Original Article

Effects of obesity on the association between common variations in the TBX5 gene and matrix metalloproteinase 9 levels in Taiwanese

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A B S T R A C T
Objectives: The TBX5 gene, a member of the T-box family, is associated with congenital heart disease, electrocardiographic parameters, and development of atrial fibrillation in the general population. This study aimed to elucidate the role of TBX5 gene polymorphisms in metabolic and inflammatory profiles possibly linked to TBX5-related pathologies.

Materials and Methods: A sample population of 597 individuals having routine health examinations was enrolled. Five tagging TBX5 single nucleotide polymorphisms (SNPs) were analyzed using polymerase chain reaction and restriction enzyme digestion or TaqMan SNP genotyping assays. Associations between genotypes/haplotypes and matrix metalloproteinase 9 (MMP9) levels were investigated using general-linear model analysis. Interactions between each genotype/haplotype, MMP9 level, and obesity status were tested using two-way analysis of variance with Golden Helix SVS Win32 7.3.1 software.

Results: After adjusting for clinical covariates, TBX5 genotypes were found to be associated with MMP9 levels (p = 0.002 and p = 0.001 for rs4113925 and rs3825214, respectively) in a dominant inheritance model. Haplotype analysis using three tag SNPs (rs11067101, rs1247973, and rs3825214) revealed a significant association between TBX5 haplotype GCG and MMP9 levels (uncorrected p = 0.0093 and the corrected false discovery rate p = 0.0435). Multivariate analysis identified that SNP rs3825214, in addition to the MMP9 and E-selectin genotypes, was independently associated with MMP9 levels (p < 0.001). Using a dominant inheritance model, subgroup and interaction analysis showed associations between the rs4113925, rs3825214, and MMP9 levels only in nonobese individuals (p = 1.04 × 10^-4 and p = 7.11 × 10^-3, respectively; interaction p = 0.009 and 0.018, respectively). Subgroup analysis showed a borderline significant association between haplotype GCG and MMP9 levels (uncorrected p = 0.020 and corrected false discovery rate p = 0.073), but with no evidence of interaction.

Conclusion: TBX5 genotypes/haplotypes are independently associated with MMP9 in Taiwanese individuals and occur predominantly in nonobese people. These associations may broaden our understanding of the mechanism underlying T-box family gene activity and related cardiovascular pathologies.

1. Introduction

T-box genes encode a family of transcriptional regulators that bind via a highly evolutionary conserved DNA-binding domain, the T-box, to cognate DNA elements (T-box-binding elements) in...
promoters to activate or repress target gene transcription in a variety of developmental contexts [1–3]. To date, more than 20 different mammalian T-box genes have been identified, many of which have orthologs in various multicellular organisms.

The T-box genes exhibit diverse patterns of spatial and temporal expression in embryonic development. Developmental functions of the T-box genes range from the specification of the primary germ layers by genes to later roles in limb development [4–6] and also include specification of cell identity during organogenesis in cardiac development [7–9]. The biologically important roles of several members of this gene family were further emphasized by clinical studies demonstrating that mutations in T-box genes are associated with numerous disease states in humans and by the observation that T-box genes are amplified in a subset of cancers [10,11].

TBX5, a member of the T-box family, is critical for forelimb development and cardiogenesis [12,12]. TBX5 independently promotes cardiac chamber formation in vertebrates [13]. Song et al [14] and Qian et al [15] also showed that four transcription factors, GATA binding protein 4 (GATA4), heart- and neural crest derivative-binding protein 4 (GATA4), myocyte-specific enhancer factor 2C, and TBX5, are capable of reprogramming adult mouse fibroblasts into functional cardiac-like myocytes in vitro and in vivo, and that expression of these factors in noncardiomyocytes enhances the function of injured hearts following myocardial infarction. Haploinsufficiency of TBX5 causes Holt–Oram syndrome, an autosomal dominant disorder characterized by upper limb malformations and cardiac septation defects. TBX5 gene mutations have also been associated with atrial septal defect, ventricular septal defect, and atrioventricular (AV) block [10,16]. TBX5 gene polymorphisms have been associated with genetic determinants of the PR interval, QRS duration, and development of atrial fibrillation in the general population [17,18]. Kim et al [19] also observed that genetic polymorphisms in the TBX5 gene are associated with the risk of metabolic syndrome. Herein, TBX5 genotypes/haplotypes were analyzed in 597 Taiwanese individuals to further elucidate the role of the TBX5 gene in metabolic and inflammatory profiles associated with TBX5-related pathologies.

