; fax: +81 25 227 0744.
E-mail address: kaz@dent.niigata-u.ac.jp (K. Yamazaki).

0003–9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.archoralbio.2011.11.008

Please cite this article in press as: Miyashita H, et al. Relationship between serum antibody titres to *Porphyromonas gingivalis* and hs-CRP levels as inflammatory markers of periodontitis. *Archives of Oral Biology* (2011), doi:10.1016/j.archoralbio.2011.11.008

Several studies have suggested chronic periodontitis as a possible risk factor for atherothrombotic vascular diseases such as cerebral infarction and ischaemic heart disease (IHD).[1–4] Atherosclerosis is an inflammatory disease initiated by injury to the vascular endothelium[5] and is a major cause of atherothrombotic vascular diseases involving plaque formation, plaque disruption, and subsequent atherothrombosis.[6,7] Regarding mechanisms of the relationship between periodontitis and atherothrombotic vascular diseases, the bacterial burden in gingival tissues that induces systemic inflammation may modulate atherogenesis in periodontitis patients. A similar association has been observed between infection with *Chlamydia pneumoniae* and *Helicobacter pylori* and atherothrombotic vascular disease.[8,9]

C-reactive protein (CRP), a plasma protein synthesised by the liver, is a sensitive and dynamic systemic marker of inflammation[10] and it was reported to be a predictor of cardiovascular risk independent of conventional cardiovascular risk factors.[11,12] A meta-analysis of 22 prospective studies confirmed that high CRP levels are associated with the risk of coronary heart disease (CHD) and ischaemic stroke.[13] On the other hand, a recent meta-analysis found that CRP levels were linearly associated with several conventional risk factors[14]; therefore, the roles of CRP in cardiovascular risk need to be assessed further by considering the effects of other modifiers of CRP levels, such as infectious diseases that sustain a low systemic grade of inflammation. Indeed, several studies have reported slightly elevated CRP levels in periodontitis patients and patients with other chronic infectious diseases.[15]

Porphyromonas gingivalis, a periodontopathic Gram-negative bacterium, has been acknowledged as a risk factor of periodontal disease and extensively analysed with regard to its role in the pathogenesis of atherogenesis.[16–20] The use of clinical parameters to define periodontal disease has been criticised to investigate the relationship between two diseases since they do not represent systemic effects elicited by the periodontal disease directly. Previous reports have demonstrated an association between its antibody titre, another systemic marker of inflammation, and the incidence of stroke[4] and IHD[21,22] in Western populations. A recent nested case–control study in a larger cohort successfully investigated the influence of systemic exposure to *P. gingivalis* on the incidence of stroke.[23] Taken together, CRP levels and antibody titres to periodontopathic bacteria are potentially surrogate markers of periodontitis in the assessment of systemic inflammatory responses that modulate the progression of atherosclerotic diseases. The relationship between CRP levels and antibody titres need to be clarified, since both markers monitor the systemic inflammatory response to periodontal infection and may affect each other potentially. However, no reports have investigated the association between CRP levels and serum antibody titres in an Asian population to date. Our previous report demonstrated that a high frequency of antibody positivity for *P. gingivalis* SU63 was observed in CHD patients, suggesting that the presence of a particular strain with high virulence may be related to the diseases.[24]

Therefore, the present study was designed to investigate whether titres of antibody to two strains of *P. gingivalis* (FDC381 and SU63) are associated with hs-CRP levels in Japanese periodontitis patients. Further, the effect of periodontal therapy on both markers was analysed in the same subjects.

