References

1. Loeys BL, Chen J, Neptune ER, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nat Genet 2005; 37: 275–281.
2. Loeys BL, Schwarze U, Holm T. Aneurysm syndromes caused by mutations in the TGF-β receptor. N Engl J Med 2006; 355: 788–798.
3. Mizuguchi T, Collod-Beroud G, Akiyama T, et al. Heterozygous TGFBR2 mutations in Marfan syndrome. Nat Genet 2004; 36: 855–860.
4. Yamawaki T, Nagaoka K, Morishige K, et al. Familial thoracic aortic aneurysm and dissection associated with Marfan-related gene mutations: case report of a family with two gene mutations. Intern Med 2009; 48: 555–558.
5. Akutsu K, Morisaki H, Okajima T, et al. Genetic analysis of young adult patients with aortic disease not fulfilling the diagnostic criteria for Marfan syndrome. Circ J 2010; 74: 990–997.
6. Muramatsu Y, Kosho T, Magota M, et al. Progressive aortic root and pulmonary artery aneurysms in a neonate with Loeys–Dietz syndrome type 1B. Am J Med Genet A 2010; 152A: 417–421.
7. Viassolo V, Lituania M, Marasini M, et al. Fetal aortic root dilation: a prenatal feature of the Loeys–Dietz syndrome. Prenat Diagn 2006; 26: 1081–1083.
8. Watanabe Y, Sakai H, Nishimura A, et al. Paternal somatic mosaicism of a TGFBR2 mutation transmitting to an affected son with Loeys–Dietz syndrome. Am J Med Genet A 2008; 146A: 3070–3074.

Surgical Experience With Aggressive Aortic Pathologic Process in Loeys-Dietz Syndrome

Yutaka Iba, MD, Kenji Minatoya, MD, Hitoshi Matsuda, MD, Hiroaki Sasaki, MD, Hiroshi Tanaka, MD, Hiroko Morisaki, MD, Takayuki Morisaki, MD, Junjiro Kobayashi, MD, and Hitoshi Ogino, MD

Departments of Cardiovascular Surgery and Bioscience and Genetics, National Cerebral and Cardiovascular Center, Osaka; Department of Cardiovascular Surgery, Tokyo Medical University, Tokyo, Japan

Background. Loeys-Dietz syndrome (LDS) is a recently recognized connective tissue disorder (CTD) caused by mutations in *transforming growth factor-beta receptor (TGFBR)1* and *TGFBR2*. Surgical outcomes of aortic repair in patients with LDS are poorly known.

Methods. We enrolled 16 patients with *TGFBR* mutations identified by gene analysis in this study. Between 1993 and 2011, they underwent 41 aortic surgical procedures. Ten patients (group D: dissection group) underwent aortic repair for acute or chronic aortic dissection as a first surgical intervention, and 6 patients (group N: nondissection group) underwent surgical treatment for aortic root dilatation. The mean follow-up period was 103.7 ± 92.3 months (range, 2– 276 months).

Results. There were no in-hospital deaths. In group N, valve-sparing root replacement (VSRR) was performed in all patients. The residual aorta in 9 patients (90%) from group D required further repairs, 3 times on average. Moreover, in 4 patients (40%), the aorta was entirely replaced in serial procedures. In group N, aortic dissection occurred in only 1 patient (17%). The aortic event-free rates at 5 years were 40% in group D and 80% in group N, respectively ($p = 0.819$). One late death due to arrhythmia occurred 1 month after VSRR. The cumulative survival rates at 5 years were 100% in group D and 83% in group N, respectively ($p = 0.197$).

Conclusions. Surgical outcomes for patients with LDS were satisfactory. Once aortic dissection occurred, the aorta expanded rapidly, requiring further operations. Therefore, early surgical intervention may improve prognosis by preventing a fatal aortic event.

(Ann Thorac Surg 2012;94:1413–7)

Loeys-Dietz syndrome (LDS) is a recently recognized connective tissue disorder (CTD) first described by Loeys and colleagues in 2005 [1] and resulting from mutations in *transforming growth factor-beta receptor (TGFBR)1* and *TGFBR2*. Phenotypic characteristics include arterial tortuosity, aortic aneurysms and dissections, ocular hypertelorism, bifid uvula, and cleft palate [1–3]. Of these characteristics, aortic lesions are considered to have the greatest influence on prognosis, similar to other CTDs such as Marfan's syndrome (MFS) or vascular-type Ehlers-Danlos syndrome. Indeed, some reports indicate that the aortic pathologic process in LDS is more aggressive and widespread than it is in MFS [2]. However surgical results and the postoperative prognosis of aortic repair in patients with LDS are not well known, although most patients require aortic operations.

In this study, we describe our surgical experience with aortic repair in patients with LDS with a severe aortic pathologic process.

Accepted for publication May 25, 2012.

Presented at the Poster Session of the Forty-eighth Annual Meeting of The Society of Thoracic Surgeons, Fort Lauderdale, FL, Jan 28–Feb 1, 2012.

Address correspondence to Dr Iba, 5-7-1 Fujishirodai, Suita, Osaka, 565-8565, Japan; e-mail: iba@hsp.ncvc.go.jp.

Patients and Methods

We performed genetic analysis in patients undergoing aortic surgical procedures at our center who were suspected of having a CTD because of young age, family history, typical annuloaortic ectasia (AAE), and so on. On the basis of the results, we enrolled 16 patients with mutations in *TGFBR1* or *TGFBR2* in this study (Table 1). Between 1990 and 2011, these patients collectively underwent 41 aortic operations. Mean age at the first operation was 31.4 ± 11.3 years. Nine patients had mutations in *TGFBR1* and 7 had mutations in *TGFBR2*. Ten patients underwent their first aortic operations after aortic dissection (group D: dissection group). In group D, indication for first surgical intervention was chronic type B aortic dissection in 6 patients and acute type A aortic dissection in 4 patients. Patients included a 20-year-old woman who at 19 weeks of pregnancy required urgent operation for acute type A aortic dissection. The first surgical intervention in the remaining 6 patients was for AAE without aortic dissection (group N: nondissection group).

Data were collected from hospital admission and outpatient medical records and telephone interviews. All patients were regularly assessed, either at our center or by a local cardiologist. The follow-up rate was 100%, and

Published by Elsevier Inc

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2012.05.111

Abbreviations and Acronyms

AAE = annuloaortic ectasia
CSF = cerebrospinal fluid
CTD = connective tissue disorder
LDS = Loeys-Dietz syndrome
MFS = Marfan's syndrome
TGFBR = transforming growth factor-beta receptor
VSRR = valve-sparing root replacement

the mean follow-up period was 103.7 ± 92.3 months (range, 2–276 months). Our institution approved this retrospective study, and patient consent for our study was obtained either at the time of operation or when the patients came as outpatients.

Continuous variables were expressed as mean ± standard deviation and compared using the Student's *t* test. Categorical data were compared using Fisher's exact test. Survival and aortic event-free rates were estimated using the Kaplan-Meier method, and differences between each group were determined by log-rank analysis. *p* values of less than 0.05 were considered significant. Statistical analysis was performed using SPSS, version 17.0 for Windows (IBM SPSS Inc, Chicago, IL).

Results

No operative or in-hospital deaths occurred in our series. During postoperative hospitalization, there were 3 cerebrovascular events. Subdural hematoma occurred in 2 patients; 1 underwent descending thoracic aorta replacement and the other underwent thoracoabdominal aortic replacement for chronic type B aortic dissection. A third patient, who underwent emergency arch repair for acute type A aortic dissection, was diagnosed with cerebral infarction with right hemiparesis. The woman who needed emergency operation for acute aortic dissection during pregnancy safely gave birth to a baby 5 months after operation. In group N, valve-sparing root replacement (VSRR) was carried out in all 6 patients, and no major complications occurred in this group during hospitalization.

Follow-up results after the aortic procedures are shown in Table 2. Nine patients (90%) in group D required further aortic operations. Patients underwent a mean of 3 aortic procedures. Moreover, the aorta in 4 patients was entirely replaced through serial aortic operations. In group N, only 2 patients (33%) underwent further aortic repair. One patient underwent elective abdominal aortic replacement for an aneurysm of the abdominal aorta and bilateral common iliac artery, whereas another patient was diagnosed with acute dissection from the aortic arch to the terminal aorta 6 years after VSRR. After this, she needed a further aortic operation for dilatation of the dissected aorta. She finally achieved total aortic replacement 1 year after aortic dissection. The overall aortic event-free rates at 5 and 10 years were 46.4% and 18.6%, respectively (Fig 1). In the follow-up, there was only 1 late death resulting from arrhythmia. This patient, who was discharged uneventfully 12 days after VSRR for AAE, died suddenly 32 days after operation. The cumulative survival rate at 5 years was 93.8% in all patients (Fig 2).

Comment

Some phenotypic characteristics of LDS, such as aortic tortuosity and skeletal abnormality, overlap with those of MFS, a representative CTD, suggesting that to date LDS has been regarded as an MFS-like disease or practically treated as MFS. However important phenotypic differences have been described [1–4]. Characteristic craniofacial findings of LDS, such as hypertelorism, cleft palate, or bifid uvula, are seldom found in patients with MFS. In addition, some patients with LDS are not so tall, and lens dislocation, which is typical in MFS, is uncommon in patients with LDS.

Table 1. Patient Characteristics

Characteristic	Group D n = 10	Group N n = 6	*p* Value
Male sex	3	4	0.152
Mean age at first operation	36.0 ± 12.2	23.7 ± 5.4	0.357
Genetic mutation			
TGFBR 1	6	3	0.696
TGFBR 2	4	3	
Mean number of aortic operations	3.0 ± 1.2	1.8 ± 1.5	0.125
Type of aortic operation			
Root replacement			
VSRR	3[a]	6	
Bentall	7[a]	0	
Arch repair	7	1	
DTAA repair	5	1	
TAAA repair	5	1	
AAA repair (infrarenal)	3	2	
Preoperative AI before VSRR			
None	0	1	
Trivial	1	2	
Mild	2	2	
Moderate	0	1	
Severe	0	0	
Postoperative AI after VSRR			
None	1	4	
Trivial	2	2	
Mild	0	0	
Moderate	0	0	
Severe	0	0	

[a] Including concomitant arch repair.

AAA = abdominal aortic aneurysm; AI = aortic insufficiency; DTAA = descending thoracic aortic aneurysm; TAAA = thoracoabdominal aortic aneurysm; *TGFBR = transforming growth factor beta receptor*; VSRR = valve-sparing root replacement.

Table 2. Surgical History of Each Patient

Group	Patients	Indication of First Operation	Maximum Diameter of Aorta (mm)	First Operation	Second Operation		Third Operation		Fourth Operation		Fifth Operation		Total Aortic Replacement
D	1	Chronic AD (B)	60	DTAA repair	VSRR (remodeling) + TAR	(49)	TAAA repair	(53)	Bentall	(159)	...		Yes
	2	Chronic AD (B)	60	AAA repair	HAR	(5)	DTAA repair	(46)	TAAA repair	(85)	Bentall + TAR	(154)	Yes
	3	Chronic AD (B)	N/A	DTAA repair	TAR	(41)	TAAA repair	(62)	Bentall + MVR	(104)	...		Yes
	4	Chronic AD (B)	45	AAA repair	...		...		...		...		No
	5	Chronic AD (B)	60	TAR	AAA repair	(110)	TAAA repair	(116)	...		...		No
	6	Chronic AD (B)	59	DTAA repair	Bentall + TAR	(13)	TAAA repair	(26)	...		...		Yes
	7	Acute AD (A)	N/A	Bentall	TAR	(132)	RedoBentall	(195)	Redo Bentall	(196)	...		No
	8	Acute AD (A)	78	HAR	VSRR	(51)	...		...		...		No
	9	Acute AD (A)	N/A	HAR	VSRR + TAR	(67)	...		...		...		No
	10	Acute AD (A)	...	TAR	DTAA repair	(1)	...		...		...		No
N	11	AAE	57	VSRR	TAR	(79)	DTAA repair		AAA repair		TAAA repair		Yes
	12	AAE	50	VSRR	...		...		...		...		No
	13	AAE	45	VSRR	...		...		...		...		No
	14	AAE	48	VSRR	...		...		...		...		No
	15	AAE	58	VSRR	...		...		...		...		No
	16	AAE	60	VSRR	AAA repair	(3)	...		...		...		No

Numbers in parentheses indicate months from first operation.

