Lack of CTGF*-945C/G Dimorphism in Thai Patients with Systemic Sclerosis

Worawit Louthrenoo[*,1], Nuntana Kasitanon[1], Ramjai Wichainun[1], Suparaporn Wangkaew[1], Waraporn Sukitawut[1], Yuka Ohnogi[2], Shoji Kuwata[3] and Fujio Takeuchi[2]

[1]*Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

[2]*Department of Internal Medicine (Allergy and Rheumatology), Faculty of Medicine, University of Tokyo, Tokyo, Japan*

[3]*Third Department of Internal Medicine, Teikyo University Chiba Medical Center, Teikyo University School of Medicine, Chiba, Japan*

Abstract: An association between connective tissue growth factor (CTGF) gene dimorphism at –945 (CTGF*-945C/G) and systemic sclerosis (SSc) has been reported with inconclusive results. We performed this study to determine whether such an association exists among Thai patients with SSc. DNA samples were taken from 50 Thai SSc patients (diffuse SSc in 39 and limited SSc in 11) and 99 healthy controls for determination of CTGF*-945C/G dimorphism by polymerase chain reaction (PCR) using specific oligonucleotide primers. The associations between the genotype frequencies, clinical manifestations and auto-antibodies were determined as well. When compared with the controls, SSc patients had no significantly higher frequencies of the GG genotype (44.0% *vs* 39.4%, p = 0.60), G allele (63.0% *vs* 65.2%, p = 0.80) or G phenotype (82.0% *vs* 90.9%, p = 1.0). There was no association between the presence of the GG genotype and clinical manifestations (pulmonary fibrosis, sclerodactyly, digital pitting scars, telangiectasia and pulmonary arterial hypertension), or the presence of auto-antibodies (anti-Scl-70, anti-SSA/Ro, and anti-RNP). In conclusion, we found no association between CTGF*-945C/G dimorphism and Thai SSc patients.

Keywords: Gene, genetic, scleroderma, polymorphism, connective tissue growth factor.

INTRODUCTION

Systemic sclerosis (SSc) or scleroderma is an autoimmune disease characterized by extensive organ fibrosis, vascular damage and immunologic dysfunctions [1]. The skin, lung, heart, kidney and gastrointestinal tract are affected mainly, resulting in significant mortality and morbidity. The pathogenesis of SSc is not understood clearly, but genetic, environmental, and immunologic factors are shown to be involved [2, 3]. Older age at disease onset, male sex, diffused skin type, and cardiac, pulmonary and renal involvements are important predictors for poor prognosis and survival rates [4, 5].

Connective tissue growth factor (CTGF) is a growth factor that is over expressed in almost all human disorders associated with tissue fibrosis [6]. It works synergistically with TGF-β to promote and sustain fibrosis. The role of CTGF in tissue fibrosis in SSc has been reviewed recently [7, 8]. Although an association between CTGF*-945C/G dimorphism (rs6918698) and SSc was documented in some studies [9, 10], it has not been confirmed by others [11-13].

We, therefore, performed this study to determine whether there is CTGF*-945C/G dimorphism in Thai patients with SSc.

*Address correspondence to this author at the Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand; Tel: +66-53-946449; Fax: +66-53-357959; E-mail: wlouthre@mail.med.cmu.ac.th

MATERIAL AND METHODS

The protocol of the study was approved by the Ethic Committee of the Faculty of Medicine, Chiang Mai University, and the study was performed in conformity with the declaration of Helsinki. Both patients and healthy controls (HC) gave their written informed consent before they entered the study.

Fifty Thai SSc patients and 99 HC were enrolled in the study. All SSc patients fulfilled the 1980 American College of Rheumatology classification criteria for the diagnosis of systemic sclerosis [14]. They were classified further as having either a limited subtype (limited cutaneous SSc [lcSSc]) or diffuse subtype (diffuse cutaneous SSc [dcSSc]) according to the extent of skin involvement, as proposed by LeRoy *et al.* [15]. The HC comprised medical personnel, who showed no signs or symptoms suggesting SSc or any other connective tissue diseases, and they were not taking any regular medications. In patients with SSc, age, sex, duration of disease, type of systemic sclerosis (diffuse cutaneous SSc [dcSSc] or limited cutanous SSc [lcSSc]), and organs involved were recorded. Duration of the disease referred to the period after the onset of symptoms other than Raynaud's phenomenon. Pulmonary hypertension (PH) was defined as estimated systolic pulmonary artery pressure (sPA) > 45 mmHg, determined by echocardiography [16]. Pulmonary artery hypertension (PAH) was defined if the mean pulmonary artery pressure exceeded > 25 mmHg, the pulmonary wedge pressure was < 15 mmHg, and pulmonary vascular resistance surpassed 3 Wood units on right heart

