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Genetic Variants in Six-Transmembrane Epithelial Antigen of Prostate 4 Increase Risk of Developing Metabolic Syndrome in a Han Chinese Population

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Background: Altered expression of six-transmembrane epithelial antigen of prostate 4 (*STEAP4*) is linked to obesity, insulin insensitivity, metabolic homeostasis, and inflammation. This study assessed *STEAP4* single nucleotide polymorphisms (SNPs) for association with a risk in developing metabolic syndrome in a Han Chinese population. *Methods:* A total of 3375 Han Chinese subjects were included in this case–control study with 1583 metabolic syndrome (MetS) patients and 1792 healthy controls. Four SNPs (rs1981529, rs2040657, rs10263111, rs12386756) were genotyped using polymerase chain reaction and MALDI-TOF-MS. The associations between the STAMP4 SNPs and MetS were then analyzed statistically. *Results:* There was no statistical difference in allele frequency of these four SNPs between the case and control populations. The genotype of rs12386756 was shown to be significantly associated with MetS ($p = 0.035$). Compared with the AA/GG genotypes, the GA genotype of rs12386756 significantly decreased the risk of developing MetS (OR = 0.77; 95% CI, 0.63–0.94; $p = 0.0098$). There was also no haplotype that could be associated with the risk of developing MetS. Furthermore, the SNP rs1981529 of *STEAP4* was associated with body–mass index, waist circumference, and systolic blood pressure, while SNP rs10263111 was associated with waist circumference and fasting glucose levels. SNP rs12386756 was associated with waist and hip circumferences. *Conclusion:* Some SNPs of the *STEAP4* gene altered the risk of developing a metabolic syndrome in the Han Chinese population. Further studies must be conducted to understand the role of the *STEAP4* gene in the pathogenesis of metabolic syndrome.

Introduction

Metabolic syndrome (MetS) is a disorder of energy utilization and storage. Patients with metabolic syndrome have a cluster of metabolic abnormalities, such as central obesity, hyperglycemia, dyslipidemia, and dysarteriotony. MetS has become a global public health problem (Cameron *et al.*, 2004; Eckel *et al.*, 2005; Grundy, 2008). People with MetS are always at a risk of developing cardiovascular disease and diabetes (Alberti *et al.*, 2009). In China, the prevalence of MetS in men and women was 13.7% and 17.8%, respectively (Yang *et al.*, 2014). Although the exact factors causing MetS have not yet been known, it has been found that people develop metabolic syndrome if they have any of the following risk factors: mental stress, obesity, sedentary lifestyle, aging, diabetes, coronary heart disease, lipodystrophy, and schizophrenia or any other psychiatric illnesses (Cameron *et al.*, 2004; Eckel *et al.*, 2005; Grundy, 2008; Alberti *et al.*, 2009; Yang *et al.*, 2014).

Recently, several studies in China have shown that genetic susceptibility is also an important risk factor for developing MetS (Edwards *et al.*, 2008). Therefore, we need to understand the interaction of genes with environmental factors and how these interactions increase a patient's susceptibility toward MetS; a better understanding of gene variants would be beneficial in this regard.

Human six-transmembrane epithelial antigen of prostate 4 (*STEAP4*) belongs to the STEAP (six-transmembrane epithelial antigen of prostate) family of proteins and resides in the Golgi apparatus of cells. *STEAP4* plays an important role in various functions of the human body, such as adipocyte development, metabolism, adipocyte differentiation, insulin sensitivity, and metabolic homeostasis (Zhang *et al.*, 2008; Guo *et al.*, 2011; Narvaez *et al.*, 2013; Matsumoto *et al.*, 2014). An altered expression of *STEAP4* was linked with a cluster of metabolic abnormalities, such as obesity, insulin insensitivity, metabolic homeostasis, and inflammation (Chen *et al.*, 2014).