AAA = abdominal aortic aneurysm; AAE = annuloaortic ectasia; AD (A) = aortic dissection, type A; AD (B) = aortic dissection, type B; DTAA = descending thoracic aortic aneurysm; HAR = hemiarch replacement; MVR = mitral valve replacement; N/A = not available; TAAA = thoracoabdominal aortic aneurysm; TAR = total arch replacement; VSRR = valve-sparing root replacement.

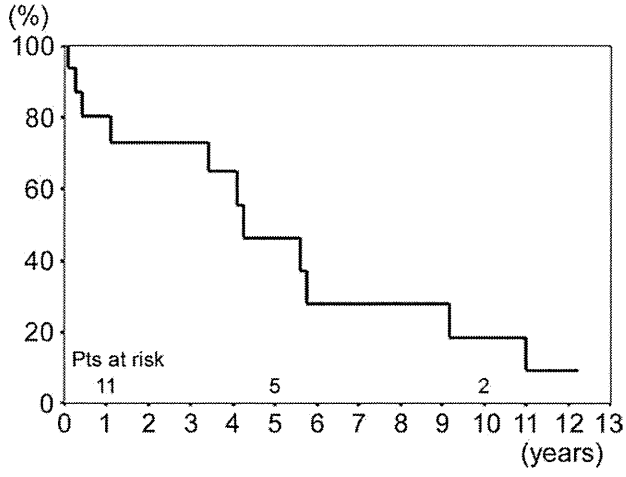


Fig 1. Freedom from aortic reintervention for all patients (Pts) after first aortic operation.

TGFBR molecules are associated with cardiovascular development and function: *TGFBR1* or *TGFBR2* mutations influence collagen deposition and elastin organization in the extracellular matrix. With respect to the histopathologic features of aortic specimens, fragmentation of elastic fibers is frequently seen in patients with both LDS and MFS. Maleszewski and colleagues [5], who examined aortic specimens of patients with both LDS and MFS in detail, showed that high collagen deposition and medial degeneration of the diffuse type with relatively little medial degeneration of the cystic type helps differentiate LDS from MFS. Indeed, in most of our patients, the pathologic findings showed diffuse medial degeneration in addition to fragmentation of elastic fibers. These changes in the media make the aortic wall fragile and lead to arterial tortuosity with aortic aneurysms and dissections.

In patients with LDS, management of aortic lesions is vital for prognosis, as it is in other CTDs. Most patients in group D, who underwent initial aortic repair after aortic dissection, needed further aortic operations because the dissected aorta of patients with LDS dilates easily. As a result, 40% of patients had their aortas replaced entirely in several rounds of aortic repair. Conversely, all patients in group N, in whom aortic lesions were detected before aortic events occurred, underwent aortic operations for AAE. For all patients in group N, VSRR with a reimplantation technique using a polyester tube graft with prefashioned pseudosinuses (Gelweave Valsalva graft; Vascutek, Renfrewshire, Scotland, UK) could be carried out successfully, and postoperative echocardiography revealed less than trivial aortic regurgitation. Patel and colleagues [6] demonstrated that midterm results of VSRR for patients with LDS are encouraging, and we also believe that it is an effective surgical option, especially for young patients.

In our series, there were 2 cases of subdural hematoma postoperatively. One occurred after descending thoracic aorta replacement. In our strategy, at the time of replacement of the descending or thoracoabdominal aorta, a cerebrospinal fluid (CSF) drainage catheter is inserted, and motor-evoked potentials are monitored for spinal cord protection. When the amplitudes of the motor-evoked potentials decrease or recover insufficiently, CSF drainage commences. Therefore the cause of the subdural hematoma might be considered to be related to rapid drainage of CSF performed for spinal cord protection. The other patient, who underwent thoracoabdominal aortic replacement for chronic type B aortic dissection, experienced subdural hematoma 2 days postoperatively, although no intracranial lesions were observed on preoperative brain computed tomography. Intracranial aneurysms have been reported as 1 of the arterial lesions related to LDS [7, 8]. In our patient, magnetic resonance or computed tomography angiography for intracranial lesions had not been performed preoperatively; however, we should pay attention to the existence of such intracranial aneurysms.

In 1 particularly interesting case (patient 11, Table 2), aortic dissection occurred from the aortic arch to the abdominal aorta about 6 years after VSRR. The patient had received regular follow-ups and a computed tomographic scan after the initial operation; the maximum diameter of the aortic arch was only 39 mm on a computed tomographic scan taken 1 month before dissection (Fig 3). After this, she underwent total aortic replacement through serial aortic repairs over 1 year. This experience may suggest that a more aggressive strategy be considered to concomitantly repair the aortic arch at the root operation for prevention of future type A aortic dissection, as Augoustides and colleagues [9] advocated. Further follow-up is therefore necessary.

Clinical strategies for aggressive aortic lesions in LDS have yet to be established because there are still few large-scale reports about surgical results and prognoses. However Williams and colleagues [10], who have much experience in aortic operations for patients with LDS, suggested that the threshold for surgical intervention in adult patients with LDS is 4 cm for the aortic root and abdominal aorta and 5 cm for the descending thoracic aorta or for rapid expansion (> 0.5 cm/year) regardless of location. This surgical approach is considered more ag-

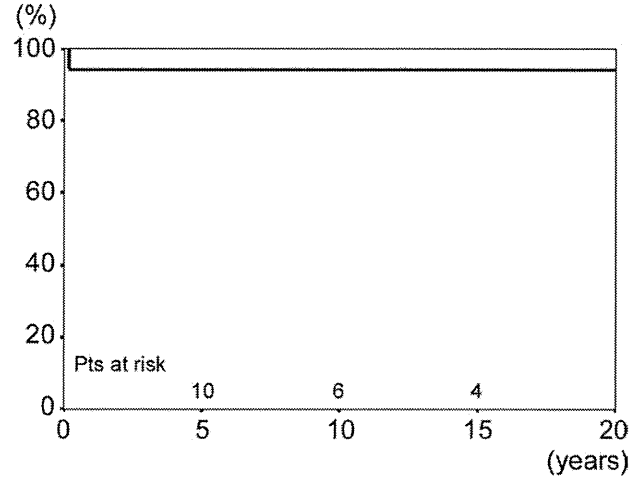


Fig 2. Kaplan-Meier survival curve for all patients (Pts) after first aortic operation.

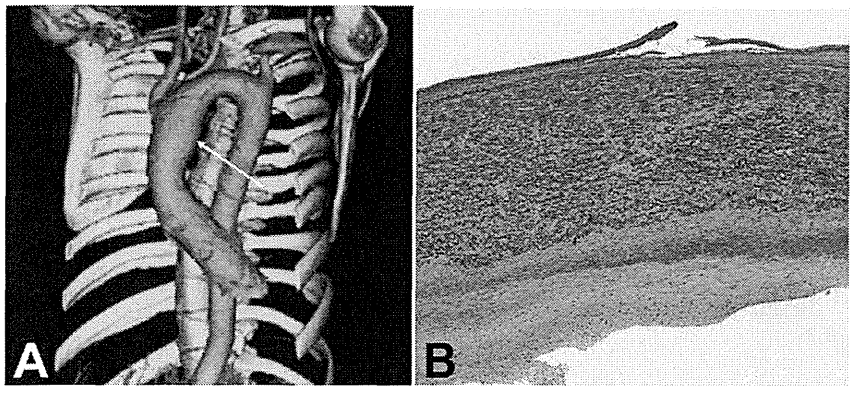


Fig 3. (A) Computed tomographic scan, obtained 1 month before aortic dissection, revealed the maximum diameter of the aortic arch to be 39 mm, with fusiform mild dilatation (arrow). (B) The histopathologic findings of the aortic wall showed diffuse medial degeneration and elastin fragmentation of media (elastica van Gieson stain ×100).

gressive than that for patients with MFS. In our experience, operative results for patients with LDS were satisfactory; moreover, once aortic dissection occurs, most patients need repeated aortic operations [11]. Before this disease was recognized, we had considered surgical indications and management of LDS in the same way as those for MFS. However for the past few years, in which we have recognized the fragile nature of aortic lesions in patients with LDS, we have been adopting a more aggressive strategy for surgical intervention. Therefore we believe that early surgical intervention may improve prognosis by preventing fatal aortic events.

Endovascular treatment should be considered a contraindication in patients with LDS because use of the native aorta as a landing zone carries the risk of potential dilatation. However a recent noteworthy case report describes hybrid therapy, combining open aortic repair and endovascular stent grafting [12].

This study has some limitations. First, our experience with this recently recognized arteriopathy is still limited. Second, this study is retrospective in nature.

In conclusion, we should recognize the aggressive aortic pathologic process in this syndrome, and a clinical management strategy should be established by accumulating further clinical experience.

References

1. Loeys BL, Chen J, Neptune ER, et al. A syndrome of altered cardiovascular craniofacial neurocognitive and skeletal development caused by mutations in TGFBR1 and TGFBR2. Nat Genet 2005;37:275–81.
2. Loeys BL, Schwartze U, Holm T, et al. Aneurysm syndrome caused by mutations in the TGF-beta receptor. N Engl J Med 2006;355:788–98.
3. Van Hemelrijk C, Renard M, Loeys B. The Loeys-Dietz syndrome: an update for the clinician. Curr Opin Cardiol 2010; 25:546–51.
4. Aalberts JJ, van der Berg MP, Bergman JE, et al. The many faces of aggressive aortic pathology: Loeys-Dietz syndrome. Neth Heart J 2008;16:299–304.
5. Maleszewski JJ, Miller DV, Lu J, Dietz HC, Halushka MK. Histopathologic findings in ascending aortas from individuals with Loeys-Dietz syndrome. Am J Surg Pathol 2009;33: 194–201.
6. Patel ND, Arnaoutakis GJ, George TJ, et al. Valve-sparing aortic root replacement in Loeys-Dietz syndrome. Ann Thorac Surg 2011;92:556–61.
7. Levitt MR, Morton RP, Mai JC, Ghodke B, Hallam DK. Endovascular treatment of intracranial aneurysms in Loeys-Dietz syndrome. J Neurointerv Surg 2011 Dec 22. [Epub ahead of print].
8. Rahme RJ, Adel JG, Bendok BR, Bebawy JF, Gupta DK, Batjer HH. Association of intracranial aneurysm and Loeys-Dietz syndrome: case illustration, management, and literature review. Neurosurgery 2011;69:e488–92.
9. Augoustides JG, Plappert T, Bavaria JE. Aortic decision-making in the Loeys-Dietz syndrome: aortic root aneurysm and a normal-caliber ascending aorta and aortic arch. J Thorac Cardiovasc Surg 2009;138:502–3.
10. Williams JA, Loeys BL, Nwakanma LU, et al. Early surgical experience with Loeys-Dietz: a new syndrome of aggressive thoracic aortic aneurysm disease. Ann Thorac Surg 2007;83: S757–63.
11. Williams ML, Wechsler SB, Hughes GC. Two-stage total aortic replacement for Loeys-Dietz syndrome. J Card Surg 2010;25:223–4.
12. Williams JB, McCann RL, Hughes GC. Total aortic replacement in Loeys-Dietz syndrome. J Card Surg 2011;26:304–8.