1. Materials and methods

1.1. Study subjects

Forty-nine patients with moderate to advanced periodontitis and 40 periodontally healthy control subjects were included in this study. The institutional review board of Niigata University Graduate School of Medical and Dental Sciences approved this study, and written informed consent was obtained from all patients before inclusion in the study. Current or past smokers were excluded from the present study, and periodontal tissue destruction was assessed as described previously.[20] Clinical examination included analysis of the plaque-control record,[25] recording of both probing depth and attachment level, and assessment of alveolar bone resorption. Probing depth and attachment level were recorded at six sites around each tooth. Alveolar bone resorption was measured radiographically on the proximal surface of each tooth. Intra- and inter-examiner (T. Nakajima, K. Tabeta, and K. Yamazaki) calibrations were performed on patients prior to initiating the baseline measurements. None of the patients had a history of periodontal treatment and none had taken antibiotics within 3 months prior to baseline examination. Serum for non-disease controls was obtained from staff members of the Niigata University Dental Hospital. None of these subjects showed periodontal pockets, loss of attachment or alveolar bone resorption.

1.2. Treatment

Initial therapy consisted of mechanical plaque control together with scaling and root planing under local anaesthesia. The effect of the initial therapy was evaluated and surgical procedures were applied for treatment of residual periodontal pockets. Either a modified Widman flap procedure or an undisplaced flap procedure was selected depending on the individual requirements. The number of sites requiring surgical procedures varied from patient to patient. After completion of surgical procedures, the patients were examined and followed up either monthly or every 3 months, depending on individual requirements. Antibacterials (a third-generation cephalosporin) were usually prescribed for 4 days after periodontal surgery. Serum samples were taken 3–4 months after completion of active therapy (scaling and root planing or periodontal surgery).

1.3. Measurement of hs-CRP

hs-CRP levels were measured by a latex particle-enhanced immunoassay procedure (SRL, Tokyo, Japan). The lower limit of the assay was 0.05 mg l^{-1}. Intra- and inter-assay CVs were less than 10%.

1.4. P. gingivalis antibody titres

Titres of antibody to *P. gingivalis* lysates were measured by enzyme-linked immunosorbent assay (ELISA) and evaluated

Please cite this article in press as: Miyashita H, et al. Relationship between serum antibody titres to *Porphyromonas gingivalis* and hs-CRP levels as inflammatory markers of periodontitis. *Archives of Oral Biology* (2011), doi:10.1016/j.archoralbio.2011.11.008

according to the method described previously, with some modifications.[26] *P. gingivalis* lysates for strains FDC381 and SU63 (Institute of Immunology Co., Ltd., Tokyo, Japan) were used as antigens for ELISA assay. The lysates (0.05 μg) were coated on 96-well ELISA plates (100 μl/well; 0.5 μg ml^{-1} in 0.05 M bicarbonate buffer, pH 9.6) and incubated overnight at 4 °C. The plates were then washed with 0.05% Tween-saline, and 100 μl of test sample diluted with 0.05% Tween-saline containing 4% goat serum (1:400–1:12800) was added and the mixture was incubated for 1 h at room temperature. After washing three times with 0.05% Tween-saline, horseradish peroxidase-conjugated goat anti-human immunoglobulin G (IgG) (Sigma, Ronkonkoma, NY, USA) was added and the samples incubated for 1 h at room temperature. Peroxidase reaction was accomplished by the addition of 75 μl/well 0.04% H_2O_2 and orthophenylenediamine (1 mg ml^{-1}) in sodium citrate buffer. Colour development was halted by the addition of 4 M sulphuric acid, and absorbance was read at a wavelength of 490 nm. Pooled sera for controls were prepared from systemically and periodontally healthy subjects with very low antibody response to *P. gingivalis*, and these were used to titrate the antibody levels into ELISA units. The subjects were then divided into tertiles based on antibody titre to *P. gingivalis* lysates.