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Moreover, previous research studies have reported that an overexpression of *STEAP4* causes increased insulin insensitivity, glucose uptake, and impaired mitochondrial functions in obese patients (Moreno-Navarrete *et al.*, 2011; Chen *et al.*, 2014) and diabetic ApoE$^{-/-}$/LDLR$^{-/-}$ mice (Han *et al.*, 2013). The expression of *STEAP4* could be regulated by a variety of inflammatory cytokines, hormones, or adipokines, such as TNFα, IL-6, IL-1β, and leptin in adipocytes (Kralisch *et al.*, 2009; Chen *et al.*, 2010; Tanaka *et al.*, 2012). In addition, recent studies have proved that *STEAP4* plays an important role in the pathogenesis of MetS: *STEAP4* primarily enhances the expression of 1,25-dihydroxy vitamin D_3, CCAAT/enhancer binding protein alpha (*C/EBPα*), and peroxisome proliferator-activated receptor gamma (*PPAR-γ*)-stimulated adipogenesis (Narvaez *et al.*, 2013; Sikkeland and Saatcioglu, 2013). All these observations indicate that *STEAP4* may be contributing to the development of MetS.

Therefore, in this study, we selected and assessed four SNPs of *STEAP4* to investigate their role in the development of metabolic syndrome in the Han Chinese population. Then, we correlated these SNPs of *STEAP4* with the clinical and biochemical characteristics of individuals with metabolic syndrome. Thus, this study thoroughly investigated the role of *STEAP4* in the development of metabolic syndrome.

Subjects and Methods

Study population

In this community-based study, we recruited a total of 3375 Han Chinese individuals and evaluated the prevalence of risk factors, such as diabetes, hypertension, hyperlipidemia, and some other chronic diseases, in these subjects. This study was conducted in 2012 on the subjects of Jilin Province, China. Among these subjects, there were 1583 individuals with MetS, they were termed as cases. The remaining 1792 individuals did not have any abnormality, so they were known as controls. The participants of this study were selected through a multistage stratified cluster sampling process that was conducted in the nine areas (both urban and rural areas) of Jilin province in China (Wang *et al.*, 2014). Metabolic syndrome was diagnosed in the participants using the definition of International Diabetes Federation (IDF) in 2009 (Alberti *et al.*, 2009); the presence of any three of the following five conditions confirmed metabolic syndrome in the patient: (1) an elevated waist circumference ≥85 cm in a Chinese male and ≥80 cm in a Chinese female; (2) elevated triglycerides ≥150 mg/dL (1.7 mM) or hypertriglyceridemia; (3) reduced levels of high-density lipoprotein cholesterol (HDL-C) <40 mg/dL (1.0 mM) in the male and <50 mg/dL (1.3 mM) in the female or being treated; (4) elevated systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg or using antihypertensive drug therapy; and (5) elevated fasting glucose ≥100 mg/dL or using antidiabetic drugs. This study was approved by the ethics committee of the School of Public Health, Jilin University, China. Each subject signed an informed consent letter before participating in this study.

The clinical and biochemical characteristics of these subjects, including hip circumference, blood pressure, and heart rate, were measured by trained personnel. Five milliliters of blood was taken from each fasting subject; this blood was phlebotomized using the nonanticoagulant plexiglass tubes and stored at −20°C for biochemical analysis.

SNP selection, DNA extraction, and genotyping

Based on previous studies, we selected four tag SNPs (rs1981529, rs2040657, rs10263111, rs12386756) using the Haploview program (http://hapmap.ncbi.nlm.nih.gov/) (Nanfang *et al.*, 2010; Zhang *et al.*, 2013). The setting parameters for minor allele frequency of these four tag SNPs were all greater than 0.05 in the Han Chinese population. The functional consequence of rs1981529 is a missense mutation, while the functional consequences of rs2040657, rs10263111, and rs12386756 are intron variants.