2. Materials and Methods

2.1. Study population

After written informed consent was obtained, the study participants, who had no known history of major systemic or cardiovascular diseases, were recruited during routine health examinations. Exclusion criteria included cancer, current renal or liver disease, and a history of myocardial infarction, stroke, or transient ischemic attacks. In addition, patients taking diabetes mellitus and/or lipid-lowering drugs during the period of blood sample collection were excluded from the analysis because previous reports revealed that these agents affect inflammation marker expression or concentrations [20–22]. A total of 597 study participants [314 men with a mean age of (± standard deviation) of 44.6 ± 10.1 years and 283 women with a mean age of 46.55 ± 9.9 years] were enrolled in the study. The clinical and biometric features of the study group are summarized in Table 1. Obesity was defined as a body mass index of 25 kg/m² or above, according to Asian criteria [23]. Current smokers were defined as individuals who smoked cigarettes regularly at the time of survey. The Ethics Committee of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, New Taipei City, Taiwan, approved the investigation.

2.2. Laboratory examination

Prior to starting the study, all participants underwent an initial screening assessment that included medical history, vital signs, and measurement of lipid variables and novel risk factors. A total of 15 mL of venous blood was collected in the morning after an overnight (8–12-hour) fast. Venous blood samples were collected from an antecubital vein with a 21-gauge needle. Serum, EDTA, sodium fluoride, and sodium citrate plasma samples were obtained by centrifugation at 3000g for 15 minutes at 4°C. Immediately after centrifugation, serum/plasma samples were frozen and stored at −80°C prior to analysis. All measurements were performed in a central laboratory.

2.3. Assays

Most markers, including serum C-reactive protein, serum amyloid A, soluble intercellular adhesion molecule 1, soluble vascular cell adhesive molecule 1, soluble E-selectin (SELE), matrix metalloproteinase (MMP) 2, and MMP9 values were transformed logarithmically before statistical testing to meet the assumption of normal distributions; however, untransformed data are shown. CRP = C-reactive protein; MMP = matrix metalloproteinase; SAA = serum amyloid A; SD = standard deviation; SELE = soluble E-selectin; SELP = soluble P-selectin; sCAM1 = soluble intercellular adhesion molecule 1; sVCAM1 = soluble vascular cell adhesive molecule 1; sTNFRII = soluble tumor necrosis factor receptor II.

Table 1 Clinical and biochemical characteristics of the study participants stratified by gender.

|                      | Total     | Men       | Women     | p          |
|----------------------|-----------|-----------|-----------|------------|
| No.                  | 597       | 314       | 283       |            |
| Age (y)              | 45.5 ± 10.1| 44.6 ± 10.1| 46.5 ± 9.9| 0.018      |
| Body mass index (kg/m²)| 24.3 ± 3.5| 25.0 ± 3.2| 23.6 ± 3.7| <0.001     |
| Current smoker (%)   | 19.4      | 33.4      | 3.9       | <0.001     |
| CRP (mg/L)           | 1.6 ± 6.1 | 1.9 ± 7.9 | 1.4 ± 3.1 | 0.065      |
| Fibrogen (μmol/L)    | 264.0 ± 69.6| 261.8 ± 71.5| 266.4 ± 67.5| 0.414      |
| SELE (μg/L)          | 53.5 ± 26.6| 60.7 ± 28.4| 45.5 ± 21.9| <0.001     |
| SELP (ng/mL)         | 140.3 ± 116.9| 155.4 ± 131.6| 123.6 ± 95.7| <0.001     |
| SAA (μmol/L)         | 6.2 ± 15.6 | 7.2 ± 19.8 | 5.1 ± 9.0  | 0.240      |
| sICAM1 (μg/L)        | 241.8 ± 112.3| 245.5 ± 121.7| 237.7 ± 112.8| 0.435  |
| sVCAM1 (μg/L)        | 491.5 ± 132.4| 495.8 ± 140.9| 486.8 ± 109.9| 0.504  |
| MMP1 (pg/mL)         | 459.4 ± 1136.2| 338.3 ± 549.8| 593.3 ± 1534.7| 0.747  |
| MMP2 (pg/mL)         | 126.8 ± 41.0| 123.7 ± 41.6| 130.2 ± 40.0| 0.016  |
| MMP9 (pg/mL)         | 144.0 ± 112.45| 155.8 ± 116.3| 130.8 ± 106.7| <0.001  |
| sTNFRII (pg/mL)      | 3269.3 ± 948.3| 3338.9 ± 996.8| 3192.2 ± 887.0| 0.061  |

Continuous variables are presented as mean ± standard deviation. SAA, CRP, sICAM1, sVCAM1, SELE, SELP, MMP1, and MMP9 values were transformed logarithmically before statistical testing to meet the assumption of normal distributions; however, untransformed data are shown.