1.5. *Statistical analysis*

Statistical differences in baseline characteristics between cases and controls were analysed by the Student's *t*-test, Mann–Whitney *U*-test and the Spearman's correlation test. The effect of periodontal treatment was compared using the paired *t*-test for periodontal status and the Wilcoxon signed-rank test for serum markers. In periodontitis patients, *P. gingivalis* antibody titres were classified into three categories according to tertiles based on the value order of antibody titres from the highest to the lowest. Associations between tertile categories and periodontal status at baseline were assessed by one-way analysis of variance by the linear trend post-test. Baseline serum markers for each tertile were compared by the Kruskal–Wallis test. Multiple regression analyses were performed to determine whether there were correlations between hs-CRP levels and independent variables. These variables were as follows: (1) categorical data: gender (0: male, 1: female), patients/controls (0: control, 1: patient), and antibody titres to *P. gingivalis* strain FDC381 and SU63 (1:3; tertile groups of EU) and (2) continuous data: ages, interleukin 6 (IL-6; pg ml^{-1}), and tumour necrosis factor alpha (TNF-α; pg ml^{-1}) for baseline. In analyses for patients after treatment, experiences of surgical treatment (1: yes, 0: no) and internal use of antibacterials (1: yes, 0: no) were included as variables. Statistical analyses were performed using standard statistical software (Statview J-4.5 application programme; SAS Institute Inc., Cary, NC, USA and SPSS 13.0 for Windows; SPSS, Inc., Chicago, IL, USA).

2. Results

2.1. *Baseline characteristics*

Baseline characteristics of cases and controls are listed in Table 1. At baseline, the condition of the periodontal tissues significantly improved following scaling and root planing along with subsequent periodontal surgery. All the procedures were completed in 6 months for scaling and root planing and in 12 months for surgical treatment. The percentage of sites showing probing depths of either >6 mm or 4–6 mm was significantly lower and the percentage of sites showing probing depths of <4 mm was significantly higher at reassessment compared with that at baseline. The percentage of sites showing a clinical attachment level of <4 mm was also higher at reassessment.

hs-CRP levels in patients before periodontal treatment were significantly higher compared with those in control subjects ($p < 0.005$), and were significantly decreased following periodontal treatment ($p < 0.005$).

Titres of antibody to *P. gingivalis* strains FDC381 and SU63 in each group are shown in Table 1. The median antibody titres to strains FDC381 and SU63 of *P. gingivalis* in patients before periodontal treatment were significantly elevated compared with those of control subjects ($p < 0.0001$), and they significantly decreased after periodontal treatment ($p < 0.0001$ and $p < 0.005$, respectively). Significantly positive correlations were observed between antibody titres to *P. gingivalis* strains FDC381 and SU63 in periodontitis patients at both baseline ($r = 0.9121$, $p < 0.0001$) and reassessment ($r = 0.8898$, $p < 0.0001$). No correlation was observed between CRP and BOP (Bleeding on Probing) at baseline examination ($r = 0.1269$, $p = 0.3899$).

2.2. *Serum antibody titres to P. gingivalis and periodontal disease status*

The relationship between antibody response to *P. gingivalis* and periodontal disease status was analysed in tertiles defined by antibody titre in periodontitis patients at baseline. As shown in Tables 2 and 3, all periodontal parameters were progressively and significantly increased with higher antibody titre to strains FDC381 and SU63, respectively ($p < 0.05$ for linear trend).

2.3. *P. gingivalis antibody titres and hs-CRP levels*

Antibody titres to both strains of *P. gingivalis* (FDC381 and SU63) and hs-CRP levels varied widely across periodontitis patients at baseline. No direct correlation was observed between titres of antibody to either *P. gingivalis* strains FDC381 or SU63 and hs-CRP levels ($r = -0.117$, $p = 0.3419$; $r = -0.06726$, $p = 0.5858$, respectively) (Fig. 1). The relationship between antibody titres to *P. gingivalis* and the hs-CRP levels was further analysed using tertile groups as defined by antibody titre to either strain of *P. gingivalis* in periodontitis patients at baseline (Tables 2 and 3). No significant difference and dose-dependent tendency was observed amongst tertiles of antibody against *P. gingivalis* strains FDC381 and SU63 ($p > 0.05$ for either strain of *P. gingivalis*, the Kruskal–Wallis test). Although median hs-CRP levels in each group decreased following periodontal treatment, a statistical difference was found only for the highest tertiles ($p < 0.05$ for either strain of *P. gingivalis*, the Wilcoxon signed-rank test). These data indicate that hs-CRP levels were independent of *P. gingivalis* antibody titres as inflammatory markers in periodontitis patients.