1874-3129/11 2011 Bentham Open

catheterization (RHC) [17]. The RHC used in this study was performed within 6 months of the echocardiography. Pulmonary fibrosis was diagnosed if a chest radiograph showed definite bibasilar pulmonary fibrotic lesions, along with bilateral basal lung crepitus on chest auscultation. In the case of doubtful pulmonary fibrosis on the chest radiograph, a high resolution computed tomography (HRCT) of the chest was performed to confirm the diagnosis. The clinical features and results of the chest radiograph; HRCT of the chest, echocardiography, and RHC performed within 6 months of the study entry (signed informed consent), were used for analysis. The blood was collected for the presence of autoantibodies and CTGF*-945C/G dimorphism on the day of the study entry.

The anti-nuclear antibody (ANA) was determined by indirect immunofluorescent methods using HEp-2 cells as a substrate by Anti-nuclear Antibody (ANA)(FA) [SRL] (TFB, Tokyo, Japan). Anti-centromere antibody was determined using MESACUP-2 TEST CENP-B (Medical and Biological Laboratories, Nagoya, Japan). Antibodies to topoisomerase I (anti-Scl70), ribonucleoprotein (RNP), Ro/SSA and La/SSB antigens were determined by the enzyme link immunosorbant assay (ELISA) using the Anti-Scl70 Antibody (E) [S], Anti-RNP Antibody (E) [S], Anti SSA/Ro Antibody (E) [S], and Anti SSB/La Antibody (E) [S] (TFB, Tokyo, Japan) test kit, respectively.

CTGF*-945C/G dimorphism was determined by the polymerase chain reaction (PCR) assay of Fonseca and Lindahl using the specific oligonucleotide primers with minor modifications [9]. In brief, CTGFG1(5'-TATATAGG CAAGGACAAGGGAC-3'), CTGFC1(5'-TATATAGGCA AGGACAAGGGAG-3') and CTGFP1(5'-CATGCTCTGA AGCATCAACAAC-3') were used for specific primers, and CTGFAPC-210(5'-ATGATGTTGACCTTTCCAGGG-3') and CTGFAPC-211(5'-TTCTGTAACTTTTCATCAGTTGC-3') for control primers. PCR was performed under the following conditions: initial denaturation for 1 min at 96 ^{o}C, 5 cycles of annealing for 45 sec at 70 ^{o}C, extension for 25 sec at 72 ^{o}C and denaturation for 30 sec at 96 ^{o}C, 21 cycles of annealing for 50 sec at 65 ^{o}C, extension for 30 sec at 72 ^{o}C and denaturation for 30 sec at 96 ^{o}C, and 4 cycles of annealing for 60 sec at 55 ^{o}C, extension for 90 sec at 72 ^{o}C and denaturation for 30 sec at 96 ^{o}C. Then, the PCR products were determined by 2% agarose gel electrophoresis.

STATISTICAL ANALYSIS

The SPSS version 16.0 (Chicago, Illinois) program was used for statistical analysis. Association analyses of the CTGF*-945C/G dimorphism with clinical features and auto-antibodies were performed with the Fisher's exact test. A p-value of less than 0.05 was considered a statistically significant difference.

