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

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, it is 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 susceptibility 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 Loey-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 r² ≥ 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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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, rs2236058 C__15953634_10, rs2236057 C__15953633_10, rs2336384 C__11461995_10, rs3766741 C__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 GeneAmp® 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 (25th/75th 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.

| 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 |
| 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.
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 [0.320–0.845], $P=0.021$) 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 [0.351–0.846], $P=0.007$) 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 (GG+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.
### Table 2. Genotype distribution of Mfn2 in case and control group.

| SNP          | Genotype (frequency, %) | χ²   | P-value |
|--------------|-------------------------|------|---------|
|              | GG                      | GT   | TT      |        |
| 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) |
| rs2336384    | Hypertension            | 24 (16.1) | 86 (57.7) | 39 (26.2) | 3.628 | 0.163 |
|              | Control                 | 42 (21.0) | 95 (47.5) | 63 (31.5) |
|              | Normotension            | 13 (26.0) | 25 (50.0) | 12 (24.0) | 2.346 | 0.310 |
|              | Control                 | 47 (19.4) | 112 (46.3) | 83 (34.3) |
| rs2295281    | Hypertension            | 15 (10.1) | 86 (57.7) | 49 (32.2) | 6.131 | 0.046 |
|              | Control                 | 27 (13.2) | 91 (44.4) | 87 (42.4) |
|              | Normotension            | 5 (10.0) | 26 (52.0) | 19 (38.0) | 2.929 | 0.231 |
|              | Control                 | 50 (20.3) | 113 (45.9) | 83 (33.8) |
| rs1703756    | Hypertension            | 1 (0.7) | 33 (22.1) | 115 (77.2) | 3.820 | 0.148 |
|              | Control                 | 7 (3.4) | 36 (17.7) | 161 (78.9) |
|              | Normotension            | 1 (2.0) | 13 (26.0) | 36 (72.0) | 1.747 | 0.417 |
|              | Control                 | 3 (1.2) | 45 (18.4) | 196 (80.4) |
| rs2236058    | Hypertension            | 32 (16.0) | 123 (61.5) | 45 (22.5) | 14.38 | 0.001 |
|              | Control                 | 118 (26.6) | 204 (46.1) | 121 (27.3) |
|              | Normotension            | 5 (10.0) | 31 (62.0) | 14 (28.0) | 9.985 | 0.007 |
|              | Control                 | 75 (30.6) | 108 (44.1) | 62 (25.3) |
| rs3766741    | Hypertension            | 166 (83.0) | 32 (16.0) | 2 (1.0) | 1.446 | 0.485 |
|              | Control                 | 362 (80.6) | 85 (18.9) | 2 (0.5) |
|              | Normotension            | 41 (82.0) | 9 (18.0) | 0 (0) | 0.204 | 0.903 |
|              | Control                 | 201 (80.7) | 44 (17.9) | 1 (0.4) |
Table 3. Allele frequency of MFN-2 in case and control group.

