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Single Nucleotide Polymorphisms (SNPs) Genotyping Reveals that Mfn2 Polymorphisms are Associated with Thoracic Aortic Dissection in Han Chinese Population

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Background: Many studies have shown that hypertension may contribute to thoracic aortic dissection (TAD). Among the factors that modulate hypertension are endoplasmic reticulum stress and vascular smooth muscle cell proliferation which are in turn modulated by mitofusion-2 (Mfn2). Specifically, we determined, in the Han Chinese population, whether single nucleotide polymorphisms (SNPs) of Mfn2 influenced the occurrence of TAD.

Material/Methods: Six tagging SNPs of Mfn2 (rs2236057, rs3766741, rs2236058, rs17037564, rs2295281, and rs2336384) were genotyped using a TaqMan assay in 200 TAD patients and 451 health individuals from the Han Chinese population.

Results: Logistic regression analysis indicated CC genotype of rs2295281 was highly linked to an increased risk of TAD (TT+CT versus CC, OR=0.540, 95% CI [0.320–0.911], *P*=0.021), implying that TT genotype and CT genotype of rs2295281 have a lower risk for TAD. Logistic regression analysis also indicated that rs2236058 was highly linked to the risk of TAD based on recessive genetic model, which indicated that the GG genotype was a protective factor against TAD (GG versus (CG+CC), OR=0.545, 95% CI [0.351–0.845], *P*=0.007). CG genotype and CC genotype of rs2236058 had a higher risk for TAD. In addition, rs2236058 was linked to the risk of TAD in the recessive genetic and homozygous models in the normotensive subgroup (GG versus (CG+CC), OR=0.298, 95% CI [0.112–0.792], *P*=0.015; GG versus CC, OR=0.528, 95% CI [0.302–0.925], *P*=0.026) but not in the hypertension subgroup.

Conclusions: Our findings showed that the occurrence of TAD in a Han Chinese population was influenced by Mfn2 polymorphisms.

MeSH Keywords: Aortic Dissection, Thoracic • Hypertension • Polymorphism, Single Nucleotide

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Background

Recent statistics show high morbidity and mortality rates due to thoracic aortic dissection (TAD) [1,2]. A mortality rate of up to 50% or more within 48 hours has been reported for untreated TAD, and this rate increases 1% to 2% per hour. When it is not diagnosed, ascending aortic dissection has a 3-month mortality rate of up to 90% [3]. Due to its many complications, the morbidity and mortality rates of TAD remain high notwithstanding the recent tremendous advancements in diagnostic and therapeutic approaches for this disease. Therefore, is it necessary to identify what may lead to TAD. The etiology of TAD is complex and heterogeneous, and previous studies clearly showed that in addition to environmental risk factors, genetic factors may also determine susceptibly to the disease. As a consequence, investigating the TAD-related pathogenic genes would be beneficial to understand the etiology of TAD and to prevent and treat the disease.

Hypertension is thought to be a key determinant of TAD development. Indeed, a history of hypertension is often reported in up to 75% of acute Stanford type A aortic dissection patients. It can cause hemodynamic changes in the aortic cavity, leading to stress changes in the vessel wall. Moreover, the weakening and destruction of the aortic wall is known to be influenced by hypertension, leading to TAD. Arteries are mainly composed of the vascular smooth muscle. In previous reports, vascular smooth muscle cells (VSMCs) were found to be closely related to multiple aortic diseases [4]. Furthermore, some studies have shown that abnormal hemodynamics and hormone levels can lead to VSMC dysfunction in some cases and result in decreased proliferation and migration abilities [4–6]. In this process, the aortic wall is ultimately thinned [7], which is considered the pathologic basis of aortic dissection.