RESULTS

This study included 99 HC (51 females and 48 males) with a mean ± SD age of 45.6 ± 15.1 years, and 50 SSc patients (45 females and 5 males), with a mean ± SD age of 48.7 ± 10.3 years. The SSc patients had a significantly greater proportion of females than the HC; 90.0% *vs* 51.5%, p = <0.001. Details of the clinical features and serological findings in the patients with dcSSc and lcSSc are shown in Table **1**. Raynaud's phenomenon was seen in all patients. Among the 50 SSc patients, 48 (39 dcSSc and 9 lcSSC) had an echocardiograph performed, and 6 (12.5%) were found to have sPA > 45 mmHg or PH. Two of these 6 patients had RHC performed, and they were found to have a mean pulmonary artery pressure of over 25 mmHg. All 6 of these patients also had pulmonary fibrosis on the chest radiograph. No patients with an echocardiographic sPA $\leq$ 45 mmHg had RHC performed. Of 34 patients (68.0%), 26 (66.7%) with dcSSc and 8 (72.7%) with lcSSc had definite pulmonary fibrosis on the chest radiograph. Twenty-four (70.6%) of these patients had HRCT of the chest performed, which confirmed the pulmonary fibrosis (usual interstitial pneumonitis – UIP). Among 16 patients with a normal chest radiograph, 2 who had HRCT performed were found to be normal. Patients with dcSSc had significantly more digital pitting scars and presence of anti-Scl70 than those with lcSSc. The frequency of anti-RNP antibody was significantly higher in lcSSc than in dcSSc (63.6% *vs* 23.1%, p = 0.024).

The genotype frequencies (GFs), allele frequencies (AFs) and phenotype frequencies (PFs) of the CTGF*-945C/G dimorphism in Thai patients with SSc and HC are shown in Table **2**. There was no difference in GFs, AFs and PFs of CTGF*-945C/G dimorphism between SSc patients and HC. In patients with SSc, there was no significant difference between GF of GG (44.0% *vs* 39.4%, p = 0.60), AF of G (63.0% *vs* 65.2%, p = 0.80), or PF of G (82.0% *vs* 90.9%, p = 1.0) when compared with the HC. When the frequencies in dcSSc and lcSSc were compared to those in the HC, there also was no significant difference in GFs, AFs, or PFs of CTGF*-945C/G dimorphism. Furthermore, there was no association of the CTGF*-945C/G alleles with any SSc clinical manifestations or the presence of auto-antibodies. Association of the CTGF*-945C/G dimorphism and presence of anti-La/SSB and anti-centromere antibodies was not performed, due to the small number of cases.

As the female sex was disproportionate among SSc patients and the HC; the GFs, AFs and PFs of CTGF*-945C/G dimorphism of the female SSc patients (n = 45) were compared to female HC (n = 51), and no significant difference was identified (data not shown). The same result was found when only females with dcSSc (n=34) were compared to those with lcSSc (n = 11), or between male HC (n=48) and female HC (n=51) (data not shown).

DISCUSSION

CTGF is a small cysteine-rich matricellular protein which is expressed in response to growth factors and other stimuli including hypoxia and shear stress [18]. The association of CTGF with tissue fibrosis has led to the investigation of possible abnormality in the CTGF gene, and the development of SSc. The first human genetic study was performed by Fonseca *et al.*, who found that the dimorphism at the CTGF*-945C/G promoter region was associated with SSc [9]. In their study, where most of the patients were white UK population, they found that the GF of GG was significantly higher in SSc patients than in their controls (30.4% *vs* 19.2%, p <0.001, OR = 2.2, 95% CI 1.5-3.2). They also found that the GG genotype was associated with

Table 1. Clinical and Laboratory Characteristics of Systemic Sclerosis (SSc) Patients

	All	dcSSc	lcSSc	p Value
N	50	39	11	
Sex, F:M	45:5	34:5	11:0	0.079
Mean ± SD age, years	48.7 ± 10.3	50.1±10.4	43.9±8.6	0.573
Mean ± SD duration of disease, years - duration from onset - duration from Dx	 7.1±4.4 5.8±4.4	 6.8±4.6 5.6±4.6	 8.1±4.0 6.8±3.6	 0.408 0.429
Telangiectasia (%)	14	15.4	9.1	1.0
Pulmonary fibrosis (%)	68	66.7	72.7	1.0
Poikiloderma (%)	50	56.4	27.3	0.171
Pulmonary hypertension[a] (%)	12.5	12.8	1.1	0.578
Sclerodactyly (%)	84	89.7	63.6	0.059
Digital pitting scar (%)	72	84.6	27.3	0.001
Dysphagia (%)	38	41.0	27.3	0.498
Gastroesophageal reflux disease (%)	34	28.2	54.5	0.151
Myositis (%)	10	7.7	18.2	0.301
Arthritis (%)	22	17.9	36.4	0.229
Anti-nuclear antibody (ANA) (%)	100	100	100	1.0
Anti-Scl70 (%)	68	84.6	9.1	<0.001
Anti-centromere (%)	2	0	9.1	0.220
Anti-RNP (%)	32	23.1	63.6	0.024
Anti-SSA (%)	34	30.8	45.5	0.475
Anti-SSB (%)	6	5.1	9.1	0.534

[a] Echocardiography was not performed in 2 lcSSc patients.