For genotyping these four SNPs, we utilized a commercial DNA extraction kit (Hangzhou, China) and extracted genomic DNA from peripheral blood lymphocytes. Then, we utilized the Assay design 3.1 (Sequenom, Inc., San Diego, CA) to design polymerase chain reaction (PCR) primers for each SNP (Table 1). SNP genotyping reactions were performed in a 384-well Spectro-CHIP using a MassARRAY nanodispenser (Sequenom, Inc.). HotStar Taq was used in PCR (Gu *et al.*, 2014). PCR amplification conditions were set for an initial cycle of 15 min at 94°C, and then the following different cycles were conducted: 45 cycles at 94°C for 20 s, 56°C for 30 s, 72°C for 60 s, and finally 72°C for 10 s. PCR products were subjected to the shrimp alkaline phosphatase reaction and single-base extension. After desalting, PCR products were subjected to MALDI-TOF-MS (Sequenom, Inc.) in the MassARRAY system for SNP genotyping.

Statistical analysis

The data were analyzed using statistical software SPSS 19.0. (SPSS, Inc., Chicago, IL). The χ^2 test or Student's *t*-test was used for statistically comparing demographic characteristics between the case and control. The Hardy–Weinberg disequilibrium (HWD) test was used to evaluate every SNP in

Table 1. Primers Used for Genotyping

SNP	*2nd-primer*	*1st primer*	*UEP_SEQ*
rs1981529	5′-ACGTTGGATGGCTCTCT GTGGATTGCTATG-3′	5′-ACGTTGGATGAGTGG TGCAGAAGTCTTGAG-3′	5′-GATCGGTGGATTGCTATG ATTATGATG-3′
rs2040657	5′-ACGTTGGATGCAAACC TGTTGGGTCATTAC-3′	5′-ACGTTGGATGTCAGA AATGGACCCAAACAC-3′	5′-GGTTTTCTCATTGACCTG AGATGCTAT-3′
rs10263111	5′-ACGTTGGATGGAATGT TTGCTTGGGTACTC-3′	5′-ACGTTGGATGTTTTAC TTGACAATGGCACG-3′	5′-TGGTTTCTCCTGAGTGCATA-3′
rs12386756	5′-ACGTTGGATGTTTGAA CCCAGGAGGTAGAG-3′	5′-ACGTTGGATGTTATA GGTGTGAACCACTGC-3′	5′-GGTAGAGGATGGCACCAC-3′

Table 2. Demographic and Clinicopathological Characteristics of Study Population

Variables	*Case* (n = *1583*)	*Control* (n = *1792*)	*t* (χ^2)	p *value*
Age (years)	49.45 ± 9.72	49.57 ± 9.40	–0.385	0.700
Gender				
Male	799 (50.5)	899 (50.2)	0.032	0.863
Female	784 (49.5)	893 (49.8)		
BMI (kg/m^2)	27.19 ± 3.18	21.89 ± 2.72	52.173	<0.001
Waist circumference (cm)	91.32 ± 8.35	75.06 ± 7.06	61.260	<0.001
Hip circumference (cm)	99.81 ± 6.31	90.75 ± 5.91	42.761	<0.001
HR (beat/min)	78.93 ± 11.11	74.15 ± 10.94	12.541	<0.001
SBP (mm Hg)	144.38 ± 18.94	120.63 ± 15.91	39.569	<0.001
DBP (mm Hg)	87.79 ± 10.91	74.71 ± 9.58	37.068	<0.001
TG (mg/dL)	3.24 ± 2.64	1.09 ± 0.55	33.670	<0.001
TC (mg/dL)	5.26 ± 1.24	4.72 ± 0.92	14.564	<0.001
LDL-C (mg/dL)	3.08 ± 0.97	2.85 ± 0.80	7.541	<0.001
HDL-C (mg/dL)	1.16 ± 0.29	1.62 ± 0.38	–39.357	<0.001
Fasting glucose (mg/dL)	6.65 ± 2.45	4.86 ± 1.05	28.018	<0.001

Data are shown as mean ± SD or frequency (% subjects); *p* values were analyzed using Student's *t*-test or nonparametric test.
BMI, body–mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, total cholesterol; TC, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

the case and control groups. To assess the association between SNPs and MetS, odd ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional binary logistic regression analysis, and the linkage disequilibrium coefficients were calculated using Haploview 4.2 software. The Akaike information criterion was applied to select the best model of inheritance for each SNP. SNP Stats program (http://bioinfo.iconcologia.net/SNPStats) (Sole *et al.*, 2006) was applied for conducting the haplotype analysis: the most frequent haplotype was considered as the reference group. The association of SNP with clinical and biochemical characteristics was assessed using one-way ANOVA or nonparametric test. A $p < 0.05$ was deemed as statistically significant.