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Artery Tortuosity Syndrome Exhibiting Early-Onset Emphysema With Novel Compound Heterozygous *SLC2A10* Mutations

Yoshiko Takahashi,[1]* Katsunori Fujii,[1] Akiko Yoshida,[2] Hiroko Morisaki,[2] Yoichi Kohno,[1] and Takayuki Morisaki[2,3]

[1]Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan

[2]Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan

[3]Department of Molecular Pathophysiology, Osaka University Graduate School of Pharmaceutical Sciences, Osaka, Japan

Manuscript Received: 23 March 2012; Manuscript Accepted: 23 October 2012

We report on a 2-year-old Japanese boy with early-onset pulmonary emphysema, exhibiting dysmorphic face, loose skin, and inguinal and Morgagni hernias. He was admitted to our hospital owing to refractory respiratory infection. On the basis of his clinical features, we investigated the *SLC2A10* gene and identified novel compound heterozygous mutations of c.417T > A and c.692G > A, leading to the diagnosis of artery tortuosity syndrome (ATS). This syndrome is an extremely rare autosomal recessive disorder characterized by tortuosity and elongation of the large and medium-sized arteries, hyperextensible skin, and diverse hernias, mostly reported from Europe and Middle Eastern countries, but not from Asia. Although chronic obstructive pulmonary disease, namely, emphysema, has not been well documented in ATS, it may be likely because TGF-beta upregulation is known to be evoked by *SLC2A10* mutations, resulting in reconstruction of pulmonary endothelial cells and emphysema. This is the first report of ATS associated with early-onset pulmonary emphysema, suggesting that patients with ATS may also require close attention for chronic obstructive pulmonary disease.

Key words: pulmonary emphysema; artery tortuosity syndrome; glucose transporter 10; *SLC2A10*; heterozygous mutation

How to Cite this Article:
Takahashi Y, Fujii K, Yoshida A, Morisaki H, Kohno Y, Morisaki T. 2013. Artery tortuosity syndrome exhibiting early-onset emphysema with novel compound heterozygous *SLC2A10* mutations.
Am J Med Genet Part A 161A:856–859.

INTRODUCTION

Arterial tortuosity syndrome (ATS, OMIM #208050) is an extremely rare autosomal recessive disorder characterized by tortuosity and elongation of the large and medium-sized arteries, propensity for aneurysm formation, vascular dissection, and pulmonary artery stenosis [Callewaert et al., 2008]. Other typical manifestations include dysmorphic features, hyperextensible skin, *cutis laxa*, hernia, skeletal abnormalities, joint hypermobility, and congenital contracture [Gardella et al., 2004; Wessels et al., 2004; Zaidi et al., 2009].

The responsible gene for ATS is *SLC2A10*, located on chromosome 20q13.1 and encoding the 541-amino-acid facilitative glucose transporter 10 (GLUT10), consisting of 12 transmembrane hydrophobic domains (TMDs) that are connected by five intracellular and six extracellular loops [Coucke et al., 2003, 2006]. To our knowledge, only 17 *SLC2A10* mutations in 32 families with ATS have been reported [Coucke et al., 2006; Drera et al., 2007; Callewaert et al., 2008; Faiyaz-Ui-Haquw et al., 2009; Zaidi et al., 2009].

Patients with ATS have been mainly reported from European and Middle Eastern countries, but not from Asia. This is the first report of an Asian ATS patient with novel compound *SLC2A10* mutations, exhibiting unusual early-onset pulmonary emphysema and recurrent respiratory infection.

CLINICAL REPORT

A 2-year-old boy, who was the third-born from healthy nonconsanguineous Japanese parents, exhibited no typical ATS manifestations. He was born at term following a normal pregnancy and delivery procedure. However, at birth, he exhibited overgrowth

*Correspondence to:
Yoshiko Takahashi, Department of Pediatrics, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260-8670, Japan. E-mail: honda-sag@umin.ac.jp
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 12 March 2013
DOI 10.1002/ajmg.a.35776

such as 4,695 g (+4 SD) in weight, 54 cm (+2.4 SD) in height, and 36.5 cm (+2.3 SD) in head circumference as macrosomia. He also exhibited pectus excavatum, and inguinal and Morgagni foramen hernias. At 2 years of age, he was admitted to our hospital for dyspnea due to respiratory syncytial virus (RSV) infection. Chest roentgenogram revealed hyperinflation of the right upper lobe and tracheal shift to the left (Fig. 1A). Computed tomography (CT) showed pulmonary emphysema in the right lobe (Fig. 1B). Pulmonary emphysema could be transiently caused by RSV infection. However, in this patient, pulmonary emphysema persisted even 1 year after he recovered from RSV infection (Fig. 1C), suggesting that it genetically happened regardless of the virus infection. Echocardiography showed normal heart ventricles, but disclosed annuloaortic ectasia (valve ring diameter: 17 mm; sinus Valsalva diameter: 27 mm; ST junction diameter: 16 mm) and tortuosity of his aortic arch. Thereafter, contrast-enhanced CT and magnetic resonance angiography (MRA) showed tortuosity and elongation of the large and medium-sized arteries (Fig. 1D,E). Other findings were saggy cheeks, hyperextensible skin (Fig. 1F), and repeated episodes of respiratory infection. Neither joint nor ocular involvement was observed.

On the basis of these clinical manifestations, we performed *SLC2A10* gene analysis after obtaining written informed consent from his parents. Sequences in the coding region of the human *SLC2A10* gene were screened using the direct sequencing method. The analysis revealed that he has two point mutations in exon 2 of *SLC2A10*, namely, c.417T > A (p.Tyr139X) and c.692G > A (p.Arg231Gln). Genetic study of his parents showed that the c.692G > A mutation was derived from his father, and the c.417T > A mutation from his mother, resulting in the compound heterozygosity in this patient (Fig. 2). No other mutation was identified in the *FBN1*, *TGFBR2*, *TGFBR1*, *ACTA2*, or *COL3A1* genes. This study was approved by local ethics committee of Chiba University Graduate School of Medicine.

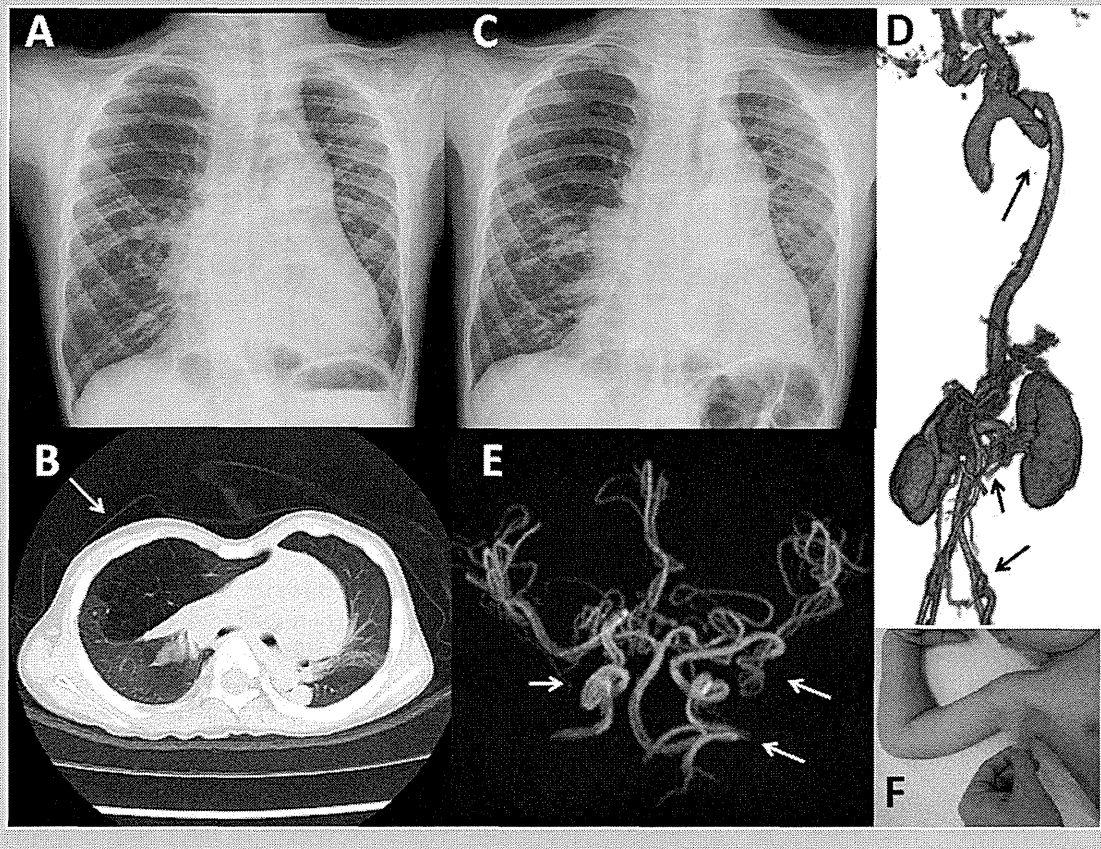


FIG. 1. Radiographic examinations and skin features of the patient. Chest roentgenogram showed hyperinflation of the right upper lobe and tracheal shift to the left (A). A CT showed pulmonary emphysema at the right pulmonary lobe (B). Chest roentgenogram showed the hyperinflation even 1 year after he recovered from respiratory syncytial virus infection (C). Contrast-enhanced CT showed tortuosity and elongation of the large and medium-sized arteries of descending and abdominal aorta (D). Magnetic resonance angiography showed tortuosity and elongation of the large and medium-sized cerebral arteries (E). His skin at the axilla was hyperextensible (F). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga]