Table 2. Association of the CTGF*-945C/G Dimorphism in Thai Healthy Controls and SSc Patients with their Clinical Manifestations and Laboratory Findings

	N	GF (%)			AF (%)		PF (%)	
		GG	CG	CC	G	C	G	C
HC	99	39 (39.4)	51 (51.5)	9 (9.1)	129 (65.2)	69 (34.8)	90 (90.9)	60 (60.6)
All SSc	50	22 (44.0)	19 (38.0)	9 (18.0)	63 (63.0)	37 (37.0)	41 (82.0)	28 (56.0)
dcSSc	39	19 (48.7)	13 (33.3)	7 (17.9)	51 (65.4)	27 (34.6)	32 (82.1)	20 (51.3)
lcSSc	11	3 (27.3)	6 (54.5)	2 (18.0)	12 (54.5)	10 (45.5)	9 (81.8)	8 (72.7)
Sclerodactyly	42	19 (45.2)	16 (38.1)	7 (16.7)	54 (64.3)	30 (35.7)	35 (83.3)	23 (54.8)
Digital pitting scar	36	13 (36.1)	15 (41.7)	8 (22.2)	41 (56.9)	31 (43.1)	28 (77.8)	23 (63.9)
Pulmonary fibrosis	34	12 (35.3)	16 (47.1)	6 (17.6)	40 (58.8)	28 (41.2)	28 (82.4)	22 (64.7)
Telangiectasia	7	4 (57.1)	3 (42.9)	0 (0)	11 (78.6)	3 (21.4)	7 (100)	3 (42.9)
Pulmonary hypertension	6	1 (16.7)	4 (66.7)	1 (16.7)	6 (50.0)	6 (50.0)	5 (83.3)	5 (83.3)
Poikiloderma	25	12 (48.0)	10 (40.0)	3 (12.0)	34 (68.0)	16 (32.0)	22 (88.0)	13 (52.0)
Gastroesophageal reflux disease	17	8 (47.1)	6 (35.3)	3 (17.6)	22 (64.7)	12 (35.3)	9 (52.9)	14 (82.4)
Dysphagia	19	11 (57.9)	6 (31.6)	2 (10.5)	28 (73.7)	10 (26.3)	17 (89.5)	8 (42.1)
Myositis	5	2 (40.0)	2 (40.0)	1 (20.0)	6 (60.0)	4 (40.0)	4 (80.0)	3 (60.0)
Sicca symptoms	5	3 (60.0)	0 (0)	2 (40.0)	6 (60.0)	4 (40.0)	3 (60.0)	2 (40.0)
Arthritis	11	5 (45.5)	5 (45.5)	1 (9.1)	15 (68.2)	7 (31.8)	10 (90.0)	6 (54.5)
Anti-Scl70	34	16 (47.1)	13 (38.2)	5 (14.7)	45 (66.2)	23 (33.8)	29 (85.3)	18 (52.9)
Anti-Ro/SSA	17	4 (23.5)	9 (52.9)	4 (23.5)	17 (50.0)	17 (50.0)	13 (76.5)	13 (76.5)
Anti-RNP	16	6 (37.5)	8 (50.0)	2 (12.5)	20 (62.5)	12 (37.5)	14 (87.5)	10 (62.5)

The comparisons were carried out in all frequencies between the SSc group and healthy controls, and between those with the presence or absence of clinical manifestations and auto-antibodies in the SSc group. No significant difference was found.