Results

Demographic characteristics and clinical and biochemical features of this study population

Table 2 displays the demographic characteristics of the study population. Table 2 also summarizes the clinical and biochemical features of this study population. Specifically, we recruited 1583 patients with metabolic syndrome and 1792 control subjects in this study. These subjects were in the age group of 25–74 years. In the case and control groups, there was no significant difference in the age ($p = 0.700$) or gender ($p = 0.863$). However, the level of HDL-C was obviously lower in the case group than that in the control group ($p < 0.001$). Moreover, compared with the control group, the levels of other parameters were significantly higher in the case group. These other parameters included body–mass index (BMI), waist circumference, hip circumference, heart rates (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TG), triglycerides (TC), low-density lipoprotein cholesterol (LDL-C), and fasting glucose levels ($p < 0.001$). Especially, our current data showed that HR is different between case and control, which is consistent with previous studies (Shigetoh *et al.*, 2009; Assoumou *et al.*, 2010; Gomez-Marcos *et al.*, 2015). This may be caused by damage in the target organs and sympathetic nervous system of MetS patients. Obesity may be another inducement (Martini *et al.*, 2001; Longo-Mbenza *et al.*, 2007).

Differences in allele and genotype frequencies of case and control groups

We performed the Hardy–Weinberg equilibrium test on subjects belonging to the case and control groups. With the

Table 3. Allele Frequency and Its Association in Case and Control Groups

							95% CI	
SNP	*Allele*	*Case*	*Control*	χ^2	p	*OR*	*Lower*	*Upper*
rs1981529	T	2779	3149	0.012	0.914	0.992	0.857	1.148
	C	387	435					
rs2040657	A	2994	3417	2.106	0.147	0.851	0.684	1.058
	T	172	167					
rs10263111	C	2519	2871	0.307	0.580	1.034	0.918	1.165
	G	647	713					
rs12386756	G	1202	1339	0.262	0.608	1.026	0.930	1.133
	A	1964	2245					

OR, odds ratio; CI, confidence interval.

Table 4. The Association and Distribution of Genotype in Case and Control Groups

SNP	Genotype	Case	Control	χ^2	p	OR	95% CI	
							Lower	Upper
rs1981529	TT	1227	1387	0.440	0.802	1.000	—	—
	CT	325	375			1.021	0.864	1.207
	CC	31	30			0.856	0.515	1.423
rs2040657	AA	1416	1626	4.265	0.119	1.000	—	—
	AT	162	165			0.887	0.706	1.114
	TT	5	1			0.174	0.020	1.493
rs10263111	CC	1012	1156	0.517	0.772	1.000	—	—
	GC	495	559			0.531	0.639	1.259
	GG	76	77			1.012	0.837	1.172
rs12386756	GG	481	561	6.698	0.035	1.000	—	—
	GA	240	217			0.775	0.622	0.966
	AA	862	1014			0.912	0.866	1.174

help of this test, we assessed the genotype distribution of the *STEAP4* gene in subjects belonging to both case and control groups. We found that all SNPs of this gene were in accordance with Hardy–Weinberg equilibrium, except rs12386756, whose genotype frequency did not conform to the HWD test. Then, we randomly selected 10% samples from the control group to repeat genotyping of rs12386756, but the result remained the same.