Please cite this article in press as: Miyashita H, et al. Relationship between serum antibody titres to *Porphyromonas gingivalis* and hs-CRP levels as inflammatory markers of periodontitis. *Archives of Oral Biology* (2011), doi:10.1016/j.archoralbio.2011.11.008

Table 1 – Clinical characteristics and serum markers of the study population.

	Periodontitis ($n = 49$)		Control ($n = 40$)
	Baseline	Re-assessment	
Age	50.8 ± 13.8	–	48.3 ± 9.9
Male/female	17/32	–	19/21
Subjects with surgery	–	25	–
Subgects with antibiotics	–	2	–
Mean PD (mm)	3.6 ± 0.9*	2.4 ± 0.5*,‡	2.0 ± 0.3
PD < 4 mm (percentage of sites)	62.4 ± 22.4*	90.9 ± 8.5*,‡	98.8 ± 3.0
PD 4–6 mm (percentage of sites)	30.2 ± 16.6*	8.5 ± 7.9*,‡	1.2 ± 2.9
PD > 6 mm (percentage of sites)	6.8 ± 7.5*	0.8 ± 1.5†,‡	0.0 ± 0.1
Mean CAL (mm)	4.1 ± 1.2*	3.6 ± 1.0*,‡	2.1 ± 0.3
CAL < 4 mm (percentage of sites)	51.9 ± 24.5*	60.0 ± 24.3*,§	97.8 ± 3.1
CAL 4–6 mm (percentage of sites)	34.7 ± 16.5*	32.2 ± 18.8*	2.2 ± 3.1
CAL > 6 mm (percentage of sites)	13.5 ± 14.9*	7.7 ± 9.9*,‡	0.0 ± 0.1
Mean BL	36.2 ± 12.9	ND	ND
BL ≥ 50% (percentage of sites)	22.1 ± 17.7	ND	ND
Number of teeth	25.4 ± 4.5†	23.9 ± 5.3*,‡	28.0 ± 2.1
hs-CRP (mg l^{-1})	0.53 (0.20–1.28)¶	0.37 (0.13–0.62)††	0.19 (0.10–0.40)
IL-6 (pg ml^{-1})	0.56 (0.33–0.79)#	0.42 (0.15–0.62)††	0.41 (0.15–0.57)
TNF-α (pg ml^{-1})	1.09 (0.77–1.48)#	1.02 (0.69–1.54)#	1.40 (0.99–2.44)
Titres of antibody to			
P. gingivalis strain FDC381	6996.8 (3318.1–18937.3)‖	4813.6 (2843.0–8228.2)‖,**	1165.7 (530.3–1874.0)
P. gingivalis strain SU63	6513.6 (3727.5–14663.7)‖	4904.3 (3307.6–10786.5)‖,††	1440.6 (820.4–3330.5)

BL, bone loss; CAL, clinical attachment level; ND, not determined; PD, pocket depth. Data are expressed as mean ± S.D. or median (IQR). Age: $p = 0.3322$ (unpaired *t*-test). Male/female: $p = 0.2208$ (chi-square test).
* $p < 0.0001$ versus controls (unpaired *t*-tests).
† $p < 0.005$ versus controls (unpaired *t*-tests).
‡ $p < 0.0001$ versus baseline (paired *t*-tests).
§ $p < 0.0005$ versus baseline (paired *t*-tests).
‖ $p < 0.0001$ versus controls (Mann–Whitney *U*-tests).
¶ $p < 0.005$ versus controls (Mann–Whitney *U*-tests).
# $p < 0.05$ versus controls (Mann–Whitney *U*-tests).
** $p < 0.0001$ versus baseline (Wilcoxon signed-ranks tests).
†† $p < 0.005$ versus baseline (Wilcoxon signed-ranks tests).