Table 3. Summary of Studies on CTGF*-945C/G Dimorphism in Patients with SSc

Author (Year)	Country (Ethnicity)	N (Healthy Control, SSc)	% GG Frequency		p Value
			Healthy Control	SSc	
Fonseca (2007)	United Kingdom (Caucasian)	1,000 (500, 500)	19.2	30.4	< 0.001
Gourh (2008)	North America (Caucasian, African-American, Hispanic-American)	1,662 (668, 994)	29.8	29.0	0.83
Kawaguchi (2009)	Japan (Japanese)	664 (269, 395)	22	32	0.002
Rueda (2009)	European ancestry (Multi-national)	4,211 (98-317, 98-369)	24.4-29.9	26.6-33.7	0.11-0.75
Granel (2010)	France (Caucasian)	510 (269, 241)	30.9	22.8	0.61
Present study	Thailand (Thai)	149 (99, 50)	44.0	39.4	0.60

the presence of alveolitis, and anti-Scl-70 and anti-centromere antibodies. Another study, which supported an association between CTGF*-945C/G dimorphism and SSc, was carried out by Kawaguchi *et al*. [10], who performed their research in Japanese patients. They found that the GF of GG was significantly higher in SSc patients than in controls (32.0% *vs* 22.0%, p = 0.0018). They also found an association between the GG genotype with diffused SSc, ILD, PAH, and the presence of anti-Scl70 and anti-U1RNP antibodies. In contrast, a study performed by Gourh *et al*. [11] in the United States, where most of the patients were white North American, African- American and Hispanic American (with a ratio of 6.6:1:1.1, respectively), found similar GFs of GG among SSc patients and controls (29.0% *vs* 29.8%, p = 0.83, OR = 1.02, 95% CI 0.9-1.2). No association with disease subtypes or the presence of anti-Scl-70, anti-centromere, or anti-RNA polymerase III was identified. A multicenter study in 7 populations (6 in Europe and 1 in North America) by Rueda *et al*. [12] also failed to confirm this association. Both SSc patients and the controls had similar GFs of GG (24.4-29.9% in SSc and 26.6-33.7% in controls). Lastly, a recent study in France by Granel *et al*. [13], who performed 7 SNPs on the CTGF gene, could not confirm that CTGF*-945C/G SNP was associated with SSc. However, they found that the female sex was a risk factor for the development of SSc, and the presence of the rs9399005 T/T SNP was found to be a protective factor for SSc, particularly the limited subtype. The discrepancy in results might be due mainly to the studies being performed in different ethnic groups. Details of previous studies on CTGF*-945C/G dimorphism in patients with SSc are summarized in Table **3**.

In this study, we found that the GF of GG in our SSc patients (44.0%) was almost equal to that seen in our controls (39.4%). In all previous studies, the GF of GG ranged from 24.4% to 32.0% in SSc, no matter whether an association was found with SSc or not [9-13]. The GF of GG in controls was similar to that with disease in studies which could not confirm this association, but it was significantly lower in studies that showed an association. We could not find a significant difference in GFs, AFs, or PFs between dcSSc and lcSSc patients, female SSc patients and female HC, or female dcSSc and female lcSSc patients. No significant association was found between the CTGF*-945C/G dimorphism and any clinical manifestations or the presence of auto-antibodies.

One might raise the question of why only 84% of our SSc patients had sclerodactyle (89.7 % in dcSSc, and 63.6% in lcSSc). The answer might be because this was a cross-sectional study, and many patients had had a long standing disease (mean duration 7 years). Many of these patients had turned into the regression phase, when the skin became soft. At this stage, the skin over dorsum of their hands was soft, and sclerodactyle was not present. The high prevalence of anti-Scl70 in dcSSc patients was not unexpected, as almost 80% of our patients had dcSS. It should be noted that approximately two thirds of our patients had pulmonary fibrosis, in both the dcSSc and lsSSc group. All of the PH patients also had pulmonary fibrosis, indicating that PH in our patients was usually secondary to lung disease. Therefore, PAH as a result of primary vasculopathy was rare in our SSc population.

There were some limitations in this study. Firstly, the number of patients was quite small. However, SSc is an uncommon disease in Thailand; therefore, the small sample size in a genetic study might not have too much impact on the statistical analysis. Secondly, there was significant disproportion of the female sex between SSc patients and the HC. However, in re-analysis using only the female SSc patients and female HC, no significant difference was found in GFs, AFs or PFs between the two groups, indicating that the female sex had no effect on genetic analysis of CTGF dimorphism. Thirdly, the diagnosis of PH was made by echocardiography (sPA > 45 mmHg). This value gave a 97% specificity and 98% positive predictive value for pulmonary hypertension. However, as most of our cases with PH had pulmonary fibrosis on the radiograph (approximately two thirds of the cases, in both dcSSc and lcSSc); therefore, PH in our SSc patients was likely to be secondary to lung disease [19], rather than primary to vasculopathy, as seen in idiopathic PAH.