As shown in Table 3, while comparing the allele distribution of these four *STEAP4* SNPs (rs1981529, rs2040657, rs10263111, and rs12386756) using the χ^2 test, we found no statistical difference in the case and control groups. The genotypic frequencies of these four SNPs of the *STEAP4* gene indicated that rs12386756 significantly increased the risk of developing a metabolic syndrome ($p=0.035$), but the other three SNPs (rs1981529, rs2040657, and rs10263111) did not show any statistical difference in genotype distribution in both the case and control groups (Table 4). Then, we performed recessive and dominant model analyses of genotype frequency by comparing the case and control groups; we found that the inheritance model for rs1981529, rs2040657, and rs10263111 is recessive, while the inheritance model for rs12386756 is overdominant. Compared with the AA/GG genotypes, the GA genotype of rs12386756 showed a significantly lower risk of developing a metabolic syndrome (adjusted OR = 0.77; 95% CI, 0.63–0.94; $p=0.0098$) (Table 5).

The data on the linkage disequilibrium analysis are shown in Figure 1. It was considered that $0.1<r^2<0.7$ is in moderate linkage disequilibrium, while $r^2<0.1$ is in limited linkage disequilibrium (Meyer *et al.* 2011). Stratified by the r^2, we calculated our data and defined that rs1981529 and rs10263111 ($r^2=0.535$), rs1981529 and rs12386756 ($r^2=0.218$), and rs10263111 and rs12386756 ($r^2=0.401$) were all considered as in moderate linkage disequilibrium, whereas other SNPs were in limited linkage disequilibrium. Table 6 illustrates that there was no significant difference in the distribution of haplotype structures of both case and control groups.

Association of genotype frequency with clinical and biochemical characteristics of individuals with MetS

We associated genotype frequency of these four tag SNPs with clinical and biochemical characteristics of individuals with metabolic syndrome (Table 7). We found that *STEAP4* SNP rs1981529 was associated with BMI (CC < CT < TT, $p=0.021$), waist circumference (CC < CT < TT, $p=0.024$), and SBP (CT < TT < CC, $p=0.042$), while the SNP rs10263111 was associated with waist circumference (GG < CG < CC, $p=0.038$) and fasting glucose levels (GG < CG < CC, $p=0.023$) of individuals with MetS. Furthermore, SNP rs12386756 was associated with waist circumference (GG < GA < AA, $p=0.024$) and hip circumference (GG < GA < AA, $p=0.014$) of subjects with MetS. However, there was no association between SNP rs2040657 and clinicobiochemical characteristics of subjects with MetS ($p>0.05$).

Discussion

Previous studies have proved that altered expression of a gene or other alterations play an important role in the

Table 5. Genotype Distribution and Odds Ratio Estimate

SNP	Genotype	Inheritance model	Case	Control	OR (95% CI)	p
rs1981529	T/T-C/T	Recessive	1552 (98%)	1762 (98.3%)	1.00	0.540
	C/C		31 (2%)	30 (1.7%)	0.85 (0.51–1.41)	
rs2040657	A/A-A/T	Recessive	1578 (99.7%)	1791 (99.9%)	1.00	0.064
	T/T		5 (0.3%)	1 (0.1%)	0.18 (0.02–1.51)	
rs10263111	C/C-C/G	Recessive	1507 (95.2%)	1715 (95.7%)	1.00	0.480
	G/G		76 (4.8%)	77 (4.3%)	0.89 (0.64–1.23)	
rs12386756	A/A-G/G	Overdominant	1343 (84.8%)	1575 (87.9%)	1.00	0.0098
	G/A		240 (15.2%)	217 (12.1%)	0.77 (0.63–0.94)	