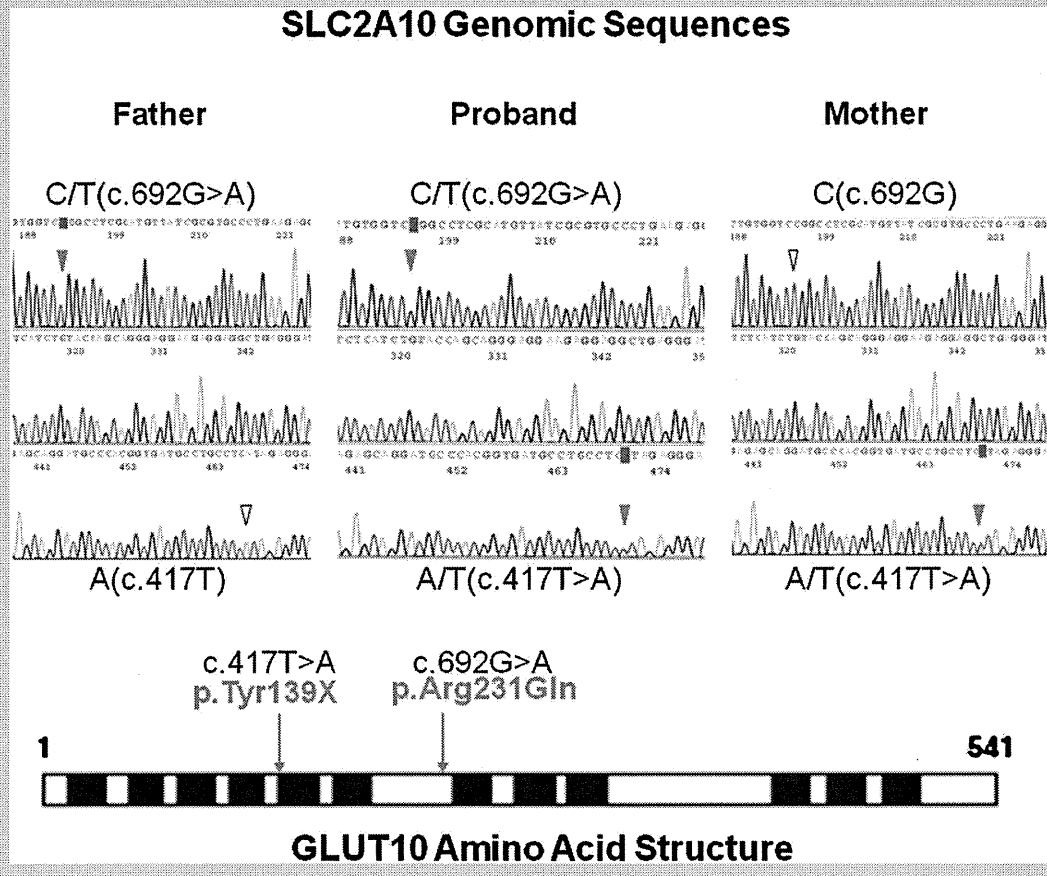


FIG. 2. *SLC2A10* genomic mutations of the patient, his father, and his mother. The patient has two point mutations in exon 2 of this gene, namely, c.692G > A (p.Arg231Gln) and c.417T > A (p.Tyr139X). Genetic study showed that the c.692G > A mutation was inherited from his father and the c.417T > A mutation from his mother. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga]

DISCUSSION

We present a 2-year-old Japanese patient with ATS and novel compound heterozygous *SLC2A10* mutations. The main clinical features of ATS include dysmorphic features, tortuosity and elongation of the large and medium-sized arteries, and connective tissue disorders. Interestingly, our patient exhibited unusual early-onset pulmonary emphysema that has not been previously reported, causing recurrent respiratory infection. To our knowledge, this is the first report of ATS from Asia, exhibiting early-onset pulmonary emphysema.

In this study, we successfully identified the patient's novel compound heterozygous mutations as c.417T > A and c.692G > A in the *SLC2A10* gene. The genotyping analysis disclosed the maternally inherited c.417T > A transition leading to p.Tyr139X as a nonsense mutation, and the paternally inherited c.692G > A transition leading to p.Arg231Gln as an amino acid substitution. The former is a novel nonsense mutation (p.Tyr139X), located on the transmembrane domain (TMD) 5 of GLUT10. Interestingly, the latter was the same amino acid substitution as already reported in a Spanish patient (p.Arg231Gln), which is located in the endofacial loop between TMDs 6 and 7 of the GLUT10 protein [Callewaert et al., 2008]. The substitution of pArg231Gln means replacement of a hydrophilic arginine residue with the hydrophilic glutamine residue, suggesting that this substitution may lead to the alteration of the protein function [Callewaert et al., 2008]. To support this idea, Ritelli et al. [2009] also reported an Italian ATS family with the amino acid substitution of p.Arg231Trp, as the consequence of the c.691C > T transition. In addition, p.Arg231Gln was predicted to be a pathogenic substitution by both sorting intolerant from tolerant (SIFT) and polymorphism phenotyping (PolyPhen). Since two families with ATS, from Spain and Italy, independently showed similar substitution of p.Arg231 with molecular statistical data, we consider that the p.Arg231Gln variant is also responsible for pathogenesis in the patient reported here.

The *SLC2A10* gene encodes the facilitative glucose transporter GLUT10, consisting of 12 TMDs that are connected by five intracellular and six extracellular loops [Coucke et al., 2003, 2006]. Of the 17 *SLC2A10* mutations so far identified in ATS, nine were

missense, four nonsense, and four small deletion mutations leading to premature termination of the protein. These were all reported from European and Middle Eastern countries, but not from Asia, suggesting that certain ethnic groups are particularly associated with this disease.

Loss of function of the transporter GLUT10 results in diminished glucose-responsive transcription of decorin, a known inhibitor of the transforming growth factor beta (TGF-beta) signaling pathway [Coucke et al., 2006]. This leads to up-regulation of the TGF-beta-responsive elements, connective tissue growth factor, and versican [Coucke et al., 2006], inhibiting proper extracellular matrix formation, in particular elastogenesis [Huang et al., 2006]. Importantly, TGF-beta superfamily members are key regulators of extracellular matrix composition and alveolar epithelial cell and fibroblast function in the lung. During both lung development and disease, TGF-betas therefore control pulmonary homeostasis by providing the structural requirements and function and proper gas exchange [Morty et al., 2009].

Prolonged alterations of TGF-beta signaling have been shown to result in structural changes in the lung that compromise gas exchange and lung function, as seen in arrested lung development, a feature of bronchopulmonary dysplasia, lung fibrosis, and chronic obstructive pulmonary disease [Morty et al., 2009]. Other human diseases causing TGF-beta signal alteration include Marfan syndrome, Urban–Rifkin–Davis syndrome, and autosomal dominant or recessive cutis laxa. All these syndromes are known to exhibit pulmonary emphysema [Berk et al., 2012]. Interestingly, pulmonary pathology of Marfan syndrome shows a consistent pattern of distal acinar emphysema, suggesting that persistent TGF-beta signal alteration causes long-standing histological changes in human lung [Dyhdalo and Farver, 2011]. Thus, it is reasonable that ATS shows pulmonary emphysema as seen in the patient reported here.

Interestingly, he exhibited overgrowth at birth that has not been reported in ATS. The *SLC2A10* gene has initially been proposed as a candidate gene for diabetes mellitus because of its glucose transporter function and chromosomal localization, consistent with a type 2 diabetes mellitus locus [McVie-Wylie et al., 2001]. However, patients with ATS usually show normal glucose metabolism [Callewaert et al., 2008], and the present patient actually has not exhibited diabetes mellitus. Since the relationship between the overgrowth in this patient and the *SLC2A10* mutations remains unknown, further studies will be necessary to elucidate the possible role of *SLC2A10* for overgrowth.

In conclusion, we are the first to present a case of ATS in Asia, namely, a Japanese boy with novel compound heterozygous *SLC2A10* mutations. The patient exhibited early-onset pulmonary emphysema that has not been previously reported, suggesting that ATS patients may require close attention to chronic obstructive pulmonary disease.

ACKNOWLEDGMENTS

We thank Dr. Naruhiko Ishiwada, Dr. Haruka Hishiki, and Dr. Koji Higashi for advice on this case.

REFERENCES

Berk DR, Bentley DD, Bayliss SJ, Lind A, Urban Z. 2012. Cutis laxa: A review. J Am Acad Dermatol 66:842.e1–842.e17.

Callewaert BL, Willaert A, Kerstjens-Frederikse WS, De Backer J, Devriendt K, Albrecht B, Ramos-Arroyo MA, Doco-Fenzy M, Hennekam RC, Pyeritz RE, Krogmann ON, Gillessen-kaesbach G, Wakeling EL, Nikzainal S, Francannet C, Mauran P, Booth C, Barrow M, Dekens R, Loeys BL, Coucke PJ, De Paepe AM. 2008. Arterial tortuosity syndrome: Clinical and molecular findings in 12 newly identified families. Hum Mutat 29:150–158.

Coucke PJ, Wessels M, Van Acker P, Gardella R, Barlati S, Willems PJ, Colombi M, De Paepe A. 2003. Homozygosity mapping of a gene for arterial tortuosity syndrome to chromosome 20q13. J Med Genet 40:747–751.

Coucke PJ, Willaert A, Wessel MW, Callewaert B, Zoppi N, De Backer J, Fox JE, Mancini GMS, Kambouris M, Gardella R, Facchetti F, Willems PJ, Forsyth R, Dietz HC, Barlati S, Colombi M, Loeys B, De Paepe A. 2006. Mutations in the facilitative glucose transporter GLUT 10 alter angiogenesis and caouse arterial tortuosity syndrome. Nat Genet 38:452–457.

Drera B, Guala A, Zoppi N, Gardella R, Franceschini P, Barlati S, Colombi M. 2007. Two novel SLC2A10/GLUT10 mutations in a patient with arterial tortuosity syndrome. Am J Med Genet Part A 143A:216–218.

Dyhdalo K, Farver C. 2011. Pulmonary histologic changes in Marfan syndrome: A case series and literature review. Am J Clin Pathol 136: 857–863.

Faiyaz-Ui-Haquw M, Zaidi SH, Al-Sanna N, Alswaid A, Momenah T, Kaya N, Al-Dayel F, Bouhoaiqah I, Saliem M, Tsui LC, Teebi AS. 2009. A novel missense and a recurrent mutation in SLA2A10 gene of patients affected with arterial tortuosity syndrome. Atherosclerosis 203:466–471.

Gardella R, Zoppi N, Assanelli D, Muiesan ML, Barlati S, Colombi M. 2004. Exclusion of candidate genes in a family with arterial tortuosity syndrome. Am J Med Genet Part A 126A:221–228.

Huang R, Merrilees MJ, Braun K, Beaumont B, Lemire J, Clowes AW, Hinek A, Wight TN. 2006. Inhibition of versican synthesis by antisense alters smooth muscle cell phenotype and induces elastic fiber formation in vitro and in neointima after vessel injury. Circ Res 98:370–377.

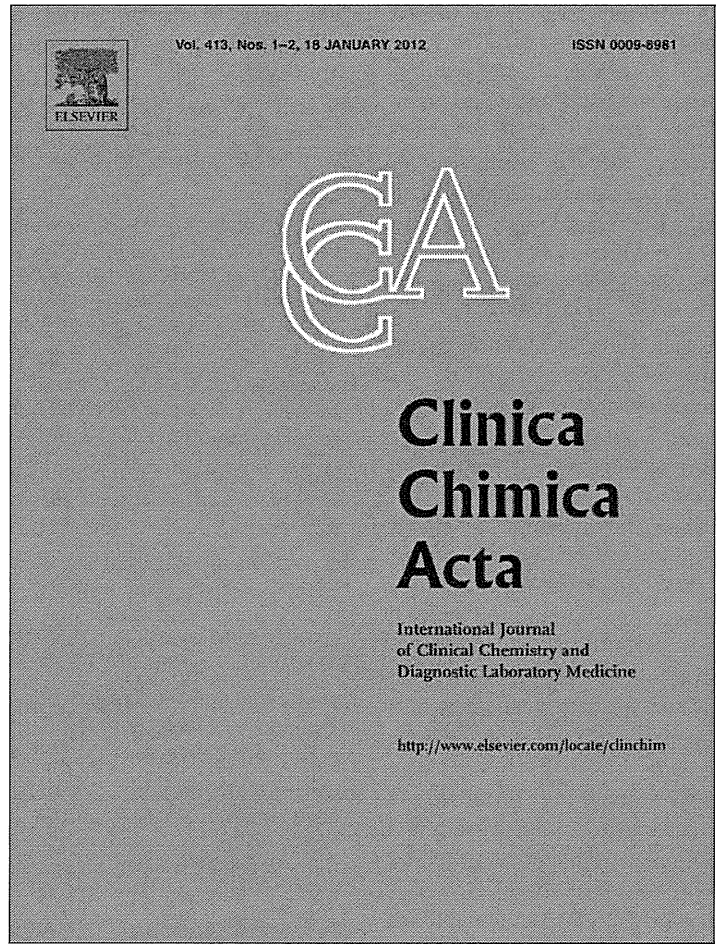
McVie-Wylie AJ, Lamson DR, Chen YT. 2001. Molecular cloning of a novel member of the GLUT family of transporters, SLC2a10 (GLUT10), localized on chromosome 20q13.1: A candidate gene for NIDDM susceptibility. Genomics 15:113–117.