We dichotomised hs-CRP levels before periodontal treatment in patients at a level of 1 mg l^{-1}, because a recent study demonstrated that the cutoff point for hs-CRP level for high risk of future development of CHD in Japanese subjects was likely to be >1.0 mg l^{-1}[27] (Table 4). A significant decrease in hs-CRP levels as a result of periodontal treatment was found in patients with hs-CRP levels >1 mg l^{-1} ($p < 0.005$).

Titres of antibody to both strains of *P. gingivalis* were higher in patients with hs-CRP levels >1 mg l^{-1} than in those with levels ≤1 mg l^{-1}; however, the difference was not statistically significant ($p > 0.05$). After periodontal treatment, antibody titres in each group were significantly decreased ($p < 0.005$ for FDC381, $p < 0.05$ for SU63).

2.4. *Correlation analyses between hs-CRP and independent variables*

To evaluate the correlation between hs-CRP levels and antibody titres to *P. gingivalis*, we performed multivariate regression analyses for possible variables. Analyses were performed separately for antibody titres to *P. gingivalis* strains FDC381 and SU63 because of colinearity. No correlation was observed between hs-CRP levels and antibody titres to *P. gingivalis* in any of the patients or control subjects. hs-CRP levels were significantly associated with age and diagnosis of periodontitis with low partial correlation coefficients in the models, including antibody titres to *P. gingivalis* strains FDC381 ($p = 0.010$, coeff. 0.278 for age; $p = 0.004$, coeff. 0.308 for patients/control, Table 5A) and SU63 ($p = 0.029$, coeff. 0.239 for age; $p = 0.001$, coeff. 0.357 for patients/control, Table 5B). IL-6 was a significant variable associated with hs-CRP in the model that included antibody titre to *P. gingivalis* strain SU63 as a variable ($p = 0.031$, Table 5B). A positive tendency of correlation between IL-6 and hs-CRP was observed in the model that included antibody titres to *P. gingivalis* strain FDC381 as a variable; however, it was not significant (Table 5A). We further analysed the effect of periodontal treatment, including experiences of periodontal surgery and internal use of antibacterials. Use of antibacterials was removed from the final model presented in Table 6 because the number of patients who received treatment without antibacterials was very small. No correlation was observed between hs-CRP and antibody titres to *P. gingivalis*. Notably, the significant association of IL-6 with hs-CRP observed at baseline disappeared after treatment in both models that included antibody titres to *P. gingivalis* strains FDC381 (Table 5A) and SU63 (Table 5B). By contrast, a negative association of TNF-α with hs-CRP was observed in the model that included antibody titres to *P. gingivalis* strain FDC381; however, the partial correlation coefficient indicated that the association was quite low (Table 5A).

Please cite this article in press as: Miyashita H, et al. Relationship between serum antibody titres to *Porphyromonas gingivalis* and hs-CRP levels as inflammatory markers of periodontitis. *Archives of Oral Biology* (2011), doi:10.1016/j.archoralbio.2011.11.008

Table 2 – Comparison of tertile distributions of clinical characteristics and antibody titre to *P. gingivalis* strain FDC381 in periodontitis patients at baseline.