2.4. Genomic DNA extraction and genotyping

Genomic DNA was extracted as previously reported [27,28]. From the published sequence of the TBX5 gene, oligonucleotide primers were generated to amplify fragments of genomic DNA containing genetic polymorphisms reported on the National Center for Biotechnology Information single nucleotide polymorphism (SNP) database [http://www.ncbi.nlm.nih.gov/SNP/]. Based on the HapMap database, five tag SNPs were chosen, which covered a haplotype region with an R² = 0.85 and minor allele frequency of >0.4 (Table 2). Genotyping for SNPs rs1247973, rs4113925, and rs3825214 was performed using polymerase chain reaction and restriction enzyme digestion. Genotyping for SNPs rs11067101 and
and smoking. The analysis of deviation from the Hardy–Weinberg equilibrium, estimation of linkage disequilibrium between polymorphisms, association between haplotypes and MMP9 levels, and inflammatory biomarkers of the study participants (stratified by gender) were tested using two-way analysis of variance. When interaction terms were significant, stratified analyses of the genetic variants of the genotypes (e.g., target genotypes affected by obesity) and MMP9 levels were performed to further investigate interactive effects while controlling for other variables including age, gender, and smoking. The analysis of deviation from the Hardy–Weinberg equilibrium, estimation of linkage disequilibrium between polymorphisms, association between haplotypes and MMP9 levels, and haplotype–obesity interactions were performed using the Golden Helix SVS Win32 7.3.1 software (Golden Helix, Bozeman, MT, USA).

3. Results

3.1. Clinical and biochemical characteristics

A summary of the characteristics, clinical profiles, and inflammatory biomarkers of the study participants (stratified by gender) is provided in Table 1. No significant deviation from the Hardy–Weinberg equilibrium was detected for the studied polymorphisms (p = 1.00, p = 0.743, p = 0.989, p = 0.895, and p = 0.917 for SNPs rs11067101, rs2236017, rs1247973, rs4113925, and rs3825214, respectively; Table 2). Two pairs of SNPs, rs2236017 and rs1247973 as well as rs3825214 and rs4113925, showed strong pairwise linkage disequilibrium (Table 3).

rs2236017 was performed using TaqMan SNP genotyping assays obtained from Applied Biosystems (Foster City, CA, USA).

3.2. Associations between TBX5 gene polymorphisms and circulating MMP9 levels

To determine whether the TBX5 genotypes affected circulating inflammatory marker levels, 11 inflammatory markers were analyzed, including C-reactive protein, fibrinogen, serum amyloid A, soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1, SELE, soluble P-selectin, MMP1, MMP2, MMP9, and soluble tumor necrosis factor receptor II. A generalized linear model was used to analyze MMP9 levels in relation to predictors of the investigated genotypes and confounders. Data on C-reactive protein, platelet aggregation, red blood cell aggregation, white blood cell aggregation, and platelet–leukocyte aggregation were logarithmically transformed prior to statistical analysis to adhere to a normality assumption. A value of p < 0.05, using two-sided tests, was considered statistically significant.

Interactions between each SNP, MMP9 level, and obesity status were tested using two-way analysis of variance. When interaction terms were significant, stratified analyses of the genetic variants of the genotypes (e.g., target genotypes affected by obesity) and MMP9 levels were performed to further investigate interactive effects while controlling for other variables including age, gender, and smoking. The analysis of deviation from the Hardy–Weinberg equilibrium, estimation of linkage disequilibrium between polymorphisms, association between haplotypes and MMP9 levels, and haplotype–obesity interactions were performed using the Golden Helix SVS Win32 7.3.1 software (Golden Helix, Bozeman, MT, USA).