Morty RE, Königshoff M, Eickelberg O. 2009. Transforming growth factor-beta signaling across ages: From distorted lung development to chronic obstructive pulmonary disease. Proc Am Thorac Soc 6:607–613.

Ritelli M, Drera B, Vicchio M, Puppini G, Biban P, Pilati M, Prioli MA, Barlati S, Colombi M. 2009. Arterial tortuosity syndrome in two Italian paediatric patients. Orphanet J Rare Dis 4:20.

Wessels MW, Catsman-Berrevoets CE, Mancini GM, Breuning MH, Hoogeboom JJ, Stroink H, Frohn-Mulder I, Coucke PJ, Paepe AD, Niermeijer MF, Willems PJ. 2004. Three new families with arterial tortuosity syndrome. Am J Med Genet Part A 131A:134–143.

Zaidi SH, Meyer S, Peltekova VD, Lindinger A, Teebi AS, Faiyaz-Ul-Haque M. 2009. A novel non-sense mutation in the SLC2A10 gene of an arterial tortuosity syndrome patient of Kurdish origin. Eur J Pediatr 168: 867–870.



Clinica Chimica Acta 413 (2012) 154–159

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Increased serum PCSK9 concentrations are associated with periodontal infection but do not correlate with LDL cholesterol concentration

Haruna Miyazawa [a,b], Tomoyuki Honda [a,b], Sayuri Miyauchi [a,b], Hisanori Domon [a], Takafumi Okui [a,b], Takako Nakajima [a,c], Koichi Tabeta [a], Kazuhisa Yamazaki [a,b,*]

[a] Center for Transdisciplinary Research, Niigata University, 5274 Gakkocho 2-ban-cho, Chu-o-ku, Niigata 951-8514, Japan
[b] Laboratory of Periodontology and Immunology, Division of Oral Science for Health Promotion, Niigata University Graduate School of Medical and Dental Sciences, 5274 Gakkocho 2-ban-cho, Chu-o-ku, Niigata 951-8514, Japan
[c] General Dentistry and Clinical Education Unit, Niigata University Medical and Dental Hospital, 5274 Gakkocho 2-ban-cho, Chu-o-ku, Niigata 951-8514, Japan

ARTICLE INFO

Article history:
Received 8 August 2011
Received in revised form 8 September 2011
Accepted 9 September 2011
Available online 6 October 2011

Keywords:
PCSK9
LDL cholesterol
Periodontitis
Chronic inflammation

ABSTRACT

Background: Periodontal disease increases the risk of atherothrombotic disease, and high concentrations of low density lipoprotein (LDL) cholesterol are considered to be involved; however, the underlying mechanisms are largely unknown. Recent studies demonstrated that proprotein convertase subtilisin/kexin type 9 (PCSK9) plays a critical role in circulating LDL cholesterol concentrations. The aim of the present study is to analyze serum PCSK9 concentrations and their relation to lipoprotein concentrations in periodontitis patients.
Methods: Sera were obtained from 40 periodontitis patients and 30 control subjects. PCSK9 concentrations, high-sensitivity C-reactive protein (hs-CRP), IL-6, TNF-α and *Porphyromonas gingivalis* antibodies were measured by ELISA, and lipid profiles were determined by a commercial laboratory.
Results: Periodontitis patients demonstrated significantly higher serum antibody titer to *P. gingivalis* and hs-CRP concentrations than control subjects, suggesting infection with *P. gingivalis* and a systemic inflammatory response. PCSK9 concentrations in periodontitis patients were significantly higher than those in control subjects. However, the concentrations of total and LDL cholesterols were not significantly different between periodontitis patients and control subjects. Moreover, no correlations were observed between PCSK9 concentrations and lipid profiles.
Conclusion: Periodontal infection upregulates PCSK9 production. However, further studies are required to elucidate how periodontal infection affects PCSK9 concentrations and subsequent lipid metabolism.

1. Introduction

Coronary heart disease (CHD) is the leading cause of death in Japan and other developed countries. Epidemiological studies suggest that individuals with periodontitis, which is a chronic infectious disease initiated by a group of periodontopathic bacteria, such as *Porphyromonas gingivalis*, have a significantly increased risk of developing CHD [1], of which the underlying mechanism is atherosclerosis [2]. Atherosclerosis is characterized by an accumulation of macrophages, lipid uptake into these cells, and the initiation of chronic inflammatory cascades [3]. Clinical and epidemiologic studies have consistently shown that high concentrations of low-density lipoprotein (LDL) cholesterol and low concentrations of HDL cholesterol are important causative factors associated with atherosclerosis and ischemic cardiovascular diseases [4].

Patients with periodontitis had higher total cholesterol, higher LDL cholesterol, and lower HDL cholesterol than healthy subjects [5–9]. We have recently uncovered an underlying mechanism by which periodontal infection induces lower HDL cholesterol in a mouse periodontitis model, where oral infection with *P. gingivalis* downregulates liver X receptors (LXRs), resulting in the suppression of an ATP-binding cassette A1-mediated HDL cholesterol generation [10]. However, little is known about the relationship between periodontal diseases and molecules affecting plasma LDL cholesterol concentrations.

The LDL receptor (LDLR) is the primary receptor for binding and internalization of plasma LDL cholesterol and regulates the plasma cholesterol concentration [11]. The number of LDLRs expressed on the surface of hepatocytes is the primary determinant of plasma cholesterol concentrations, and is therefore strictly regulated. Transcription of the LDLR gene is controlled by cellular cholesterol concentrations through the sterol responsive element-binding protein (SREBP) [12] and LXRs [13]. Additionally, posttranscriptional regulation of LDLR expression is also a major determinant of lipoprotein metabolism.

* Corresponding author. Tel./fax: +81 25 227 0744.
E-mail address: kaz@dent.niigata-u.ac.jp (K. Yamazaki).

0009-8981/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2011.09.023

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protein convertase that posttranscriptionally promotes the degradation of LDLRs in hepatocytes [14]. PCSK9 is mainly expressed in the liver, intestine, and kidney, and it is secreted into plasma [15]. It does not itself degrade LDLRs, but binds to LDLRs at epidermal growth factor-like repeat A sites on the surface of hepatocytes. This decreases the recycling of the LDLRs to the cell surface and promotes their lysosomal degradation [16], which results in a decreased number of LDLRs and increased plasma LDL concentrations.

Strong validation for the role of PCSK9 in modulating LDL cholesterol concentrations was highlighted by human genetic studies. Gain-of-function mutations in the PCSK9 gene are associated with elevated serum LDL cholesterol concentrations and premature CHD [17], whereas loss-of-function mutations are associated with low plasma LDL cholesterol [17] and a striking reduction of CHD incidence [18]. Furthermore, it has been shown that the reduction of plasma LDL cholesterol concentrations absolutely depends on the presence of hepatic LDLR, and that the infusion of a monoclonal antibody to PCSK9 results in increased hepatic LDLR expression [19].

It has been well-recognized that infection and inflammation induce marked changes in lipid and lipoprotein metabolism [20]. However, the effect of periodontal disease on serum concentrations of PCSK9 has not yet been reported. Based on these observations, we hypothesized that periodontal infection stimulates PCSK9 secretion, thereby increasing the circulating LDL cholesterol concentrations and concomitantly increasing the risk for CHD.

2. Materials and methods

2.1. Patients

Forty patients with moderate to advanced periodontitis diagnosed based on the clinical criteria [21], and 30 periodontally healthy control subjects took part 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. Smoking habits were classified as either "ever" (current and past smokers) or "never". Periodontal tissue destruction was assessed as previously described [22]. Clinical examination included the probing depth, attachment level and alveolar bone resorption. Probing depth and attachment level were recorded at 6 sites around each tooth. Alveolar bone resorption was measured on the proximal surface of each tooth on a radiograph [23]. Intra- and interexaminer (T.H., T.N., K.T. and K.Y.) calibrations were performed on patients prior to initiating the baseline measurements. All of the patients had no history of periodontal treatment and had not taken antibiotics within the 3 months prior to the baseline examination. For the non-diseased controls, sera were obtained from staff members of the Niigata University Dental Hospital. None of these subjects had periodontal pockets, loss of attachment or alveolar bone resorption.

2.2. Serum PCSK9, lipoprotein, hs-CRP and anti-P. gingivalis antibody levels

Sera were obtained prior to oral examination and stored at −80 °C until assayed, and serum PCSK9 concentrations were determined by ELISA (R&D Systems, Inc., Minneapolis, MN). Serum cholesterol and triglyceride profiles were analyzed at Skylight Biotech Inc. (Akita, Japan). Serum high-sensitivity C-reactive protein (hs-CRP) was measured with nephelometry, a latex particle-enhanced immunoassay (NA Latex CRP kit, Dade Behring, Tokyo, Japan). *P. gingivalis*-specific IgG antibody levels were measured by ELISA according to a previously described method [24].

2.3. Statistical analysis

A Kolmogorov–Smirnov test was used to evaluate whether the data sets have parametric distribution. Based on the results, the differences of clinical and biochemical parameters between patients and controls were analyzed using the Mann–Whitney *U*-test, χ^2-tests (gender and smoking), and unpaired *t*-test where appropriate (age and BMI). In periodontitis patients, hs-CRP and PCSK9 concentrations were classified into three categories according to tertiles based on the value order of their concentrations from the lowest to the highest. Associations between tertile categories and other markers were assessed by Kruskal–Wallis test. Linear correlations were obtained using Spearman's rank correlation coefficient analysis. Stepwise multiple regression analysis was used for determining independent predictors of PCSK9 concentrations. Factors that were not significant in the univariate analysis were excluded from this analysis. Statistical analyses were performed using standard statistical software (GraphPad Prism, GraphPad Software Inc., La Jolla, CA and StatView J-5.0 application program, SAS Institute Inc., Cary, NC). $P<0.05$ was considered statistically significant.

3. Results

3.1. Clinical profiles

The clinical profile of the study population is shown in Table 1. The mean age of the periodontitis patients was slightly higher than that of control subjects. There were more female periodontitis patients than male. There was no difference in BMI values between the 2 groups. The hs-CRP concentrations in patients were significantly higher than

Table 1
Clinical profiles and serum markers of study population.