Mitofusion-2 (Mfn2), is a recently identified gene that is localized to chromosome 1p36.22. Spanning 4.16 kb of genomic DNA, the gene encodes a protein containing 661 amino acids. The expression of Mfn2 produces a protein found on the mitochondrial membrane which participates in processes such as regulating mitochondrial networks and fusion [8]. Inadequate regulation of mitochondrial fusion and fission results in a series of problems such as apoptosis disorders and energy metabolic dysfunctions [9]. During the proliferation of VSMCs, Mfn2 is a negative phase regulatory factor in the signaling pathway of extracellular single-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) [8,10]. Notably, several studies have shown that Mfn2 gene polymorphisms are predicts the risk of essential hypertension in the Han Chinese population [11–13]. Therefore, we inferred that Mfn2 may be associated with the risk of TAD as no study has examined this relationship. Thus, the relationship between Mfn2 and the risk of TAD in a Han Chinese population was herein studied.

Material and Methods

Patients

This study was approved by the ethics committee of Beijing Anzhen Hospital of Capital Medical University. Signed written informed consents were obtained from all participants before the study. We assigned all participants into 2 categories: the control group and TAD group. All participants were of Han Chinese ancestry without intermarriages. Participants were enrolled at the Beijing Anzhen Hospital of Capital Medical University, Beijing, China. TAD was diagnosed on the basis of computed tomography angiography (CTA) and other imaging examinations according to the new guidelines for TAD diagnosis [14]. These guidelines have been recommended by several academic authorities, including the American Heart Association. There were several exclusion criteria, including familial aortic dissection, i.e., one or more family members had TAD; Ehlers-Danlos, Marfan, and Loeys-Dietz syndromes, and other genetic defect syndromes; aortic arch constriction, aortic wall hematoma, and other aortic diseases; diabetes mellitus; primary kidney disease; endocrine or cancers disease. Furthermore, control participants were required to undergo at least one imaging examination, such as aortic CTA, MRI, echocardiography or aortic imaging, to make a clear diagnosis excluding TAD and other related diseases. Individuals in the TAD group and control group were not family members.

An individual with a mean diastolic blood pressure (DBP) ≥90 mmHg and/or a mean systolic blood pressure (SBP) ≥140 mmHg was considered hypertensive. In addition, patients who regularly used antihypertensive medicine were considered hypertensive. SBP and DBP were measured at the first and fifth Korotkoff sounds, respectively. Data relating to drinking and smoking habits were recorded during the interview. Those who smoked >100 cigarettes were designated as smokers, while those who drank >12 times in a year were designated as drinkers [15,16]. Our study was performed in compliance with the Declaration of Helsinki, and all participants signed an informed consent form. Appropriate permission was granted by the Ethics Committee of our hospital.

Genotyping and identification of SNP

We chose common single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) >0.10 in Mfn2 in the Han Chinese population dataset from the International HapMap Project SNP database (*http://www.hapmap.org/*. We selected the tag SNPs, meaning the remaining common SNPs with an $r^2 \geq 0.85$, by Haploview 4.2 software (*http://www.broad.mit.edu/mpg/haploview*). Based on these criteria, we selected 6 tag SNPs of Mfn2 (rs2236057, rs17037564, rs2295281, rs3766741, rs2236058, and rs2336384).

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Table 1. Characteristics of Controls and TAD.

Variables	Controls (n=451)	TAD (n=200)	*P*-value
Age (years)	47.94±5.54	47.33±10.81	0.448
Gender (Male/Female)	278/173	155/45	<0.001
SBP (mmHg)	124.17±17.15	139.22±26.04	<0.001
DBP (mmHg)	80.44±13.93	81.43±17.79	0.485
BMI (kg/m²)	25.82±5.81	25.35±3.74	0.297
CREA (mmol/L)	74.39±12.24	93.95±18.37	<0.001
UREA (mmol/L)	5.19±1.33	6.76±3.55	<0.001
TCHO (mmol/L)	5.10±0.89	4.22±0.98	<0.001
TG (mmol/L)	1.72±0.34	1.75±0.99	0.715
LDL-C (mmol/L)	3.18±0.81	2.81±0.79	<0.001
HDL-C (mmol/L)	1.22±0.48	0.90±0.26	<0.001
Hypertension (n, %)	205 (45.5%)	149 (74.5%)	<0.001
Smokers (n ,%)	152 (33.9%)	96 (48.0%)	0.001
Drinkers (n, %)	227 (50.6%)	43 (21.5%)	<0.001

Continuous variables were expressed as means ± standard deviations when normally distributed and as median (interquartile range) when asymmetrically distributed. BMI – body mass index; CREA – creatinine; DBP – diastolic blood pressure; EH – essential hypertensive patients; HDL-C – high-density lipoprotein; HR – heart rate; LDL-C – low-density lipoprotein; NT – normotensive subjects; SBP – systolic blood pressure; TCHO – total cholesterol; TG – triglyceride; UREA – urea nitrogen.