Table 2

| SNP No. | Primer sequence | PCR size and RE | Allele | Location | Hardy–Weinberg p |
|---------|-----------------|-----------------|--------|----------|-----------------|
| rs11067101 | TaqMan SNP genotyping assays | 361 bp | A/G | Intron 1 | 1 |
| rs2236017 | TaqMan SNP genotyping assays | 271 bp | C/T | Intron 6 | 0.743 |
| rs1247973 | [F5]’-TATACTTCCAGCATACTCGG-3’ | Nal III | C/T | Intron 7 | 0.989 |
| rs4113925 | [F5]’-GACATCAGTGGCGAACAGA-3’ | Sal I | C/T | Intron 7 | 0.895 |
| rs3825214 | [F5]’-ATTGTAAGGCTTCCTTTAATAT-3’ | A/G | Intron 7 | 0.917 |

PCR = polymerase chain reaction; RE = restriction enzyme; SNP = single nucleotide polymorphism.

3.3. TBX5 haplotypes and MMP9 levels

As SNP regression demonstrated that multiple sites within the TBX5 gene significantly affected MMP9 levels, haplotypes were

Table 4

| TBX5 genotypes | MMP9 levels | p* |
|----------------|-------------|----|
| rs11067101 AA | 143.79 ± 117.19 (112) | 0.255 |
| AG | 137.21 ± 110.52 (283) | |
| GG | 155.93 ± 114.72 (176) | |
| rs2236017 AA | 139.08 ± 112.34 (395) | 0.073 |
| AG | 155.93 ± 114.72 (176) | |
| TT | 155.69 ± 128.78 (54) | 0.728 |
| rs1247973 AA | 140.87 ± 116.82 (257) | 0.002 |
| AG | 147.69 ± 107.70 (249) | |
| CT | 143.45 ± 118.90 (311) | 0.376 |
| rs4113925 AA | 141.49 ± 110.44 (156) | 0.846 |
| AG | 141.49 ± 110.44 (156) | 0.013 |
| CC | 161.14 ± 141.38 (130) | 0.002 |
| rs3825214 AA | 128.78 ± 117.69 (127) | 0.013 |
| AG | 153.55 ± 119.56 (276) | 0.002 |
| GG | 153.55 ± 119.56 (276) | 0.019 |

Note: Data are presented as mean ± standard deviation (N). MMP9 = matrix metalloproteinase 9; N = number of subjects; SD = standard deviation.

* The p value is adjusted for age, sex, body mass index, and smoking status.
inferred to capture possible allelic associations. In the present investigation, two pairs of SNPs, rs2236017 and rs1247973 as well as rs3825214 and rs4113925, showed strong pairwise linkage disequilibrium; thus, three tag SNPs (rs11067101, rs1247973, and rs3825214) were enrolled for haplotype analysis. Eight common haplotypes (≥ 5% frequency) were derived from these three SNPs, accounting for all inferred haplotypes. Haplotype analysis revealed a significant association between the TBX5 haplotype GCG and MMP9 levels (uncorrected p = 0.0093 and corrected false discovery rate p = 0.0435) (Table 5).

3.4. Stepwise regression analysis of MMP9 levels using a general linear model in the study population

In addition to the independent variables associated with MMP9 levels, as reported previously by Wu et al [29], TBX5 gene variants, gender, and body mass index were further used for multivariate analysis. In a stepwise regression analysis, the TBX5 SNP rs3825214 genotype with a dominant model, in addition to age, gender, smoking status, fibrinogen levels, and SELE SNP rs5368, was independently associated with MMP9 levels (p < 0.05; Table 6).

3.5. Interactions between TBX5 genotypes/haplotypes, obesity, and MMP9 levels

After adjusting for age, gender, and smoking status, subgroup and interaction analyses revealed associations between rs4113925, rs3825214, and MMP9 levels only in nonobese individuals, using a dominant inheritance model (p = 1.04 × 10⁻⁴ and p = 7.11 × 10⁻⁵, respectively; interaction p = 0.0088 and interaction p = 0.0183, respectively; Fig. 1).

Subgroup analysis showed a borderline significant association between the haplotype GCG and MMP9 levels (uncorrected p = 0.020 and corrected false discovery rate p = 0.073), but with no evidence of interaction (interaction p = 0.409) (data not shown).