	Titres of antibody to *P. gingivalis* strain FDC381		
	3rd tertile (>12,500) ($n = 17$)	2nd tertile (4100–12,500) ($n = 16$)	1st tertile (<4100) ($n = 16$)
Age	43.2 ± 14.1[§]	54.9 ± 13.6	54.8 ± 10.6
Male/female	6/11	4/12	7/9
Periodontal status at baseline			
Mean PD (mm)	4.1 ± 1.0[‡]	3.4 ± 0.7	3.3 ± 0.6
PD ≥ 4 mm (percentage of sites)	48.3 ± 22.8[§]	31.9 ± 19.3	30.1 ± 17.3
Mean CAL (mm)	5.0 ± 1.4[†]	3.8 ± 0.9	3.6 ± 0.5
CAL ≥ 4 mm (percentage of sites)	64.4 ± 23.4[‡]	39.8 ± 22.7	38.5 ± 18.3
Mean BL	44.0 ± 14.2[†]	36.2 ± 9.7	28.1 ± 9.2
BL ≥ 50% (percentage of sites)	34.3 ± 20.0[*]	20.2 ± 12.8	10.9 ± 10.4
Number of teeth	25.1 ± 4.9	26.4 ± 3.3	24.8 ± 5.2
Periodontal status at reassessment			
Mean PD (mm)	2.3 ± 0.5[‖]	2.5 ± 0.4[‖]	2.4 ± 0.5[‖]
PD ≥ 4 mm (percentage of sites)	10.5 ± 9.7[‖]	9.2 ± 7.3[‖]	8.2 ± 8.6[‖]
Mean CAL (mm)	4.2 ± 1.2[‡,¶]	3.4 ± 0.7[**]	3.2 ± 0.5[**]
CAL ≥ 4 mm (percentage of sites)	53.8 ± 27.7[‡,**]	34.8 ± 20.1	29.2 ± 16.3
Number of teeth	22.8 ± 5.9[**]	24.9 ± 4.2[#]	24.0 ± 5.7[#]
Serum markers at baseline			
hs-CRP level (mg l^{-1})	0.48 (0.13–1.32)	0.31 (0.19–0.81)	0.78 (0.40–4.84)
Titres of antibody to			
P. gingivalis strain FDC381	35934.1 (18937.3–66100.2)[††]	6797.6 (4887.3–9209.8)	1384.0 (759.3–3189.3)
P. gingivalis strain SU63	42798.3 (9658.4–55106.2)[††]	6394.3 (4422.8–8123.3)	772.2 (487.9–3748.5)
Serum markers at reassessment			
hs-CRP level (mg l^{-1})	0.31 (0.12–0.57)[§§]	0.26 (0.10–0.43)	0.44 (0.35–0.72)
Titres of antibody to			
P. gingivalis strain FDC381	16283.7 (8228.2–57048.6)[††,‡‡]	4587.2 (3675.7–5952.9)[‡‡]	1366.9 (689.6–2754.4)
P. gingivalis strain SU63	18778.9 (5051.1–46579.6)[††,§§]	4721.7 (3836.3–8404.8)[§§]	1790.0 (499.4–3505.0)

BL, bone loss; CAL, clinical attachment level; PD, pocket depth. Data are expressed as mean ± S.D. or median (IQR).
[*] $p < 0.0001$ amongst tertiles (one-way ANOVA followed by the linear trend post-test).
[†] $p < 0.0005$ amongst tertiles (one-way ANOVA followed by the linear trend post-test).
[‡] $p < 0.005$ amongst tertiles (one-way ANOVA followed by the linear trend post-test).
[§] $p < 0.05$ amongst tertiles (one-way ANOVA followed by the linear trend post-test).
[‖] $p < 0.0001$ versus baseline (paired *t*-tests).
[¶] $p < 0.0005$ versus baseline (paired *t*-tests).
[#] $p < 0.005$ versus baseline (paired *t*-tests).
[**] $p < 0.05$ versus baseline (paired *t*-tests).
[††] $p < 0.0001$ amongst tertiles (Kruskal–Wallis tests).
[‡‡] $p < 0.005$ versus baseline (Wilcoxon signed-ranks tests).
[§§] $p < 0.05$ versus baseline (Wilcoxon signed-ranks tests).

3. Discussion

In the present study, no apparent association was found between antibody levels to *P. gingivalis* and hs-CRP levels, although the increase of *P. gingivalis* antibody titres and hs-CRP levels in patients was ameliorated by periodontal treatment. hs-CRP levels and antibody titres to *P. gingivalis* exhibited a tendency towards a negative association. Although we could not find reports of data similar to this observation, we speculate that high antibody titres may function on suppressing acute phase responses, such as CRP production. The present study indicates the different roles of antibody titres to *P. gingivalis* and hs-CRP levels as inflammatory markers of periodontitis in the evaluation of host immune response.