We used ethylene diamine tetra-acetic acid (EDTA)-anticoagulated vacutainer tubes to collect blood samples. Using the common phenol-chloroform method, we isolated genomic DNA from peripheral blood leukocytes, which were kept at -80°C. TaqMan assays were then performed using the selected SNPs. The probes and primers used for SNP genotyping for rs2295281 C__16189654_10, rs2236058C__15953634_10, rs2236057C__15953633_10, rs2336384 C__11461995_10, rs3766741C__25606040_10, and rs17037564 C__32800152_10 were purchased from Applied Biosystems Assay (Foster City, CA, USA). The assay reactions contained TaqMan EXPress Master Mix (2×), working stock of SNP Genotyping Assay (20×) and genomic DNA in a final volume of 5 μL. We used a GeneAmpR PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) for amplification, and the reaction protocol was as follows: initial denaturation and activation at 95°C for 1 minute, followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds. A negative control well was included in each 384-well plate which carried 2 samples without DNA and 2 duplicate samples (control). The plates were scanned using an ABI HT 7900 machine, and the Allelic Discrimination of SDS 2.0 was used for the end-point analysis. All the operators were trained professionally.

Statistical analysis

Statistical Product and Service Solutions (SPSS) (Version 21.0) (IBM, Armonk, NY, USA) was applied for data analysis. Data with normal distribution is shown as mean ± standard deviation and that with non-normal distribution is shown as median ($25^{th}/75^{th}$ quartiles). Categorized data are presented as percentages. Data from different groups were compared using the chi-square test, Student's *t*-test, and the Mann-Whitney U test. All analyses were 2-tailed, and $P<0.05$ was defined as indicating statistical significance. Hardy-Weinberg equilibrium (HWE) was performed as previously reported [17]. The chi-square test was used to compare the allelic and genotypic frequencies between the TAD group and the control group. Logistic regression analysis was applied to explore the relationship between each Mfn2 SNP and TAD risk under different genetic models (recessive, dominant, and additive models) subsequent to adjustment for confounding factors. Furthermore, we calculated the 95% confidence intervals (95% CIs) and odds ratios (ORs).

Results

Characteristics of the participants

The number of participants included in this study was 200 TAD cases (45 females and 155 males; mean age 47.33±10.81) and 451 controls (173 females and 278 males; mean age 47.94±5.54). Table 1 shows the clinical data of all participants. All participants in the control group and the TAD group were age-matched. Among all the individuals, we found that SBP,

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urea nitrogen (UREA), creatinine (CREA), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and total cholesterol (TCHO) were remarkably higher in TAD patients relative to the controls. Hypertension and smoking were found to be remarkably higher in total TAD participants relative to the control group. The following parameters were not significantly different between the control and the TAD participants: body mass index (BMI), age, and DBP. The incidence of drinking was lower in the control group relative to the TAD group.

Distribution and detection of SNPs

A total of 6 SNPs with a MAF >0.10 in Mfn2 were selected as the tag SNPs of the gene, and Mfn2 was genotyped in all participants in strict accordance with the methodical steps described. Among all participants, 100.0% of the rs2295281 samples, 98.8% of the rs2236058 samples, 98.6% of the rs2336384 samples, 99.7% of the rs3766741 samples, and 99.5% of the rs17037564 samples were successfully genotyped. No variants of rs2236057 were detected in the TAD group.

