Original Article
Impact of thrombospondin-2 gene variations on the risk of thoracic aortic dissection in a Chinese Han population

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Abstract: Objective: Genetic factors play an important role in thoracic aortic dissection (TAD) etiology and thrombospondin-2 gene (THBS2) polymorphisms may be involved. This study tried to examine the single-nucleotide polymorphisms (SNP) rs8089 of THBS2 for their association with TAD susceptibility in Chinese Han population. Methods: The rs8089 SNP of THBS2 was genotyped in 112 subjects who were diagnosed as TAD and in 184 age- and gender-matched matched controls. Results: The THBS2 rs8089 SNP was associated with increased TAD susceptibility for allele level comparison (P < 0.0001), and for dominant model (P = 0.0073) or extreme genotype model (P = 0.0459) in Chinese Han Population. But for the recessive model, no statistical difference was found (P = 0.099), which may be resulted from the relatively small sample size and low genotype frequency. Conclusion: In conclusion, the present study suggested that the THBS2 rs8089 variant was associated with TAD, with the G allele representing a risk factor in a Chinese Han population.

Keywords: Thrombospondin-2, gene polymorphisms, thoracic aortic dissection

Introduction

Thoracic aortic dissection (TAD), characterized by a tear in the intimal layer of the aorta, aortic aneurysms formation and separation of the arterial wall, is a fatal cardiovascular disease [1]. The thoracic aortic aneurysms tend to be asymptomatic and usually are not noticed before thoracic aortic dissection (TAD) occurs [2]. Nowadays, despite the rapid progress of the current diagnostic and treatment techniques, it is still associated with high morbidity and mortality [3-5], making prevention and early diagnosis critical for survival.

Chronic inflammation, chronic hypertension, dyslipidemia, increased neoangiogenesis, enhanced oxidative stress, and extracellular matrix (ECM) degradation involved in the pathological process of TAD [3, 6, 7]. However, the cause of TAD has not been clearly proven, with both environmental and genetic factors involved. Previous studies on the genetic basis of TAD were only focused on its relation to system-ic connective tissue disorders such as the Marfan syndrome and the Ehlers-Danlos syndrome. However, recent studies showed that up to 19% of individuals with non-syndromic TAD referred for surgery have a familial background, with several genetic loci identified to be associated with nonsyndromatic TAD [8-12]. Nevertheless, the genetic factors that affect susceptibility to this sporadic TAD is still poorly understood though several revealed genetic factors have been found in several populations [13-17].

The thoracic aortic aneurysms and TAD develop as a result of progressive weakening of the aortic wall, including medial degeneration (degeneration and fragmentation of elastic fibers), smooth muscle cells depletion, increased expression and tissue localization of elastin- and collagen-degrading enzymes, and an accumulation of basophilic ground substances [18]. Namely, it means the histological appearance of TAD may be influenced by the balance of vascular smooth muscle cells (VSMCs), ECM pro-

Table 1. Summary of the basic characteristics of the groups

Clinical characteristics	AS patients	Controls	P-value
No.	112	184	
Age (years)	28.3 ± 9.2	27.1 ± 6.8	n.s
Female/male	88/24	136/48	n.s
Smoking	71	84	n.s
Diabetes	23	34	n.s
Hypertension	97	162	n.s
Dyslipidaemia	68	81	n.s

teins proteolytic enzymes, and their inhibitors [19-23].

The thrombospondin-2 gene (THBS2) is a multi-functional protein that plays an autocrine role in the control of smooth muscle cell growth [24]. THBS2 also plays a role in organization of the extracellular matrix, as suggested by the observation that disruption of Thbs2 results in abnormalities of fibroblasts, connective tissue, and blood vessels [25]. Furthermore, a twofold increase in MMP-2 activity was found to contribute to the adhesive defect observed in THBS2-null fibroblasts [26]. Given above, it is interesting if THBS2 may play a role in the pathologies of TAD. A previous study that involved 1351 hypertensive individuals (88 patients with TAD and 1263 controls) demonstrated the THBS2 SNP rs8089 is a risk factor for TAD in hypertensive patients in Japanese population. However, no replicated studies were performed no matter in Japanese or other populations.