Systemic elevations of antibody are frequently observed as a manifestation of bacterial infection. Antibody titres to periodontopathic bacteria have been shown to be elevated in periodontitis patients[28,29] and were decreased by periodontal treatment.[30,31] Furthermore, IgG antibody titres to selected periodontal species could serve as surrogate markers of clinical periodontal status in epidemiological studies.[32] Titres of antibody to periodontopathic bacteria, especially *P. gingivalis*, reflect current and past infection status as being a useful systemic biomarker for periodontitis in the patients who have not received periodontal therapy.

Regarding the relationship between periodontitis and atherothrombotic diseases, previous reports have demonstrated an association between titres of antibodies to *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* with the incidence of stroke[4] and IHD.[21,22] The positive relationship shown by those data suggests that antibody titres to periodontopathic bacteria may directly represent the effect of systemic inflammation, which may possibly modulate vascular inflammation in the progression of atherothrombotic diseases. Our previous report demonstrated that high frequency of antibody positivity for *P. gingivalis* strain SU63 was observed in CHD patients but not in periodontitis patients, suggesting that the presence of a particular strain with high virulence may be related to the disease.[24] It is possible that

Please cite this article in press as: Miyashita H, et al. Relationship between serum antibody titres to *Porphyromonas gingivalis* and hs-CRP levels as inflammatory markers of periodontitis. *Archives of Oral Biology* (2011), doi:10.1016/j.archoralbio.2011.11.008

Table 3 – Comparison of tertile distributions of clinical characteristics and antibody titre to P. gingivalis strain SU63 in periodontitis patients at baseline.

	Titres of antibody to *P. gingivalis* strain SU63		
	3rd tertile (>9600) (n = 17)	2nd tertile (4350–9600) (n = 16)	1st tertile (<4350) (n = 16)
Age	45.1 ± 14.3	53.8 ± 13.1	54.0 ± 12.9
Male/female	4/13	6/10	7/9
Periodontal status at baseline			
Mean PD (mm)	4.0 ± 1.1*	3.6 ± 0.8	3.3 ± 0.7
PD ≥ 4 mm (percentage of sites)	46.4 ± 23.7*	34.1 ± 18.1	29.9 ± 18.8
Mean CAL (mm)	4.6 ± 1.4*	4.2 ± 1.2	3.6 ± 0.7
CAL ≥ 4 mm (percentage of sites)	57.7 ± 24.7*	49.7 ± 25.6	35.6 ± 18.4
Mean BL	40.9 ± 13.3*	38.0 ± 13.1	29.6 ± 9.9
BL ≥ 50% (percentage of sites)	28.1 ± 19.5*	23.2 ± 18.5	14.6 ± 12.3
Number of teeth	26.5 ± 3.5	25.1 ± 4.8	24.6 ± 5.2
Periodontal status at reassessment			
Mean PD (mm)	2.4 ± 0.4†	2.4 ± 0.5‡	2.4 ± 0.4†
PD ≥ 4 mm (percentage of sites)	10.5 ± 8.0†	9.7 ± 10.8†	7.7 ± 6.6‡
Mean CAL (mm)	3.8 ± 1.1‡	3.9 ± 1.1‖	3.2 ± 0.5§
CAL ≥ 4 mm (percentage of sites)	45.5 ± 25.7*,§	45.1 ± 28.5	28.6 ± 13.0
Number of teeth	24.4 ± 4.2‖	23.4 ± 6.1‖	23.8 ± 5.7‖
Serum markers at baseline			
hs-CRP level (mg l^{-1})	1.06 (0.12–1.46)	0.34 (0.25–0.64)	0.64 (0.33–4.84)
Titres of antibody to			
P. gingivalis strain FDC381	35934.1 (12590.5–66100.2)¶	6797.6 (4537.1–10174.7)	1384.0 (759.3–3455.4)
P. gingivalis strain SU63	42798.3 (14663.7–55106.2)¶	6396.3 (5282.1–6816.7)	772.2 (487.9–3527.1)
Serum markers at reassessment			
hs-CRP level (mg l^{-1})	0.31 (0.10–0.57)**	0.35 (0.15–0.46)	0.42 (0.31–0.68)
Titres of antibody to			
P. gingivalis strain FDC381	14302.2 (6646.6–45533.6)¶,#	4587.2 (3395.3–5700.2)**	1366.9 (689.6–3316.9)
P. gingivalis strain SU63	18778.9 (9407.1–46579.6)¶,**	4735.8 (3869.8–6250.3)	1752.9 (499.4–3343.0)