The genotype frequencies for all 5 SNPs were in line with HWE in the control group (rs2336384, P=0.318, F=0.047; rs2295281, P=0.249, F=0.054; rs17037564, P=0.059, F=0.096; rs2236058, P=0.096, F=0.079; rs3766741, P=0.203, F=0.060), indicating that the results of this study have a representative genetic group. The genotype distribution and allele frequency of each locus are shown in Tables 2 and 3, respectively. Univariate analysis showed that the genotype distribution of rs2295281 and rs2236058 differed significantly between the TAD group and control group (P<0.05). These findings indicated that these 2 sites might be linked to the risk of TAD. But rs2336384, rs17037564, or rs3766741 were not associated with the risk of TAD.

Because we found that polymorphisms of Mfn2 were associated with hypertension in a previous study, we performed subgroup analysis according to whether patients had hypertension. Univariate analysis indicated that the genotype distribution of rs2295281 in the hypertension subgroup was remarkably linked to TAD risk (P=0.046). In the normotensive subgroup, the locus and the risk of aortic dissection were not correlated. These results indicated a significant effect of the interaction between this polymorphism and hypertension on TAD. The genotype distribution and allele frequency of the rs2236058 (P=0.007) locus were strongly correlated with TAD in the normotensive subgroup, and CG genotype and CC genotype (P=0.034) may be at higher risk of developing aortic dissection. This locus was not found to be associated with TAD in the hypertension subgroup.

Association analyses

Because the effects of genes on the phenotype of a disease usually follow a certain genetic pattern, such as additive genetic models, dominant genetic models, recessive genetic models, or homozygous models, we analyzed the correlation between different polymorphic *loci* of Mfn2 and the susceptibility to TAD. To exclude the effects of possible confounding factors on the correlation analysis of gene mutation sites with TAD, we used logistic regression to correct the following factors to prevent bias, including sex, age, BMI, hypertension history, TCHO, triglyceride, drinking, and smoking. The outcomes from the regression analysis are shown in Tables 4–6.

CC genotype of rs2295281 indicated risk for TAD

We found that rs2295281 was strongly correlated with TAD risk based on dominant genetic model, which indicated that the CC genotype was a risk factor for TAD (TT+CT versus CC, OR=0.540, 95% CI [0.320–0.911], P=0.021) and that TT genotype and CT genotype of rs2295281 had a lower risk for TAD. For the subgroup analysis, there was no correlation between this polymorphism and TAD risk in either the hypertension subgroup or the normotensive group.

GG genotype of rs2236058 was a protective factor against TAD

We found that rs2236058 was strongly correlated with the risk of TAD under the recessive genetic model, which indicated that the GG genotype was a protective factor against TAD (GG versus (CG+CC), OR=0.545, 95% CI [0.351–0.845], P=0.007) and that CG genotype and CC genotype of rs2236058 had an elevated risk for TAD. Hypertension-based subgroup analysis showed that rs2236058 was linked to the risk of TAD in the recessive genetic model and the homozygous model in the normotensive subgroup (GG versus (CG+CC), OR=0.298, 95% CI [0.112–0.792], P=0.015; GG versus CC, OR=0.528, 95% CI [0.302–0.925], P=0.026). However, rs2236058 was not found to be associated with TAD in the hypertension group. These findings implied that the rs2236058 polymorphism might influence the occurrence of TAD independently of hypertension.

The rs2336384 polymorphism was not correlated with TAD

We found that rs2336384 was neither correlated to the risk of TAD in the hypertension patient subgroup nor in the non-hypertension participant subgroup.

Association between rs3766741 polymorphism and TAD

Since there were only 2 participants carrying genotype GG in each group, we explored rs3766741 in the dominant genetic model only and found that it was not correlated with the risk of TAD (OR=0.443, 95% CI [0.062–3.167], P=0.405), either in the hypertension subgroup or in the normotensive subgroup.

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Table 2. Genotype distribution of Mfn2 in case and control group.