In conclusion, we could not confirm the presence of CTGF*-945C/G dimorphism in Thai SSc patients.

ACKNOWLEDGEMENT

This study was partly supported by a grant from the Ministry of Education, Culture, Sport, Science and Technology of Japan, and the Faculty of Medicine, Chiang Mai University Endowment Fund.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

REFERENCES

[1] Varga J, Denton CP. Systemic sclerosis and the scleroderma-spectrum disorders. In: Firestein GS, Budd RC, Harris ED, McInnes IB, Ruddy S, Sergent JS, Eds. Kelley's textbook of rheumatology. Philadelphia: Saunders Elsevier 2009: pp. 1311-51.

[2] Agarwal SK, Tan FK, Arnett FC. Genetics and genomic studies in scleroderma (systemic sclerosis). Rheum Dis Clin North Am 2008; 34: 17-40; v.

[3] Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. N Engl J Med 2009; 360: 1989-2003.

[4] Ruangjutipopan S, Kasitanon N, Louthrenoo W, Sukitawut W, Wichainun R. Causes of death and poor survival prognostic factors in thai patients with systemic sclerosis. J Med Assoc Thai 2002; 85: 1204-9.

[5] Karassa FB, Ioannidis JP. Mortality in systemic sclerosis. Clin Exp Rheumatol 2008; 26: S85-93.

[6] Shi-Wen X, Leask A, Abraham D. Regulation and function of connective tissue growth factor/CCN2 in tissue repair, scarring and fibrosis. Cytokine Growth Factor Rev 2008; 19: 133-44.

[7] Abraham DJ, Krieg T, Distler J, Distler O. Overview of pathogenesis of systemic sclerosis. Rheumatology (Oxford) 2009; 48(Suppl 3): iii3-7.

[8] Leask A, Denton CP, Abraham DJ. Insights into the molecular mechanism of chronic fibrosis: the role of connective tissue growth factor in scleroderma. J Invest Dermatol 2004; 122: 1-6.

[9] Fonseca C, Lindahl GE, Ponticos M, *et al*. A polymorphism in the CTGF promoter region associated with systemic sclerosis. N Engl J Med 2007; 357: 1210-20.

[10] Kawaguchi Y, Ota Y, Kawamoto M, *et al*. Association study of a polymorphism of the CTGF gene and susceptibility to systemic sclerosis in the Japanese population. Ann Rheum Dis 2009; 68: 1921-4.

[11] Gourh P, Mayes MD, Arnett FC. CTGF polymorphism associated with systemic sclerosis. N Engl J Med 2008; 358: 308-9; author reply 9.

[12] Rueda B, Simeon C, Hesselstrand R, *et al*. A large multicentre analysis of CTGF -945 promoter polymorphism does not confirm association with systemic sclerosis susceptibility or phenotype. Ann Rheum Dis 2009; 68: 1618-20.

[13] Granel B, Argiro L, Hachulla E, *et al*. Association between a CTGF gene polymorphism and systemic sclerosis in a French population. J Rheumatol 2010; 37: 351-8.

[14] Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980; 23: 581-90.

[15] LeRoy EC, Black C, Fleischmajer R, *et al*. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988; 15: 202-5.

[16] Mukerjee D, St George D, Knight C, *et al*. Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. Rheumatology (Oxford) 2004; 43: 461-6.

[17] Badesch DB, Abman SH, Simonneau G, Rubin LJ, McLaughlin VV. Medical therapy for pulmonary arterial hypertension: updated ACCP evidence-based clinical practice guidelines. Chest 2007; 131: 1917-28.

[18] de Winter P, Leoni P, Abraham D. Connective tissue growth factor: structure-function relationships of a mosaic, multifunctional protein. Growth Factors 2008; 26: 80-91.

[19] Simonneau G, Robbins IM, Beghetti M, *et al*. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol 2009; 54: S43-54.

Received: July 18, 2011 Revised: August 13, 2011 Accepted: August 22, 2011