	Periodontitis (n = 40)	Control (n = 30)
Age	52.1 ± 11.9§	46.1 ± 6.3
Male/Female	13/27	16/14
Smoking status (never smoker/ever smoker)	33/7¶	30/0
BMI (kg/m^2)	22.8 ± 3.2	22.2 ± 1.9
Mean PD (mm)	3.4 (3.0–3.9)*	1.9 (1.9–2.1)
PD<4 mm (percentage of sites)	69.0 (53.4–81.1)*	100 (98.9–100)
PD 4–6 mm (percentage of sites)	24.4 (13.3–41.6)*	0.0 (0.0–0.6)
PD>6 mm (percentage of sites)	4.7 (2.3–8.1)†	0.0 (0.0–0.0)
Mean CAL (mm)	3.7 (3.3–4.7)*	2.1 (1.9–2.3)
CAL<4 mm (percentage of sites)	56.5 (44.2–73.5)*	98.8 (97.9–100)
CAL 4–6 mm (percentage of sites)	31.7 (22.3–43.9)*	1.1 (0.0–2.1)
CAL>6 mm (percentage of sites)	8.7 (3.1–15.5)*	0.0 (0.0–0.0)
Mean BL	34.6 (28.5–44.9)	ND
BL ≥ 50% (percentage of sites)	17.4 (8.8–34.0)	ND
Number of teeth	26.0 (24.8–27.3)†	28.0 (26.3–29.8)
Antibody titers to		
P. gingivalis FDC381	7171.7 (3338.0–15009.8)*	635.6 (426.5–1701.8)
P. gingivalis Su63	6157.1 (3660.7–15773.6)*	1354.1 (793.8–2653.5)
hs-CRP (mg/l)	0.35 (0.17–1.08)‡	0.16 (0.09–0.29)
IL-6 (pg/ml)	0.52 (0.29–0.68)	0.42 (0.26–0.64)
TNF-α (pg/ml)	1.09 (0.77–1.41)‡	1.60 (0.89–2.68)
TC (mg/dl)	194.0 (166.4–223.1)	191.6 (174.1–214.3)
LDL-c (mg/dl)	104.8 (87.9–125.9)	96.1 (74.3–108.7)
HDL-c (mg/dl)	53.8 (45.0–69.6)‡	62.3 (56.6–66.9)
Triglyceride (mg/dl)	91.1 (70.4–127.4)	82.4 (55.8–115.9)
PCSK9 (ng/ml)	321.6 (245.5–404.7)*	185.8 (141.0–214.7)

Data are expressed as mean ± S.D. or median (IQR).
*$P<0.0001$, †$P<0.005$, ‡$P<0.05$ vs control (Mann–Whitney *U*-tests).
§$P<0.05$ vs control (unpaired *t*-tests).
¶$P<0.05$ (χ^2-tests).
PD, pocket depth; CAL, clinical attachment level; BL, bone loss; ND, not determined; TC, total cholesterol; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high-density lipoprotein-cholesterol.

in control subjects ($P<0.05$, Mann–Whitney U-test). Contrarily, the mean TNF-α concentrations in periodontitis patients were significantly lower than in controls. No difference was observed for IL-6 concentrations. Antibody titers for the 2 strains of *P. gingivalis* were significantly greater in periodontitis patients than control subjects.

3.2. Serum PCSK9 concentrations and lipid profile

The PCSK9 concentrations in periodontitis patients were significantly higher than control subjects. There was no significant difference in the median total cholesterol and LDL cholesterol concentrations between periodontitis patients and control subjects (Table 1). The median concentration of LDL cholesterol was greater in periodontitis patients than in control subjects although there was no significant difference.

3.3. Relationship between serum PCSK9 concentrations and LDL cholesterol concentrations

Although a close relationship between PCSK9 and LDL cholesterol has been reported, we failed to detect such an association in both periodontitis patients and control subjects. No correlation was also observed between PCSK9 and total cholesterol (Fig. 1).

3.4. Relationships between PCSK9 concentrations and total cholesterol, LDL cholesterol, and HDL cholesterol concentrations

Tertile distributions of PCSK9 concentrations were analyzed for total cholesterol, LDL cholesterol, and HDL cholesterol concentrations. Periodontitis patients with higher PCSK9 concentrations tended to have lower median concentrations of all lipid markers although no significant difference was observed (Fig. 2).

3.5. Relationships between hs-CRP, PCSK9, LDL cholesterol, and HDL cholesterol concentrations

Tertile distributions of hs-CRP are compared in Fig. 3. Periodontitis patients with higher hs-CRP tended to have higher LDL cholesterol, and patients with lower hs-CRP tended to have higher HDL cholesterol. However, no obvious trend was observed for PCSK9.

3.6. Effect of possible confounding factors on the serum PCSK9 concentrations

In order to evaluate the association of the periodontitis with increased concentrations of PCSK9 while adjusting for possible confounding factors, we further performed a multivariate regression analysis. In the multivariate regression model, mean pocket depth only remained significantly associated with serum PCSK9 concentrations ($\beta=0.317$, $P=0.01$) after adjustment for age, smoking status, HDL cholesterol, hs-CRP, and TNF-α (Table 2).

4. Discussion

Activating mutations in the PCSK9 gene result in severe familial hypercholesterolemia and accompanying increased cardiovascular risks, so other factors that elevate circulating PCSK9 concentrations could also be considered as risk factors for CHD. This case–control study is the first to show that serum PCSK9 concentrations were

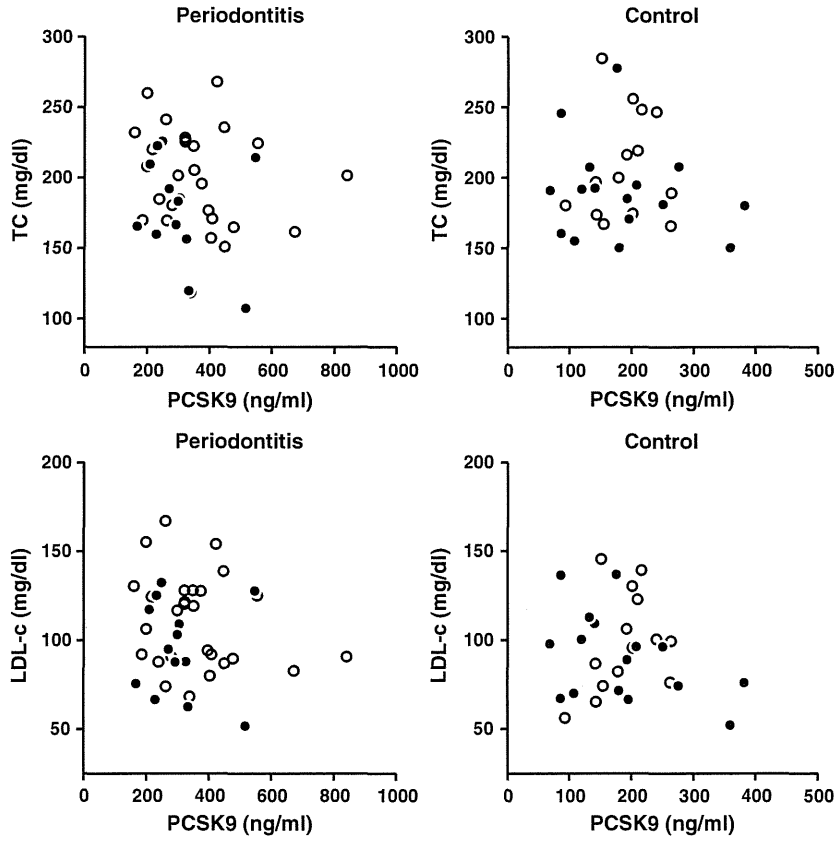


Fig. 1. Relationship between serum PCSK9 concentrations and the lipid profile of periodontitis patients and control subjects. Males and females are shown as closed circles and open circles, respectively. Spearman's rank correlation coefficient analysis demonstrated that none of the markers demonstrated significant correlations with PCSK9. Periodontitis patients (male): TC, Spearman $r=-0.3077$, $P=0.3064$; LDL-c, Spearman $r=-0.1264$, $P=0.6808$. Periodontitis patients (female): TC, Spearman $r=-0.2656$, $P=0.1806$; LDL-c, Spearman $r=-0.2238$, $P=0.2618$. Control (male): TC, Spearman $r=-0.1412$, $P=0.6020$; LDL-c, Spearman $r=-0.3529$, $P=0.1800$. Control (female): TC, Spearman $r=0.1209$, $P=0.6806$; LDL-c, Spearman $r=0.4022$, $P=0.1540$.

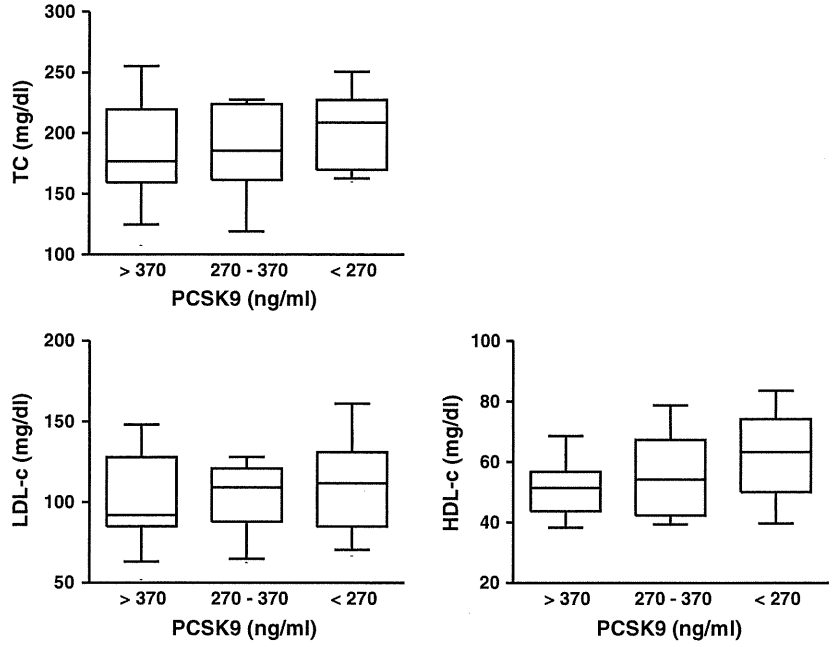


Fig. 2. Each lipid and lipoprotein marker in periodontitis patients by tertiles of PCSK9. Box plots represent medians and the 25%ile and 75%ile as boxes and the 10%ile and 90%ile as whiskers. Total, LDL, and HDL cholesterol concentrations tended to be higher in subjects with lower PCSK9 concentrations. However, none of these was statistically significant.

significantly higher in patients with chronic periodontitis, who were otherwise healthy, when compared to healthy controls.

In this study population, periodontitis patients and controls demonstrated a slight difference in the mean age. Although there is no information regarding the relationship between ages and circulating PCSK9 concentrations, we analyzed PCSK9 concentrations according to age because age is a common risk factor for both atherosclerosis and periodontitis. In multivariate regression analysis it was indicated that among the confounding factors including ages only mean pocket depth representing presence and severity of periodontitis illustrates the elevated concentrations of PCSK9. However, since PCSK9 concentrations tended to be increased with increasing age (data not shown), aging may have some effect on the serum PCSK9 concentrations.

Theoretically, PCSK9 elevation should induce the degradation of LDLR, resulting in decreased clearance of cholesterol from circulation and elevated concentrations of LDL cholesterol. However, contradictory to our initial hypothesis, elevated concentrations of PCSK9 were not associated with elevated concentrations of LDL cholesterol.