SNP			Genotype (frequency, %)			χ^2	*P*-value
			GG	GT	TT		
rs2336384	Total	Case	38 (19.0)	111 (55.5)	51 (25.5)	4.724	0.094
		Control	89 (20.1)	207 (46.8)	146 (33.1)		
	Hypertension	Case	24 (16.1)	86 (57.7)	39 (26.2)	3.628	0.163
		Control	42 (21.0)	95 (47.5)	63 (31.5)		
	Normotension	Case	13 (26.0)	25 (50.0)	12 (24.0)	2.346	0.310
		Control	47 (19.4)	112 (46.3)	83 (34.3)		
			TT	CT	CC		
rs2295281	Total	Case	20 (10.0)	112 (56.0)	68 (34.0)	8.479	0.014
		Control	77 (16.9)	204 (45.2)	170 (37.9)		
	Hypertension	Case	15 (10.1)	86 (57.7)	49 (32.2)	6.131	0.046
		Control	27 (13.2)	91 (44.4)	87 (42.4)		
	Normotension	Case	5 (10.0)	26 (52.0)	19 (38.0)	2.929	0.231
		Control	50 (20.3)	113 (45.9)	83 (33.8)		
			GG	AG	AA		
rs1703756	Total	Case	2 (1.0)	47 (23.5)	151 (57.5)	3.499	0.174
		Control	10 (2.2)	81 (18.1)	357 (79.7)		
	Hypertension	Case	1 (0.7)	33 (22.1)	115 (77.2)	3.820	0.148
		Control	7 (3.4)	36 (17.7)	161 (78.9)		
	Normotension	Case	1 (2.0)	13 (26.0)	36 (72.0)	1.747	0.417
		Control	3 (1.2)	45 (18.4)	196 (80.4)		
			GG	CG	CC		
rs2236058	Total	Case	32 (16.0)	123 (61.5)	45 (22.5)	14.38	0.001
		Control	118 (26.6)	204 (46.1)	121 (27.3)		
	Hypertension	Case	27 (18.1)	92 (61.1)	31 (20.8)	5.696	0.058
		Control	43 (21.7)	96 (48.5)	59 (29.8)		
	Normotension	Case	5 (10.0)	31 (62.0)	14 (28.0)	9.985	0.007
		Control	75 (30.6)	108 (44.1)	62 (25.3)		
			CC	CG	GG		
rs3766741	Total	Case	166 (83.0)	32 (16.0)	2 (1.0)	1.446	0.485
		Control	362 (80.6)	85 (18.9)	2 (0.5)		
	Hypertension	Case	124 (83.2)	23 (15.5)	2 (1.3)	1.961	0.375
		Control	161 (79.3)	41 (20.2)	1 (0.5)		
	Normotension	Case	41 (82.0)	9 (18.0)	0 (0)	0.204	0.903
		Control	201 (80.7)	44 (17.9)	1 (0.4)		

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Table 3. Allele frequency of MFN-2 in case and control group.

SNP			Allele (frequency, %)		χ^2	*P*-value
			G	T		
rs2336384	Total	Case	187 (46.75)	213 (53.25)	1.14	0.286
		Control	385 (43.6)	499 (56.4)		
	Hypertension	Case	134 (44.9)	164 (55.1)	0.003	0.955
		Control	179 (44.8)	221 (55.2)		
	Normotension	Case	51 (26.0)	49 (50.0)	3.501	0.061
		Control	206 (40.9)	298 (59.1)		
			T	C		
rs2295281	Total	Case	152 (38.0)	248 (62.0)	0.27	0.601
		Control	355 (39.5)	543 (60.5)		
	Hypertension	Case	116 (38.7)	184 (61.3)	0.812	0.368
		Control	145 (35.4)	265 (64.6)		
	Normotension	Case	36 (36.0)	64 (64.0)	1.814	0.178
		Control	21 (43.3)	27 (56.7)		
			G	A		
rs1703756	Total	Case	51 (12.75)	349 (7.25)	0.58	0.445
		Control	101 (11.3)	795 (88.7)		
	Hypertension	Case	35 (11.7)	263 (88.3)	0.042	0.837
		Control	50 (12.3)	358 (87.7)		
	Normotension	Case	15 (15.0)	85 (85.0)	1.724	0.189
		Control	51 (10.5)	43989.5)		
			G	C		
rs2236058	Total	Case	186 (46.8)	212 (53.2)	0.942	0.332
		Control	440 (49.7)	446 (50.3)		
	Hypertension	Case	145 (48.7)	153 (51.3)	0.497	0.481
		Control	182 (45.9)	214 (54.1)		
	Normotension	Case	41 (42.0)	59 (58.0)	4.512	0.034
		Control	25 (52.7)	23 (47.3)		
			C	G		
rs3766741	Total	Case	364 (91.0)	36 (9.0)	0.26	0.607
		Control	809 (90.1)	89 (9.9)		
	Hypertension	Case	271 (90.9)	27 (9.1)	0.450	0.502
		Control	363 (89.4)	43 (10.6)		
	Normotension	Case	91 (91.0)	9 (9.0)	0.012	0.913
		Control	44 (90.7)	46 (9.3)		