Accordingly, the aims of this study were to determine whether the THBS2 SNP rs8089 were associated with TAD in a Chinese Han population.

Methods

The study was approved by the ethics committee of Jining First People's Hospital, and informed consent was obtained from patients and control participants.

Study population

A total of 112 patients diagnosed with TAD and 184 age- and gender-matched healthy controls were recruited in this study. All subjects included in this study were Chinese Han population. The TAD diagnosis was confirmed by noninva-

sive imaging such as transesophageal echocardiography, helical computed tomography (CT), or magnetic resonance imaging (MRI) [27]. A complete clinical history was obtained from all subjects. Patients with Marfan syndrome, Ehlers-Danlos syndrome, Loeys-Dietz syndrome, traumatic aneurysms, aortic coarctation, or familial history of TAD were excluded from the study. The clinical examination and radiological assessment were performed by two independent examiners who were blinded to the clinical information. Disagreements were resolved through discussion and consensus. Hypertension was diagnosed as a systolic blood pressure ≥ 140 mmHg, a diastolic blood pressure ≥ 90 mmHg, or treatment with anti-hypertensive medication. Dyslipidemia was defined as total cholesterol > 6.5 mmol/L or treatment for elevated blood lipids. Diabetes was defined as a fasting plasma sugar level > 7.8 mmol/L, a glucose level > 11.1 mmol/L 2 h after oral glucose challenge, or ongoing treatment of diabetes. Clinical data, such as fasting insulin levels and glucose levels, were measured routinely for these individuals.

Genotyping

DNA samples were obtained from all the participants from peripheral blood with the Chelex-100 method [28]. The SNP was then genotyped using Taqman assay (Applied Biosystems 7500, ABI, Foster City, CA) and dual-labeled probes in real-time PCR. The primers and probes were designed and synthesized by Sigma (Sigma-Proligo, The Woodlands, TX). Genotyping was performed by independent laboratory personnel who were blinded to the study, and three authors independently reviewed the genotyping results, data entry, and statistical analyses. In addition, we randomly selected 5% samples of case and control subjects for reproducibility tests at least twice in different days and yielded a 100% concordant.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL, USA), version 16.0 for Windows. The demographic and clinical data were presented as Mean ± SD and compared between groups by the Student's t-tests. The genotype and allelic frequencies were evaluated by Hardy-Weinberg equilibrium and compared by the Chi-square test. The

Table 2. Genotype and allele distributions of the THBS2 SNP rs8089 for the cases and controls

Group	Allele (%)					H-WE			
	TT	GT	GG	GT + GG	GT + TT	GG	T	G	
Control	135	43	6	49	178	6	85.1	14.9	0.273
Case	65	38	9	47	103	9	75.0	25.0	/
OR (95% CI)	/	/	3.12 (1.06 to 9.13)	1.99 (1.21 to 3.28)	/	2.59 (0.90 to 7.49)	/	1.90 (1.52 to 2.39)	/
P	/	/	0.0459	0.0073	/	0.099	/	< 0.0001	/

association between the THBS2 SNP rs8089 polymorphism and TAD susceptibility was assessed under the following genetic models, which were treated as a dichotomous variable: (i) G-allele versus T-allele for allele level comparison; (ii) GT + GG versus TT for a dominant model of the G allele; (iii) GG versus GT + TT for a recessive model of the G-allele; and (iv) GG versus TT for the extreme genotype. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics

Demographic data of the population studied and the number of individuals in each group were shown in **Table 1**. There were no significant differences between groups in terms of the demographic data like age and gender.