It has been reported that the presence of periodontal pockets was positively associated with higher LDL and total cholesterol in men [25]. Löesche et al. showed that total and LDL cholesterols were

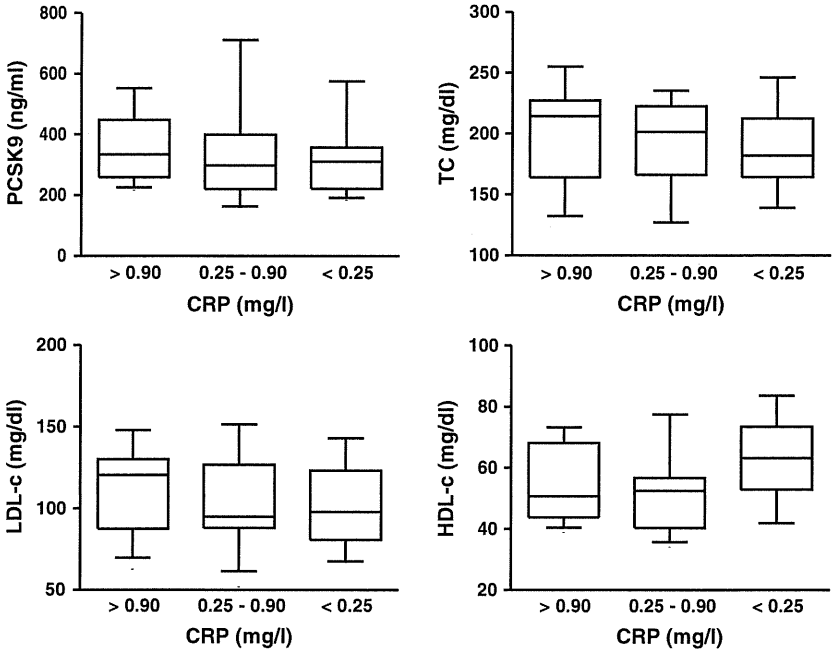


Fig. 3. PCSK9 serum concentrations and each lipid and lipoprotein marker in periodontitis patients by tertiles of hs-CRP. Box plots represent medians and the 25%ile and 75%ile as boxes and the 10%ile and 90%ile as whiskers. PCSK9, total cholesterol and LDL cholesterol concentrations tended to be higher in subjects with higher hs-CRP concentrations, while HDL cholesterol concentrations tended to be lower with higher hs-CRP concentrations. However, none of these was statistically significant.

Table 2
Multiple regression analysis for the associations between PCSK9 and evaluated variables.

	Standard β	t	P-value
Age	0.225	1.868	NS
Smoking	0.001	0.013	NS
HDL-c	−0.069	−0.543	NS
hs-CRP	−0.117	−1.008	NS
TNF-α	−0.176	−1.541	NS
Mean PD	0.317	2.655	0.010

Ages, HDL-c (mg/dl), TNF-α (pg/ml), and mean PD (mm) were used as continuous variables, whereas smoking (0: never smoker, 1: ever smoker) and hs-CRP (1 to 3: tertile groups of hs-CRP concentration) were used as categorized variables.
$R = 0.511$, adjusted $R^2 = 0.191$, $F = 3.710$ ($P = 0.003$).

significantly higher in patients with moderate periodontitis (age 50–60 years) compared with age- and sex-matched controls [26]. Furthermore, intensive periodontal therapy composed of subgingival mechanical debridement with an adjunctive local delivery of minocycline significantly decreased total and LDL cholesterols compared to baseline [27]. The underlying mechanisms for elevated LDL cholesterol concentrations in periodontitis patients have not yet been elucidated.

Contrarily, it has been reported that infection or inflammation decreases serum cholesterol as a result of decreases in both LDL and HDL cholesterols in primates, including humans [20]. In addition, human plasma LDL cholesterol is known to decrease during the acute phase response, possibly because inflammation causes a dysregulation of LDLR expression, and TNF-α and IL-1 override the suppression of LDLR induced by a high intracellular concentration of cholesterol [28,29]. In fact, periodontal infection induces an acute phase response as evidenced by elevated CRP concentrations in periodontitis patients [30,31].

We found that there was no difference in the total cholesterol concentrations between periodontitis patients and controls and that the median concentrations of LDL cholesterol were tended to be higher in periodontitis patients than in controls. These data are consistent with a cross-sectional study in a Japanese population [8]. The reason for the absence of correlation between PCSK9 and LDL cholesterol concentrations is not known. It is speculated that elevated PCSK9 concentrations due to periodontal infection seen in our study may not have a significant effect on the number of LDLRs that affect the circulating cholesterol concentrations. However, it is not reasonable to consider that the PCSK9 concentrations in periodontitis patients are not high enough to elevate LDL cholesterol because the range of the concentrations of PCSK9 in our population overlaps with that of the Japanese familial hypercholesterolemia patients having PCSK9 mutations [32].

Although little is known regarding whether infection/inflammation affects the concentration of circulating PCSK9 and its underlying mechanisms, one study has shown that inflammatory stimuli such as LPS and zymosan markedly increased hepatic PCSK9 mRNA expression, which resulted in decreased LDLR protein expression [33].

Despite a failure to detect an association between PCSK9 and LDL cholesterol concentrations in periodontitis patients in this study, there was a positive association between periodontal infection and serum PCSK9 concentrations. The effect of infection and inflammation on lipid and lipoprotein metabolism is complex, and contrasting results are often demonstrated between rodents and primates.

Acknowledgment

This study was supported by the Japan Society for the Promotion of Science (23390476 and 23659974 for KY, 20295426 for KT, and 21390555 for TN), the Young Researcher Overseas Visits Program for Vitalizing Brain Circulation (S2203) and the Promotion of Niigata University Research Project.

References

[1] Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. J Gen Intern Med 2008;23:2079–86.
[2] Rosamond W, Flegal K, Friday G, et al. Heart disease and stroke statistics—2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2007;115:e69–171.
[3] Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999;340:115–26.
[4] Lusis AJ. Atherosclerosis. Nature 2000;407:233–41.
[5] D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J 2006;151:977–84.
[6] Ramirez-Tortosa MC, Quiles JL, Battino M, et al. Periodontitis is associated with altered plasma fatty acids and cardiovascular risk markers. Nutr Metab Cardiovasc Dis 2010;20:133–9.
[7] Rufail ML, Schenkein HA, Koertge TE, et al. Atherogenic lipoprotein parameters in patients with aggressive periodontitis. J Periodontal Res 2007;42:495–502.
[8] Shimazaki Y, Saito T, Yonemoto K, Kiyohara Y, Iida M, Yamashita Y. Relationship of metabolic syndrome to periodontal disease in Japanese women: the Hisayama Study. J Dent Res 2007;86:271–5.
[9] Yamazaki K, Honda T, Domon H, et al. 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;149:445–52.
[10] Maekawa T, Takahashi N, Tabeta K, et al. Chronic oral infection with *Porphyromonas gingivalis* accelerates atheroma formation by shifting the lipid profile. PLoS One 2011;6:e20240.
[11] Russell DW, Schneider WJ, Yamamoto T, Luskey KL, Brown MS, Goldstein JL. Domain map of the LDL receptor: sequence homology with the epidermal growth factor precursor. Cell 1984;37:577–85.
[12] Yokoyama C, Wang X, Briggs MR, et al. SREBP-1, a basic-helix–loop–helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell 1993;75:187–97.
[13] Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science 2009;325:100–4.
[14] Lambert G, Charlton F, Rye KA, Piper DE. Molecular basis of PCSK9 function. Atherosclerosis 2009;203:1–7.
[15] Seidah NG, Benjannet S, Wickham L, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. Proc Natl Acad Sci U S A 2003;100:928–33.
[16] Zhang DW, Lagace TA, Garuti R, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. J Biol Chem 2007;282:18602–12.
[17] Abifadel M, Varret M, Rabes JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 2003;34:154–6.
[18] Cohen JC, Boerwinkle E, Mosley Jr TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006;354:1264–72.
[19] Chan JC, Piper DE, Cao Q, et al. A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates. Proc Natl Acad Sci U S A 2009;106:9820–5.
[20] Khovidhunkit W, Kim MS, Memon RA, et al. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res 2004;45:1169–96.
[21] Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997;24:72–7.
[22] Yamazaki K, Ohsawa Y, Tabeta K, et al. Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. Infect Immun 2002;70:2492–501.
[23] Schei O, Waerhaug J, Lovdal A, Arno A. Alveolar bone loss as related to oral hygiene and age. J Periodontol 1959;30:7–16.
[24] Tabeta K, Tanabe N, Yonezawa D, et al. Elevated antibody titers to *Porphyromonas gingivalis* as a possible predictor of ischemic vascular disease. J Atheroscler Thromb 2011;18:808–17.
[25] Katz J, Flugelman MY, Goldberg A, Heft M. Association between periodontal pockets and elevated cholesterol and low density lipoprotein cholesterol levels. J Periodontol 2002;73:494–500.
[26] Lösche W, Karapetow F, Pohl A, Pohl C, Kocher T. Plasma lipid and blood glucose levels in patients with destructive periodontal disease. J Clin Periodontol 2000;27:537–41.
[27] D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. J Dent Res 2005;84:269–73.
[28] Ruan XZ, Varghese Z, Fernando R, Moorhead JF. Cytokine regulation of low-density lipoprotein receptor gene transcription in human mesangial cells. Nephrol Dial Transplant 1998;13:1391–7.
[29] Ruan XZ, Varghese Z, Powis SH, Moorhead JF. Dysregulation of LDL receptor under the influence of inflammatory cytokines: a new pathway for foam cell formation. Kidney Int 2001;60:1716–25.

[30] Nakajima T, Honda T, Domon H, et al. Periodontitis-associated up-regulation of systemic inflammatory mediator level may increase the risk of coronary heart disease. J Periodontal Res 2010;45:116–22.
[31] Yamazaki K, Honda T, Oda T, et al. Effect of periodontal treatment on the C-reactive protein and proinflammatory cytokine levels in Japanese periodontitis patients. J Periodontal Res 2005;40:53–8.
[32] Noguchi T, Katsuda S, Kawashiri MA, et al. The E32K variant of PCSK9 exacerbates the phenotype of familial hypercholesterolaemia by increasing PCSK9 function and concentration in the circulation. Atherosclerosis 2010;210:166–72.
[33] Feingold KR, Moser AH, Shigenaga JK, Patzek SM, Grunfeld C. Inflammation stimulates the expression of PCSK9. Biochem Biophys Res Commun 2008;374: 341–4.

Journal of
PERIODONTAL RESEARCH

J Periodont Res 2012; 47: 402–408

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2011.01441.x

Short communication

Oral infection with *Porphyromonas gingivalis* and systemic cytokine profile in C57BL/6.KOR-*ApoE*^{shl} mice

S. Miyauchi[1,2,3], T. Maekawa[1,2,3], Y. Aoki[1,2,3], H. Miyazawa[1,2,3], K. Tabeta[1], T. Nakajima[1,4], K. Yamazaki[1,2]

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

Miyauchi S, Maekawa T, Aoki Y, Miyazawa H, Tabeta K, Nakajima T, Yamazaki K. Oral infection with Porphyromonas gingivalis *and systemic cytokine profile in C57BL/6.KOR-*ApoE^{shl} *mice. J Periodont Res 2012; 47: 402–408. © 2011 John Wiley & Sons A/S*

Background and Objective: Periodontal infection affects atherosclerotic diseases, such as coronary heart diseases. Mouse models have revealed that oral infection with *Porphyromonas gingivalis* induces changes in inflammatory- and lipid metabolism-related gene expression, regardless of the development of atherosclerotic lesions. However, the serum protein expression profile in the oral infection model has not been investigated. The present study aimed to analyse the effect of oral infection with *P. gingivalis* on the expression levels of multiple cytokines in the serum in apolipoprotein E-deficient mice by using a cytokine antibody array.

Material and Methods: C57BL/6.KOR-*Apoe*^{shl} mice were orally infected with *P. gingivalis* five times at 3 day intervals and were then killed. Splenocytes were isolated and analysed for proliferative activity and immunoglobulin G (IgG) production in response to *in vitro* restimulation with *P. gingivalis*. The expression levels of various cytokines in the sera were analysed using a mouse antibody array glass chip.

Results: Splenocytes from *P. gingivalis*-infected mice demonstrated significantly greater proliferation and IgG production in response to *P. gingivalis* compared with those from sham-infected mice. Antibody array analysis revealed the selective upregulation of matrix metalloproteinase 3, intercellular adhesion molecule 1, insulin-like growth factor binding protein 2 and chemokine (C-X-C motif) ligand 7 and the downregulation of interleukin-17, tumor necrosis factor-α and L-selectin.