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Table 4. Logistic regression to correct the association of MFN-2 gene polymorphisms with TAD.

SNP	Models	Genotype	OR (95%CI)[a]	P[a]
rs2336384	Additive	GG *vs.* GT *vs.* TT	0.876 (0.687–1.117)	0.287
	Dominant	(GG+GT) *vs.* TT	1.045 (0.679–1.607)	0.842
	Recessive	GG *vs.* (GT+TT)	0.707 (0.483–1.034)	0.074
	Homozygote	GG *vs.* TT	0.818 (0.498–1.343)	0.427
rs2295281	Additive	TT *vs.* CT *vs.* CC	0.930 (0.728–1.187)	0.561
	Dominant	(TT+CT) *vs.* CC	0.540 (0.320–0.911)	0.021
	Recessive	TT *vs.* (CT+CC)	1.198 (0.839–1.709)	0.320
	Homozygote	TT *vs.* CC	0.649 (0.368–1.144)	0.134
rs17037564	Additive	GG *vs.* AG *vs.* AA	1.143 (0.804–1.625)	0.456
	Dominant	(GG+AG) *vs.* AA	0.565 (0.119–2.686)	0.473
	Recessive	GG *vs.* (AG+AA)	1.238 (0.826–1.857)	0.301
	Homozygote	GG *vs.* AA	0.473 (0.102–2.184)	0.327
rs2236058	Additive	GG *vs.* CG *vs.* CC	0.906 (0.709–1.156)	0.426
	Dominant	(GG+CG) *vs.* CC	1.317 (0.884–1.963)	0.176
	Recessive	GG *vs.* (CG+CC)	0.545 (0.351–0.845)	0.007
	Homozygote	GG *vs.* CC	0.861 (0.662–1.119)	0.861
rs3766741	Dominant	(CG+GG) *vs.* CC	0.443 (0.062–3.167)	0.405

Table 5. Logistic regression to correct the association of MFN-2 gene polymorphisms with TAD in normotensive group.

SNP	Models	Genotype	OR (95%CI)[a]	P[a]
rs2336384	Additive	GG *vs.* GT *vs.* TT	0.678 (0.434–1.061)	0.089
	Dominant	(GG+GT) *vs.* TT	0.563 (0.269–1.178)	0.127
	Recessive	GG *vs.* (GT+TT)	0.625 (0.298–1.308)	0.212
	Homozygote	GG *vs.* TT	0.689 (0.440–1.080)	0.104
rs2295281	Additive	TT *vs.* CT *vs.* CC	0.750 (0.470–1.197)	0.228
	Dominant	(TT+CT) *vs.* CC	0.472 (0.172–1.298)	0.146
	Recessive	TT *vs.* (CT+CC)	0.815 (0.422–1.575)	0.543
	Homozygote	TT *vs.* CC	0.670 (0.390–1.151)	0.147
rs17037564	Additive	GG *vs.* AG *vs.* AA	1.361 (0.697–2.657)	0.367
	Dominant	(GG+AG) *vs.* AA	2.633 (0.228–3.408)	0.438
	Recessive	GG *vs.* (AG+AA)	1.335 (0.642–2.779)	0.439
	Homozygote	GG *vs.* AA	1.684 (0.494–5.739)	0.404
rs2236058	Additive	GG *vs.* CG *vs.* CC	0.663 (0.432–1.017)	0.060
	Dominant	(GG+CG) *vs.* CC	0.826 (0.451–1.902)	0.835
	Recessive	GG *vs.* (CG+CC)	0.298 (0.112–0.792)	0.015
	Homozygote	GG *vs.* CC	0.528 (0.302–0.925)	0.026
rs3766741	Dominant	(CG+GG) *vs.* CC	0.996 (0.998–1.004)	0.652

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Table 6. Logistic regression to correct the association of MFN-2 gene polymorphisms with TAD in hypertensive group.