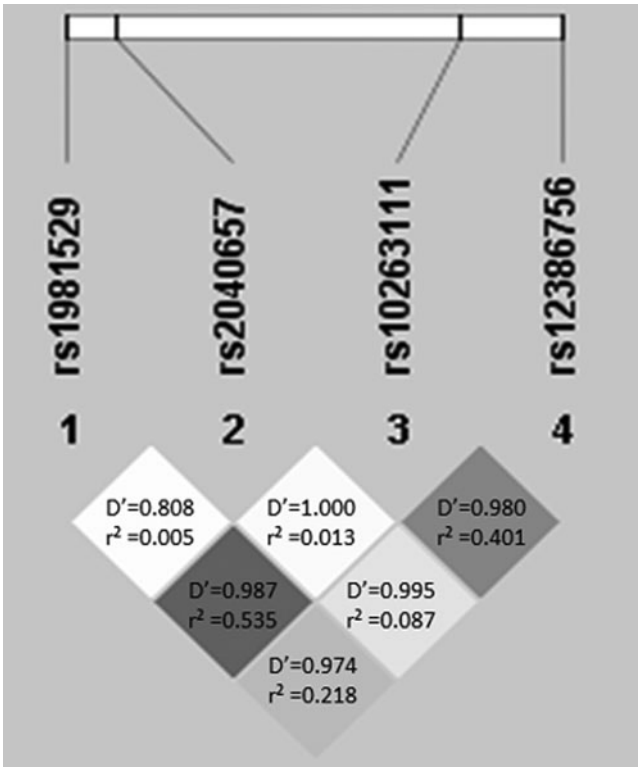

FIG. 1. Linkage disequilibrium analysis of these SNPs.

development of metabolic syndrome in human beings (Edwards *et al.*, 2008; Duvnjak and Duvnjak, 2009; Narvaez *et al.*, 2013; Kolehmainen *et al.*, 2015). Previous studies have indicated that the *STEAP4* gene plays a pivotal role in regulating obesity and insulin resistance (Wellen *et al.*, 2007; Chen *et al.*, 2010). Therefore, research studies that investigate the alterations of the *STEAP4* gene could help us in understanding that these alterations increase the risk of developing a metabolic syndrome in subjects. In this study, to determine how alterations in *STEAP4* SNPs increased the risk of developing metabolic syndrome in human beings, we genotyped four tag SNPs (rs1981529, rs2040657, rs10263111, and rs12386756) that cover the whole *STEAP4* gene. Based on our data, we inferred that *STEAP4* rs12386756 has a lower risk of developing metabolic syndrome in this Han Chinese population. However, the other three SNPs of the *STEAP4* gene did not exhibit such an association. Furthermore, we also associated SNPs of the *STEAP4* gene with clinical and biochemical characteristics of individuals with MetS. We found that SNP rs1981529 of the *STEAP4* gene was associated with BMI, waist circumference, and SBP, while SNP rs10263111 was associated with waist circumference and fasting glucose levels of subjects with MetS. Furthermore, SNP rs12386756 was associated with waist and hip circumferences of subjects with MetS. Based on these findings, we can conclude that further study of the *STEAP4* gene could help us in understanding the pathogenesis of metabolic syndrome in human beings. Thus, scientists would then be able to develop a novel strategy for controlling metabolic syndrome.

In this study, SNP rs12386756 is inherited by an overdominant model. Moreover, we have found for the first time that SNP rs12386756 caused the risk of developing metabolic syndrome in subjects; this finding has not been reported in previous studies. In our study, we found that subjects with rs12386756 GA genotype had a 0.77-fold lower risk of developing a metabolic syndrome than those with the AA/GG genotype, indicating that heterozygote genotype GA is a protective genotype in MetS. However, a previous study conducted by Zhang *et al.* (2013) showed no association between rs12386756 and MetS, whereas another study reported by Miot *et al.* (2010) showed that two parameters in the MetS, including triglyceride and fasting glucose levels, had an association with rs12386756 genotype. Although we do not know the factors contributing to this discrepancy, the sample size could be one of the factors causing this discrepancy in the results of the two research studies. SNP rs12386756 is localized in intron region of the *STEAP4* gene, so further studies must be conducted to determine whether rs12386756 genotype affects the expression of the *STEAP4* gene.