4. Discussion

This investigation analyzed the association between TBX5 gene variants and metabolic profiles and inflammatory marker levels in Taiwanese individuals. Our data revealed significant associations between TBX5 genotypes/haplotypes and MMP9 levels. The association with MMP9 levels is independent of our previously reported MMP9 and SELE gene variants. Interactions between obesity, TBX5 genetic variants, and MMP9 levels were also noted. These data

Table 5

| Haplotype | Frequency (%) | MMP9 level | p₁ | p₂ |
|-----------|--------------|------------|----|----|
| H1        | GCA          | 19.3       | -0.0970 | 0.4078 | 0.6493 |
| H2        | ATA          | 17.9       | -0.0291 | 0.0184 | 0.0720 |
| H3        | GTA          | 14.3       | 0.0145  | 0.7880 | 0.8589 |
| H4        | GGC          | 11.5       | -0.0659 | 0.0093 | 0.0435 |
| H5        | GTG          | 10.8       | -0.2063 | 0.1882 | 0.4298 |
| H6        | ATG          | 9.3        | 0.0495  | 0.7609 | 0.8447 |
| H7        | ACA          | 8.5        | 0.0575  | 0.6541 | 0.8244 |
| H8        | ACC          | 8.4        | 0.0886  | 0.1816 | 0.4395 |

MMP9 = matrix metalloproteinase 9; p₁ = uncorrected p value; p₂ = corrected false discovery rate p value; SNP = single nucleotide polymorphisms. 

SNP1, rs11067101; SNP2, rs1247973; SNP3, rs3825214. Coefficients and p values were estimated based on haplotype trend regression analysis implemented in the HelixTree program. The selected haplotype was compared to all unselected haplotypes, adjusted for age, sex, smoking, and body mass index.

Table 6

| Variable | Beta | R² | p |
|----------|------|----|---|
| Gender   | -0.051 | 0.022 | 0.022 |
| Age      | -0.003 | 0.001 | 0.001 |
| BMI      | <0.001 | 0.003 | 0.939 |
| Current smoker | 0.087 | 0.028 | 0.002 |
| Fibrinogen | 0.001 | <0.001 | <0.001 |
| Glucose  | 0.001 | <0.001 | 0.056 |
| SELE rs5368 TT genotype | -0.155 | 0.044 | <0.001 |
| MMP9 rs2274756 GA genotype | 0.161 | 0.082 | 0.049 |
| TBX5 rs3825214 AA genotype | -0.061 | 0.021 | 0.004 |

BMI = body mass index; MMP9 = matrix metalloproteinase 9; SELE = soluble E-selectin.

Cumulative R²: Multiple linear regression, adjusted for age, gender, smoking status, BMI, fibrinogen, fasting plasma glucose, SELE rs5368 genotype, MMP9 rs2274756 genotype, and TBX5 rs3825214 genotype.

Fig. 1. Interactive effect of MMP9 levels on the association between TBX5 genotypes and obesity status. (A) After adjusting for clinical covariates, the major allele of rs4113925 of the TBX5 gene was found to be associated with decreased MMP9 levels in nonobese individuals (p = 1.04 × 10⁻⁴). Interaction analysis revealed an interaction between obesity status and the rs4113925 genotype (interaction p = 0.0088 for the dominant model, after adjustment for age, gender, and smoking status). (B) After adjusting for clinical covariates, the major allele of rs3825214 of the TBX5 gene was found to be associated with decreased MMP9 levels in nonobese individuals (p = 7.11 × 10⁻⁵). Interaction analysis revealed an interaction between obesity status and the rs4113925 genotype (interaction p = 0.0183 for the dominant model, after adjustment for age, gender, and smoking status). MMP9 = matrix metalloproteinase 9.
provide the first evidence that TBX5 is a transcriptional regulator that may modulate the expression of MMP9. These results also expand our understanding of the underlying mechanism of the association between TBX5 and cardiovascular diseases.

TBX5 is a transcriptional regulator in which mutations result in various congenital heart diseases. Heart formation requires a highly balanced network of transcriptional gene activation. TBX5 also plays an important role in postnatal maturation of the AV node. AV bundle, and left and right bundle branch patterning [30]. However, there is no TBX5 binding site on the MMP9 promoter region; thus, it is unlikely that TBX5 alone directly regulates the expression of MMP9. TBX5 commonly functions with other transcription factors, including NK2 transcription factor-related locus (NKX2-5). In a previous investigation, Hiroi et al. [31] revealed that two different types of cardiac transcriptional factors, TBX5 and NKX2-5, directly bound to the promoter of the gene for cardiac-specific natriuretic peptide precursor type A (NPPA) in tandem, and both showed synergistic activation that induced cardiac development. TBX5 also acts cooperatively with NKX2-5 to regulate the expression of short stature homeobox 2 and bone morphogenetic protein 4, which are essential for the formation of the pacemaker region of the developing heart [32]. Co-occupancy of a chromatin region by multiple transcription factors has also been shown to identify a distinct set of developmentally relevant enhancers, including GATA4, NKX2-5, TBX5, serum response factor, and myocyte-enhancer factor 2A [33]. An NKX2-5-binding site was identified on the MMP9 promoter region by in silico analysis (http://mbs.cbrc.jp/research/db/TFSEARCH). Thus, it is possible that TBX5 may act with NKX2-5 to regulate the expression of MMP9. Munshi et al. [34] demonstrated that a distal enhancer for connexin 30.2, a gap junction protein required for normal AV delay in mice, is necessary and sufficient to direct expression to the developing AV conduction system. This enhancer requires TBX5 and GATA4 for proper expression in the conduction system. These results suggest the importance of TBX5 in association with other transcription factors in regulating transcription and cardiac development.