| SNP                | Allele (frequency, %) | \( \chi^2 \) | P-value |
|--------------------|-----------------------|--------------|---------|
|                    | G                     | T            |         |
| Total              | Case                  | 187 (46.75)  | 213 (53.25) | 1.14   | 0.286 |
|                    | Control               | 385 (43.6)   | 499 (56.4)  |        |       |
| rs2336384          | Hypertension          | 134 (44.9)   | 164 (55.1)  | 0.003  | 0.955 |
|                    | Case                  | 21 (63.6)    | 12 (36.4)   |        |       |
|                    | 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                  |                       |              |            |        |       |
| Total              | Case                  | 152 (38.0)   | 248 (62.0)  | 0.27   | 0.601 |
|                    | Control               | 355 (39.5)   | 543 (60.5)  |        |       |
| rs2295281          | Hypertension          | 116 (38.7)   | 184 (61.3)  | 0.812  | 0.368 |
|                    | Case                  | 21 (63.6)    | 12 (36.4)   |        |       |
|                    | 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                  |                       |              |            |        |       |
| Total              | Case                  | 51 (12.75)   | 349 (7.25)  | 0.58   | 0.445 |
|                    | Control               | 101 (11.3)   | 795 (88.7)  |        |       |
| rs1703756          | Hypertension          | 35 (11.7)    | 263 (88.3)  | 0.042  | 0.837 |
|                    | Case                  | 51 (10.5)    | 4989 (90.5) |        |       |
| Normotension       | Case                  | 15 (15.0)    | 85 (85.0)   | 1.724  | 0.189 |
|                    | Control               | 51 (10.5)    | 4989 (90.5) |        |       |
| C                  |                       |              |            |        |       |
| Total              | Case                  | 186 (46.8)   | 212 (53.2)  | 0.942  | 0.332 |
|                    | Control               | 440 (49.7)   | 446 (50.3)  |        |       |
| rs2236058          | Hypertension          | 145 (48.7)   | 153 (51.3)  | 0.497  | 0.481 |
|                    | Case                  | 25 (52.7)    | 14 (47.3)   |        |       |
| Normotension       | Case                  | 41 (42.0)    | 59 (58.0)   | 4.512  | 0.034 |
|                    | Control               | 25 (52.7)    | 23 (47.3)   |        |       |
| G                  |                       |              |            |        |       |
| Total              | Case                  | 364 (91.0)   | 36 (9.0)    | 0.26   | 0.607 |
|                    | Control               | 809 (90.1)   | 89 (9.9)    |        |       |
| rs3766741          | Hypertension          | 271 (90.9)   | 27 (9.1)    | 0.450  | 0.502 |
|                    | Case                  | 91 (91.0)    | 9 (9.0)     |        |       |
| Normotension       | Case                  | 91 (91.0)    | 9 (9.0)     | 0.012  | 0.913 |
Table 4. Logistic regression to correct the association of MFN-2 gene polymorphisms with TAD.

| SNP          | Models         | Genotype               | OR (95%CI)      | P  |
|--------------|----------------|------------------------|-----------------|----|
| 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 (0.819–1.709) | 0.210 |
|              | Homozygote     | TT vs. CC              | 0.649 (0.368–1.144) | 0.134 |
| rs17037564   | Additive       | GG vs. AG vs. AA       | 0.906 (0.709–1.152) | 0.426 |
|              | Dominant       | GG vs. (AG+AA)         | 1.238 (0.826–1.857) | 0.301 |
|              | Recessive      | GG vs. (AG+AA)         | 1.208 (0.817–1.757) | 0.271 |
|              | Homozygote     | GG vs. AA              | 0.473 (0.102–2.184) | 0.327 |
| rs2236058    | Additive       | GG vs. CG vs. CC       | 0.545 (0.351–0.845) | 0.007 |
|              | Dominant       | GG vs. (CG+CC)         | 1.317 (0.884–1.963) | 0.176 |
|              | Recessive      | GG vs. (CG+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)      | P  |
|--------------|----------------|------------------------|-----------------|----|
| rs2336384    | Additive       | GG vs. GT vs. TT       | 0.678 (0.434–1.061) | 0.089 |
|              | Dominant       | (GG+GT) vs. TT         | 0.563 (0.269–1.788) | 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 (0.470–1.219) | 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 |
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.

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### 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 hypertension, 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...
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 underrepresent 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 rs236058 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.

References:

1. Wang G, Zhai S, Li T et al: Mechanism and management of retrograde type A aortic dissection complicating TEVAR for type b aortic dissection. Ann Vasc Surg, 2016; 32: 111–18
2. Tolenaar JL, Froehlich W, Jonker FH et al: Predicting in-hospital mortality in acute type B aortic dissection: Evidence from International Registry of Acute Aortic Dissection. Circulation, 2014; 130: 545–50
3. Pape LA, Awaïs M, Wozniacki EM et al: Presentation, diagnosis, and outcomes of acute aortic dissection: 17-year trends from the International Registry of Acute Aortic Dissection. J Am Coll Cardiol, 2015; 66: 350–58
4. Lacolley P, Regnault V, Nicoletti A et al: The vascular smooth muscle cell in arterial pathology: A cell that can take on multiple roles. Cardiovasc Res, 2012; 95: 194–204
5. Korshunov VA, Schwartz SM, Berk BC: Vascular remodeling: Hemodynamic and biochemical mechanisms underlying Glagov’s phenomenon. Arterioscler Thromb Vasc Biol, 2007; 27: 1722–28
6. Gerhoffer WT: Mechanisms of vascular smooth muscle cell migration. Circ Res, 2009; 101: 24–31
7. Milewicz DM, Guo DC, Tran-Fadulu V et al: Genetic basis of thoracic aortic aneurysms and dissections: Focus on smooth muscle cell contractile dysfunction. Annu Rev Genomics Hum Genet, 2008; 9: 283–302
8. de Brito OM, Scorrano L: Mitofusin-2 regulates mitochondrial and endoplasmic reticulum morphology and tethering: The role of Ras. Mitochondrion, 2009; 9: 222–26
9. Guo X, Chen K, Guo Y et al: Mitofusin 2 triggers vascular smooth muscle cell apoptosis via mitochondrial death pathway. Circ Res, 2007; 101: 1113–22
10. Yu H, Guo Y, Mi L et al: Mitofusin 2 inhibits angiotensin II-induced myocardial hypertrophy. J Cardiovasc Pharmacol Ther, 2011; 16: 205–11
11. Wang Z, Liu Y, Liu J et al: HSG/Mfn2 gene polymorphism and essential hypertension: A case-control association study in Chinese. J Atheroscler Thromb, 2011; 18: 24–31
12. Wang Z, Liu Y, Liu J et al: A novel 5’-uncoding region –1248 A>G variation of mitofusin-2 gene is associated with hypertension in Chinese. Yonsei Med J, 2013; 54: 603–8
13. Li M, Zhang B, Li C et al: The association of mitofusin-2 gene polymorphisms with susceptibility of essential hypertension in northern Han Chinese population. Int J Med Sci, 2016; 13: 39–47
14. Rogers AM, Hermann LK, Booher AM et al: Sensitivity of the aortic dissection detection risk score, a novel guideline-based tool for identification of acute aortic dissection at initial presentation: Results from the international registry of acute aortic dissection. Circulation, 2011; 123: 2213–18
15. Gu D, Su S, Ge D et al: Association study with 33 single-nucleotide polymorphisms in 11 candidate genes for hypertension in Chinese. Hypertension, 2006; 47: 1147–54
16. Ge D, Huang J, He J et al: Beta2-adrenergic receptor gene variations associated with stage-2 hypertension in northern Han Chinese. Ann Hum Genet, 2005; 69: 36–44
17. Li Y, Gu S, Wu Q et al: No association of ERCC1 C8092A and T19007C polymorphisms to cancer risk: A meta-analysis. Eur J Hum Genet, 2007; 15: 967–73
18. Xia Y, Wu Y, He X et al: Effects of mitofusin-2 gene on cell proliferation and chemotherapy sensitivity of MCF-7. J Huazhong Univ Sci Technolog Med Sci, 2008; 28: 185–89
19. de Brito OM, Scorrano L: Mitofusin 2: A mitochondria-shaping protein with signaling roles beyond fusion. Antioxid Redox Signal, 2008; 10: 621–33
20. Sebastian D, Hernandez-Alvarez MI, Segales J et al: Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. Proc Natl Acad Sci USA, 2012; 109: 5523–28
21. Ngoh GA, Papanicolaou KN, Walsh K: Loss of mitofusin 2 promotes endoplasmic reticulum stress. J Biol Chem, 2012; 287: 20321–32
22. Chen KH, Guo X, Ma D et al: Dysregulation of HSG triggers vascular proliferative disorders. Nat Cell Biol, 2004; 6: 872–83
23. Jezek P, Plecita-Hlavata L: Mitochondrial reticulum network dynamics in relation to oxidative stress, redox regulation, and hypoxia. Int J Biochem Cell Biol, 2009; 41: 1790–804