BL, bone loss; CAL, clinical attachment level; PD, pocket depth. Data are expressed as mean ± S.D. or median (IQR).
* $p < 0.05$ amongst tertiles (one-way ANOVA followed by the linear trend post-test).
† $p < 0.0001$ versus baseline (paired t-tests).
‡ $p < 0.0005$ versus baseline (paired t-tests).
§ $p < 0.005$ versus baseline (paired t-tests).
‖ $p < 0.05$ versus baseline (paired t-tests).
¶ $p < 0.0001$ amongst tertiles (Kruskal–Wallis tests).
# $p < 0.0005$ versus baseline (Wilcoxon signed-ranks tests).
** $p < 0.05$ versus baseline (Wilcoxon signed-ranks tests).

P. gingivalis strain SU63 has specific biological effects that may induce specific host responses. However, hs-CRP levels were similar in the tertile analysis of antibodies to *P. gingivalis* strains FDC381 and SU63 in the present study. For this study population, we have no information about antibody titres to other *P. gingivalis* strains. Other strains with high virulence to the host may be probable candidates for further research in this area to establish a correlation between antibody titres to them and CRP in the serum. The finding indicates that a population who may be at risk for CHD can be predicted by the

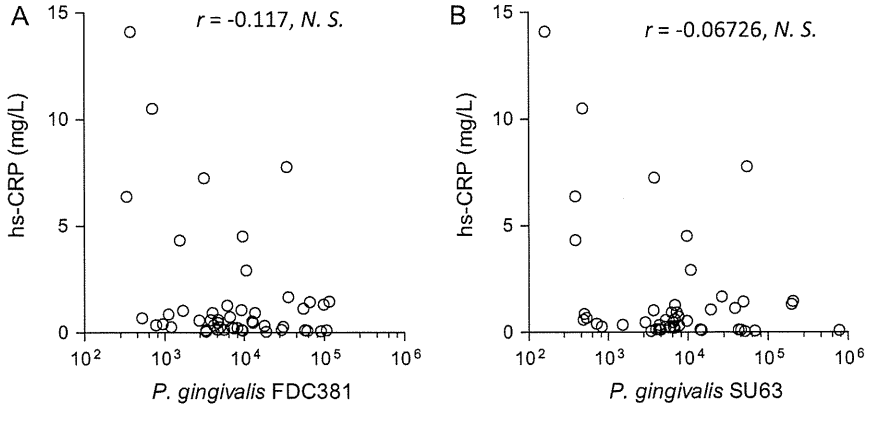

Fig. 1 – Correlations between systemic inflammatory mediators and antibody titre to *P. gingivalis* in periodontitis patients at baseline (n = 49). (A) *P. gingivalis* strain FDC381 versus hs-CRP levels, (B) *P. gingivalis* strain SU63 versus hs-CRP levels. Spearman's correlation testing was performed for statistical analysis.

Please cite this article in press as: Miyashita H, et al. Relationship between serum antibody titres to *Porphyromonas gingivalis* and hs-CRP levels as inflammatory markers of periodontitis. *Archives of Oral Biology* (2011), doi:10.1016/j.archoralbio.2011.11.008