SNP	Models	Genotype	OR (95%CI)[a]	P[a]
rs2336384	Additive	GG *vs.* GT *vs.* TT	0.983 (0.716–1.350)	0.914
	Dominant	(GG+GT) *vs.* TT	0.781 (0.482–1.265)	0.315
	Recessive	GG *vs.* (GT+TT)	1.328 (0.754–2.341)	0.326
	Homozygote	GG *vs.* TT	1.017 (0.731–1.416)	0.921
rs2295281	Additive	TT *vs.* CT *vs.* CC	1.204 (0.865–1.677)	0.272
	Dominant	(TT+CT) *vs.* CC	0.820 (0.412–1.632)	0.572
	Recessive	TT *vs.* (CT+CC)	1.532 (0.975–2.408)	0.064
	Homozygote	TT *vs.* CC	1.038 (0.718–1.501)	0.843
rs17037564	Additive	GG *vs.* AG *vs.* AA	0.974 (0.616–1.542)	0.912
	Dominant	(GG+AG) *vs.* AA	0.235 (0.028–1.873)	0.183
	Recessive	GG *vs.* (AG+AA)	1.107 (0.655–1.873)	0.703
	Homozygote	GG *vs.* AA	0.495 (0.171–1.436)	0.196
rs2236058	Additive	GG *vs.* CG *vs.* CC	1.141 (0.826–1.578)	0.424
	Dominant	(GG+CG) *vs.* CC	1.647 (0.986–2.752)	0.057
	Recessive	GG *vs.* (CG+CC)	0.813 (0.470–1.409)	0.461
	Homozygote	GG *vs.* CC	1.072 (0.766–1.500)	0.685
rs3766741	Dominant	(CG+GG) *vs.* CC	0.418 (0.037–4.690)	0.479

Discussion

The analysis of tagging SNPs is an important method for comprehensive detection of susceptibility genes associated with disease progression. In our study, we used the Haploview software to identify 5 tagging SNPs in Mfn2, which were subjected to genotyping. Based on univariate analysis, genotype distribution of rs2295281 in the hypertension subgroup was strongly linked to TAD risk (*P*=0.046). Logistic regression analysis revealed that rs2295281 was strongly correlated with the risk of TAD under the dominant genetic model, which indicated that genotype CC is a risk factor for TAD and that T allele carriers have a lower risk for TAD.

This project is the first genetic study on the correlation between Mfn2 and TAD using SNPs. Although there have not been any investigations on the correlation between Mfn2 and TAD, there are several studies on Mfn2 and hypertension. According to our analysis, 3 groups investigated the correlation between polymorphisms of Mfn2 and essential hypertension. Wang et al. [11] investigated the association between 7 SNPs in intron 2 and essential hypertension and revealed that rs2236055, rs4846085, rs1474868, rs2336384, and rs873457 were strongly correlated with essential hypertension in a Chinese population. Later on, Wang et al. [12] investigated the SNPs in the 5'-untranslated region (UTR) of Mfn2 and found a correlation between the -1248A>G variant in the MFN-2 gene and hypertension in a Chinese population. These outcomes suggest that Mfn2 polymorphisms participate in the initiation of hypertension. In another study, Li et al. [13] investigated the relationship between the tagging SNPs in Mfn2 and the risk of hypertension. Consistent with Wang et al. [11] findings, they found that the rs2336384 polymorphism was strongly correlated with essential hypertension in a northern Han Chinese population. In addition, rs3766741, rs2236058, and rs2236057 polymorphisms were strongly correlated with essential hypertension. Despite the fact that the SNPs investigated in previous studies were different from those of this study, the outcomes imply that Mfn2 polymorphisms are correlated with essential hypertension risk.