Association of THBS2 polymorphism rs8089 with TAD

As expected, the distribution of the genotypes of SNPs of THBS2 rs8089 gene conformed to the Hardy-Weinberg equilibrium and the genotyping success rate was 100%. **Table 2** listed the genotyped and allele distributions of the THBS2 rs8089 for the cases and controls. The genotype frequencies of the THBS2 rs8089 T/G polymorphism were 58.0% (TT), 33.9% (GT) and 8.0% (GG) in TAD patients, and 73.4% (TT), 23.4% (GT) and 3.3% (GG) in controls (P = 0.0151). For allele level comparison, the THBS2 rs8089 G allele was associated with an increased risk of TAD in terms of the frequency of allele comparison (G vs. T: OR = 1.90; 95% CI = 1.52 to 2.39, P < 0.0001). For a dominant model of the G allele, the GT + GG genotypes were associated with the risk for TAD (GT + GG vs. TT, OR = 1.99, 95% CI = 1.21 to 3.28, P = 0.0073). For a recessive model of the G allele, the GG homozygote genotype was not associated with susceptibility to TAD (GG vs. GT + TT.

OR = 2.59, 95% CI = 0.90 to 7.49, P = 0.099). For the extreme genotype, the GG genotypes were associated with the risk for TAD (GG vs. TT, OR = 3.12, 95% CI = 1.06 to 9.13, P = 0.0459).

Discussion

The present study demonstrated that the THBS2 rs8089 SNP was associated with increased TAD susceptibility for allele level comparison, and for dominant model or extreme genotype model in Chinese Han population. But for the recessive model, no statistical difference was found, which may be resulted from the relatively small sample size and low genotype frequency.

Aortic dissection is among the cardiovascular diseases with the highest morbidity and mortality rates. Previous understanding of the pathogenesis of aortic aneurysms focused on the biomechanical factors like hemodynamics and wall mechanics, but recently the genetic risk factors attached more attention [29]. As mentioned in the introduction section, TAD may be introduced by the unbalance of vascular smooth muscle cells (VSMCs), ECM proteins proteolytic enzymes, and their inhibitors (TIMPs). For example, the proteinases of the MMPs family were found able to destruct the elastic media, deteriorate the mechanical properties of the artery wall, and finally contribute to the pathogenesis of TAD [30-33]. And recent studies demonstrated that SMCs participate in remodeling of the aortic wall by production of MMPs and TIMPs in aortic media [31, 34]. And the genetic variants may contribute to the amount and function of the VSMCs, MMPs, and TIMPs, thus influence the progression of the TAD formation.

THBS2 belongs to the thrombospondin family, is a disulfide-linked homotrimeric glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. It is a multifunctional protein with

autocrine ability to control the growth of SMCs [24]. Increased expression of THBS2 is also found in human hypertrophied hearts, indicating the potential role of THBS2 in SMCs [35]. Moreover, the THBS2 may be also involved in organization of the extracellular matrix, as suggested by the observation that disruption of THBS2 in mice results in a complex phenotype characterized by abnormalities of fibroblasts, connective tissue, and blood vessels that associated with Ehlers-Danlos syndrome type IV [25]. Furthermore, THBS2 is capable of binding both the pro and mature forms of MMP-2, leading to a twofold increase in MMP-2 activity was found to contribute to the adhesive defect observed in THBS2-null fibroblasts [26, 35, 36].

The most important limitation of the present study is the relatively small sample size. A single center case-control study is not sufficient to fully interpret the relationship between THBS2 polymorphisms and susceptibility to TAD. And as the difference between the groups with the recessive model was not statistically significant, which may be due to the relatively small sample size. Further study with multiple population and larger sample size is needed. Also our investigation is only a genetic association study and the precise impact of this polymorphism on protein function has not been confirmed by molecular biology techniques.

In conclusion, the present study suggested that the THBS2 rs8089 variant was associated with TAD, with the G allele representing a risk factor in Chinese Han population.

Disclosure of conflict of interest

None.

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