Several lines of evidence suggest that MMP9 may be associated with some TBX5-related pathologies. Both TBX5 and MMP9 have been associated with atrial and AV conduction pathologies with active fibrotic processes. Progressive cardiac conduction disease (PCCD), the most common cause of acquired, permanent, third-degree AV block, is characterized by a slow, progressive loss of AV conduction fibers. Increased myocardial collagen turnover with increased plasma MMP9 levels was observed in patients with PCCD [35]. Cardiac remodeling induced by MMP9 appears to play a role in the pathomechanism of AV conduction delay and ventricular dilation in hyperhomocysteinemia [36]. Polymorphisms of the TBX5 gene have also been associated with genetic determinants of the PR interval and QRS duration, markers of cardiac conduction. Atrial fibrosis in atrial fibrillation is characterized by severe alterations in collagen I and III synthesis/degredation associated with disturbed MMP/tissue inhibitor of metalloproteinase systems [37]. Tumor necrosis factor triggers expression and activation of MMPs, including MMP9, contributing to adverse myocardial remodeling in atrial fibrillation [38]. A higher risk of atrial fibrillation was also noted with TBX5 gene variants [17,18]. Thus, it is possible that TBX5 polymorphisms may act through MMP9, which results in TBX5-related pathologies.

The possibility that MMP9 is an intermediate between TBX5 and cardiovascular pathologies is further supported by the study of TBX20, a closely related T-box family member of TBX5. Both TBX5 and TBX20 are crucial lineage deciding players in early myocardial dichotomy with specification of chamber and nonchamber myocardium in the forming vertebral heart [39]. TBX20 mutations also contribute to various congenital heart diseases in Chinese patients, including atrial septal defect, total anomaly of pulmonary venous return, and tetralogy of Fallot [40]. Similar to TBX5, cardiac TBX20 has been shown to directly interact with NKX2-5, GATA4, and GATA5 in the regulation of gene expression in the developing heart [13]. More importantly, TBX20 has been shown to increase MMP9 and MMP13 expression in primary avian endocardial cells, which suggests an important role in extracellular matrix remodeling and promoting cell proliferation in mesenchymal valve precursor populations in the endocardial cushion during embryonic development [41]. It is interesting that our data showed a significant association between TBX5 gene polymorphisms and MMP9 levels in Taiwanese individuals. Further study is necessary to elucidate the role of the TBX20 gene in regulating MMP9 levels.

Interactions between genotypes/adiposity and lipid variables/inflammatory markers have been demonstrated previously [27,29,42,43]. The present data provide further evidence that the interaction between obesity and TBX5 genotypes/haplotypes affects MMP9 levels. Although there is no association between the MMP9 level and obesity, it is likely that an increased inflammatory status with adiposity may affect the role of genetic effects. Our data suggested that the genetic background associated with MMP9 levels might differ between obese and nonobese individuals.

Major limitations of the study include the use of a single population with a modest sample size and the cross-sectional design. Replication in a second cohort with a larger sample size and prospective design would improve the strength of the genetic association and interaction analyses. When the false discovery rate was applied for multiple tests in haplotype analysis, the statistical significance of a portion of our results became marginal. However, a significant p value was obtained in the multivariate analysis, suggesting that the association between the TBX5 polymorphisms and MMP9 levels may not be due to chance.

In conclusion, the significant associations between TBX5 genotypes/haplotypes and MMP9 levels observed in this investigation suggest that MMP9 is involved in TBX5-related pathology. Our results provide an insight into the regulation and functional mechanism underlying cardiac development and PCCD. The regulation of TBX5-induced MMP9 expression might represent a new therapeutic target for PCCD.

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