Essential hypertension is the main pathogenic factor in TAD, and many of the gene polymorphisms associated with essential hypertension are also associated with TAD, such as MTHFR 677C/T and MMP9. Therefore, we inferred that Mfn2 may be correlated with the risk of TAD. Recently, several research groups have investigated the Mfn2 gene and may present evidence for the relationship between Mfn2 and TAD. Furthermore, other reports show that Mfn2 modulates endoplasmic reticulum stress, insulin resistance, and proliferation of VSMCs [18–21]. In addition, hypertension can cause hemodynamic changes in

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the aortic cavity, triggering stress response in the vessel wall. It also contributes to the weakness and destruction of the aortic wall. We speculate that hypertension is an independent determinant of TAD for the following reasons: 1) Mfn2 not only exists in the outer membrane of mitochondria but also modulates mitochondrial fusion and exists in the endoplasmic reticulum, regulating endoplasmic reticulum morphology [19]. 2) Some *in vitro* experiments showed that Mfn2 knockout in fibroblasts in embryonic mice could increase the stress effect of the endoplasmic reticulum and accelerate apoptosis of the endoplasmic reticulum [21], the medial degeneration leading to bands of cellular loss in the aortic media. Other oxidative factors produced by mitochondria are thought to lead to atherosclerosis and hypertension [22]. 3) Insulin resistance modulates other atherosclerotic conditions, including hypertension, via stimulating protein kinase C (PKC) and advanced glycation end-products (AGEs)-RAGE axis leading to accumulation of AGEs.

Our study also indicated that rs2236058 was strongly correlated with the risk of TAD under the recessive genetic model, indicating that the GG genotype was a protective factor in TAD (GG versus (CG+CC), OR=0.545, 95% CI [0.351–0.845], *P*=0.007). CG genotype and CC genotype of rs2236058 are more susceptible to TAD. In addition, rs2236058 was strongly correlated with the risk of TAD in the recessive genetic model and homozygous model in the normotensive subgroup (GG versus (CG+CC), OR=0.298, 95% CI [0.112–0.792], *P*=0.015; GG versus CC, OR=0.528, 95% CI [0.302–0.925], *P*=0.026) but not in the hypertension subgroup. Interestingly, some patients with TAD have no history of hypertension. In *in vitro* experiments, the overexpression of Mfn2 significantly inhibited the proliferation of serum-activated VSMCs, which inhibited the cell cycle by inhibiting the ERK/MAPK pathway [18,19,23]. This inhibition may cause the dysfunction of VSMCs, leading to the remodeling of the vessel wall. The vessel wall will then become progressively thinner, which is the pathological basis for the formation of TAD. This hypothesis may explain the relationship between Mfn2 and TAD.

All participants belonged to the ethnic Han population so as to minimize population stratification. Moreover, we excluded individuals with Ehlers-Danlos, Loeys-Dietz, and Marfan syndromes, and other genetic defect syndromes with the aim of avoiding selection bias. Nevertheless, there were some limitations to this study that should be mentioned. First, the data presented here should be interpreted with the understanding that the prognostic data obtained during the follow-up period was not sufficient to enable assessment of the relationship between the polymorphisms and TAD, which may limit or under-represent the role of Mfn2 polymorphisms in the occurrence of hypertension. Second, this study only focused on 5 of the most common tagging SNPs, but other SNPs, such as low frequency SNPs, were not examined. Additional studies should determine the mechanisms that orchestrate the effects of the positive SNPs on the functions of Mfn2 in the pathogenesis of TAD.

Conclusions

We showed that rs2295281 and rs2236058 in Mfn2 were strongly correlated with TAD in a Han Chinese population. These results provide a clue for the mechanism underlying TAD, and additional large sample, functional studies on Mfn2 in TAD are needed to verify the exact mechanisms. These findings should additionally be tested in different populations worldwide.

Conflict of interest

None.

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