Conclusion: These data demonstrate that oral infection with *P. gingivalis* induces alterations in systemic cytokine production. These cytokines could play roles in the development not only of periodontitis but also of atherosclerosis.

Dr Kazuhisa Yamazaki, DDS, PhD, Professor, Laboratory of Periodontology and Immunology, Division of Oral Science for Health Promotion, Niigata University Graduate School of Medical and Dental Sciences, 5274 Gakkocho 2-ban-cho, Chu-o-ku, Niigata 951-8514, Japan
Tel: +81 25 227 0744
Fax: +81 25 227 0744
e-mail: kaz@dent.niigata-u.ac.jp

Key words: animal model; cytokine; periodontal medicine; *Porphyromonas gingivalis*

Accepted for publication October 16, 2011

Recent evidence suggests that periodontal infection not only induces periodontal tissue destruction but also adversely affects systemic health (1). The conditions affected by periodontal disease include atherothrombotic diseases, such as coronary heart disease, and cerebrovascular disease, diabetes and preterm birth. Recent studies suggest that certain chronic infections may be considered novel and potentially modifiable risk factors (2). A number

of molecules have been identified as risk factors and risk markers for atherosclerosis. In particular, acute-phase proteins, such as C-reactive protein and serum amyloid A (SAA), have received considerable attention because elevated levels of these molecules not only were observed in coronary heart disease patients but also predicted future development of the disease. Furthermore, in addition to the induction of acute-phase proteins, chronic infection status conferred a markedly increased risk of atherosclerosis development, even in the absence of other vascular risk factors (3).

Our previous study demonstrated that oral infection with *Porphyromonas gingivalis* in ApoE-deficient C57BL/6.KOR-*Apoe*^{shl} (B6.Apoeshl) mice clearly induced the elevation of SAA (the mouse equivalent of human C-reactive protein), with a concomitant increase in the number of atherosclerotic lesions (4). Gene expression analysis clarified that the expression of the molecules related to inflammation was elevated in the aorta, and the expression of lipid metabolism-related genes, particularly those involved in cholesterol efflux, was impaired in the liver. As direct invasion of bacteria into the systemic circulation was not observed in this model, changes in gene expression could be attributable to immune and/or inflammatory responses to the organism.

In this regard, the B6.Apoeshl mouse is a useful model for investigating the effect of the periodontitis-induced systemic inflammatory response on atherogenesis, because the atherosclerotic lesion that is accelerated by oral infection with *P. gingivalis* develops in B6.Apoeshl mice but not in wild-type mice (4).

Animal studies that have attempted to elucidate the effect of periodontal infection on a systemic condition have mainly examined the pathological changes, the inflammatory markers (5–7) and gene expression (4,8) in the aorta and liver. To the best of our knowledge, there are no data on the protein profile in the oral infection model. Almost all cell functions are executed by proteins, which cannot be studied by examining DNA and RNA alone. Experimental analysis clearly shows a disparity between the relative expression levels of mRNAs and their corresponding proteins (9); therefore, it is critical to analyse the protein profile.

To gain further insight into the mechanisms by which periodontal infection affects systemic conditions, we analysed the effect of *P. gingivalis* oral infection on the expression levels of multiple cytokines in the serum of B6.Apoeshl mice by using a cytokine antibody array.

Material and methods

Mice

All animal studies were performed in accordance with the policies of the Institutional Animal Care and Use Committee at Niigata University. Six-week-old male C57BL/6 (wild-type) mice and spontaneously hyperlipidemic male B6.Apoeshl mice (10,11) were obtained from Japan SLC, Inc. (Shizuoka, Japan). The mice were maintained in specific pathogen-free conditions and fed regular chow and sterile water until the commencement of infection at 8 wk of age.

Bacterial culture

P. gingivalis strain W83 was cultured in modified Gifu Anaerobic Medium (GAM) broth (Nissui, Tokyo, Japan) in an anaerobic jar (Becton Dickinson Microbiology System, Cockeysville, MD, USA) in the presence of an AnaeroPack^{TM} (Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan) for 48 h at 37°C. Bacterial suspensions were prepared in phosphate-buffered saline without Mg^{2+}/Ca^{2+} using established growth curves and spectrophotometric analysis. The number of colony forming units was standardized using optical density (600 nm).

Oral infection

The murine experimental periodontitis model was developed according to Baker *et al.* (12), with slight modifications. The animals received the antibiotics sulfamethoxazole and trimethoprim at final concentrations of 700 and 400 μg/mL, respectively, in water bottles *ad libitum* for 10 d, and this treatment was followed by 3 d without antibiotics. The experimental group was then infected. A total of 10^9 colony forming units of live *P. gingivalis* suspended in 100 μL of phosphate-buffered saline with 2% carboxymethyl cellulose (Sigma-Aldrich, St Louis, MO, USA) was given to each mouse via a feeding needle. This suspension was given five times at 3 d intervals. The control group received the same pretreatment and was sham infected without the *P. gingivalis*. Two days after the final treatment, the mice were killed by CO_2 inhalation, and their tissues were removed.

Serum SAA levels

Blood samples were collected by cardiac puncture before killing, and 200 μL of serum were separated after centrifugation, and the SAA was measured using a commercial ELISA kit (Invitrogen, Carlsbad, CA, USA).

Proliferation of spleen cells and production of immunoglobulin G and interleukin-6

The proliferation and immunoglobulin G (IgG) production of splenocytes isolated from *P. gingivalis*-infected and sham-infected mice were assayed by using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) and ELISA (Pierce Biotechnology, Rockford, IL, USA), respectively, according to the manufacturers' instructions. Briefly, cells (5×10^5 per well) were stimulated with the soluble antigen of *P. gingivalis* W83 (Institute of Immunology Co., Ltd, Tokyo, Japan) at concentrations of 100 and 1000 ng/mL for 2, 4 and 6 d. At each time point, aliquots (70 μL) were removed for cytokine ELISAs, and the residual cultures were used for cell counting. Phytohemagglutinin (PHA)-P (Sigma-Aldrich, St Louis, MO, USA) was used at a concentration of 10 μg/mL and served as a positive control.

Levels of IgG specific for *P. gingivalis* W83 in the culture supernatant were determined by ELISA, as described previously (4), and interleukin (IL)-6 levels were determined by an

ELISA kit (Thermo Fisher Scientific, Waltham, MA, USA).

Antibody array

The expression levels of various cytokines in the sera from infected and sham-infected mice were analysed using a mouse antibody array glass chip (RayBio Mouse Cytokine Antibody Array G series 1000; RayBiotech Inc., Norcross, GA, USA). Incubation and washes were performed according to the manufacturer's instructions. Briefly, chip arrays were blocked at room temperature for 30 min before being incubated with 150 μL of each sample at room temperature for 16 h. Glass chips were then washed and incubated with biotin-conjugated primary antibody and fluorescent dye-conjugated streptavidin according to the manufacturer's instructions. Fluorescence detection and analysis were performed using a GenePix 4000B laser scanner (Molecular Devices, Sunnyvale, CA, USA). A list of the cytokines examined is shown in Table 1.

Statistical analysis

GRAPHPAD Prism (GraphPad Software, Inc., La Jolla, CA, USA) was used for the analysis. Parametric data were analysed using Student's paired or unpaired *t*-test. Nonparametric data were analysed by Mann–Whitney *U*-test. A probability value of < 0.05 was considered statistically significant.

Results and Discussion

Oral infection with *P. gingivalis* induced a significant elevation in the serum SAA level compared with sham-infected mice ($p < 0.01$; Fig. 1). Furthermore, splenocytes from *P. gingivalis*-infected mice demonstrated significantly greater proliferation (Fig. 2A), as well as IgG (Fig. 2B) and IL-6 production (Fig. 2C) in response to *in vitro* restimulation with *P. gingivalis* antigens compared with those from sham-infected mice.

These data suggest that oral inoculation with *P. gingivalis* induces systemic inflammation and modulation of the immune response without apparent bacteremia (data not shown). This conclusion is consistent with additional data showing that the selective upregulation or downregulation of several molecules in the sera of infected mice was detectable on the microarray membranes. Among the examined molecules listed in Table 1, matrix metalloproteinase 3 (MMP-3), intercellular adhesion molecule 1 (ICAM-1),

Table 1. List of 96 examined cytokines

Axl
B lymphocyte chemoattractant (BLC) (CXCL13)
Basic fibroblast growth factor (bFGF)
CD30
CD30 ligand
CD40
Cytokine-responsive gene (CRG) -2 (CXCL10)
Cutaneous T cell attracting chemokine (CTACK) (CCL27)
CXCL16
Dipeptidyl peptidase (DPP) IV/CD26
Dtk
E-Selectin
Eotaxin (CCL11)
Eotaxin-2 (CCL24)
Fas ligand
Fcγ receptor IIB
Fms-related tyrosine kinase 3 (Flt-3) ligand
Fractalkine (CX3CL1)
Glucocorticoid-induced TNF-receptor (GITR) (TNFRSF18)
Granulocyte colony stimulating factor (GCSF)
Granulocyte-macrophage colony-stimulating-factor (GM-CSF)
Hepatocyte growth factor (HGF) receptor
Intercellular adhesion molecule (ICAM) -1
Interferon γ
Insulin-like growth factor binding protein (IGFBP) -2
IGFBP-3
IGFBP-5
IGFBP-6
Insulin-like growth factor (IGF) -I
IGF-II
Interleukin (IL) -1α
IL-1β
IL-2
IL-3
IL-3 receptor β
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-12 p40/p70
IL-12 p70
IL-13
IL-15
IL-17
IL-17B receptor
Interferon-inducible T-cell alpha chemoattractant (I-TAC) (CXCL11)
Keratinocyte-derived chemokine (KC) (CXCL1)
Leptin
Leptin receptor
LPS-inducible CXC chemokine (LIX) (CXCL5)
Lungkine (CXCL15)
L-Selectin
Lymphotactin (XCL-1)
Monocyte chemoattractant protein (MCP) -1 (CCL2)
MCP-5 (CCL12)
Macrophage colony-stimulating factor 1 (M-CSF)
Macrophage-derived chemokine (MDC) (CCL22)
Monokine induced by gamma interferon (MIG) (CXCL9)
Macrophage inflammatory protein (MIP) -1α (CCL3)
MIP-1γ (CCL9)
MIP-2 (CXCL2)
MIP-3α (CCL20)
MIP-3β (CCL19)
Matrix metalloproteinase (MMP) -2
MMP-3
Osteopontin
Osteoprotegerin
Platelet factor (PF) -4 (CXCL4)
P-Selectin
Pro-MMP-9
Regulated upon activation normal T-cell expressed and secreted (RANTES) (CCL5)
Resistin
Stem cell factor (SCF)
Stromal cell-derived factor (SDF) -1α (CXCL12)
Sonic hedgehog (Shh) -N
Thymus and activation-regulated chemokine (TARC) (CCL17)
T-cell activation protein (TCA) -3 (CCL1)
Thymus expressed chemokine (TECK) (CCL25)
Thymus CK-1 (CXCL7)
Tissue inhibitor of metalloproteinase (TIMP) -1
TIMP-2
Tumor necrosis factor (TNF) -α
Soluble TNF (sTNF) receptor I
sTNF receptor II
Thyroid peroxidase (TPO)
TNF-related activation-induced cytokine (TRANCE) (TNFSF11)
TROY (TNFRSF19)
Thymic stromal lymphopoietin (TSLP)
Vascular cell adhesion molecule (VCAM) -1
Vascular endothelial growth factor (VEGF)
VEGF receptor 1
VEGF receptor 2
VEGF receptor 3
VEGF-D