Furthermore, our current data indicate that allele and genotype frequencies of rs1981529, rs2040657, and rs10263111 did not cause MetS in this cohort of individuals. This result was in good agreement with that of Miot *et al.* (2010), but contradicted the result of the study conducted by Nanfang *et al.* (2010). Nanfang *et al.* have reported that rs1981529 is significantly associated with MetS phenotype in females of Uygur population. Their data indicate that people with different genders and nationalities may have different lifestyles, so research studies conducted on different nationalities may have contradictory results.

In this study, we found that T allele carriers of rs1981529 are associated with a high BMI and waist circumference in subjects. On the other hand, CC carriers exhibit the highest level of association with SBP. In other words, T allele may be a risk factor for higher BMI and waist circumference, but it may be a protective factor for SBP. SNP rs10263111 C allele is associated with an increase in waist circumference and

Table 6. Associations Between *STEAP4* Haplotypes and the Risk of MetS

					Frequency				
	rs1891529	*rs2040657*	*rs10263111*	*rs12386756*	*Total*	*Case*	*Control*	*OR (95% CI)*	p
1	T	A	C	A	0.6205	0.6177	0.6229	1.00	—
2	T	A	C	G	0.1271	0.1235	0.1304	1.13 (0.96–1.33)	0.14
3	C	A	G	G	0.1191	0.1196	0.1186	0.95 (0.81–1.10)	0.48
4	T	A	G	G	0.0799	0.0821	0.0780	0.90 (0.75–1.09)	0.28
5	T	T	C	G	0.0496	0.0537	0.0460	0.81 (0.65–1.02)	0.08
Rare	*	*	*	*	0.0038	0.0034	0.0041	1.20 (0.48–3.01)	0.70

Rare: haplotype frequencies <0.01.
*: six other rare haplotypes.

Table 7. Association of SNPs with Clinical and Biochemical Characteristics

rs1981529	*TT*	*CT*	*CC*	*F*	p
BMI (kg/m^2)	27.27 ± 3.20	27.04 ± 3.07	25.76 ± 3.10	3.886	0.021
Waist circumference	91.57 ± 8.54	90.62 ± 7.50	88.31 ± 8.52	3.728	0.024
SBP (mm Hg)	144.24 ± 18.94	144.10 ± 18.56	152.84 ± 21.45	3.168	0.042
rs10263111	*CC*	*CG*	*GG*	*F*	p
Waist circumference	91.61 ± 8.62	91.03 ± 7.84	89.23 ± 7.53	3.270	0.038
Fasting glucose (mg/dL)	6.75 ± 2.60	6.50 ± 2.19	6.10 ± 1.62	3.769	0.023
rs12386756	*GG*	*GA*	*AA*	*F*	p
Waist circumference	90.49 ± 7.61	91.27 ± 8.48	91.79 ± 8.67	3.739	0.024
Hip circumference	99.14 ± 6.26	99.71 ± 6.31	100.20 ± 6.42	4.253	0.014

fasting glucose levels, indicating that C allele is a risk factor for developing larger waist circumferences and higher fasting glucose levels. SNP rs12386756 A allele is a risk factor for developing larger waist and hip circumferences. These data are novel and have not been reported in previous studies, so further studies must be conducted to verify these data. Moreover, studies must also investigate the underlying mechanisms of these risk factors that cause MetS. Since the three SNPs of the *STEAP4* gene were associated with the risk of developing a larger waist circumference in subjects, we concluded that the *STEAP4* gene causes the development of metabolic syndrome. The reason why some of these SNPs associated with altered clinical or chemical parameters, but not with the risk of the syndromes, is not clear. MetS is a complex disease with a cluster of metabolic abnormalities and several different conditions of diagnosis, the interactions of several conditions or some unclear factors might be the reason; thus, further study is needed to confirm our current data.

However, this cohort of subjects belonged to the Han population in northern China, so their living conditions, traditions, customs, lifestyle, and some other demographic characteristics are unique; these demographic characteristics would be different in people belonging to other races. For example, most food preparations of the Han Chinese population contain excessive salt (Gu *et al.*, 2014). Thus, these demographic characteristics of the Han Chinese population could be considered as a limitation of our study. Moreover, we only assessed SNPs of the *STEAP4* gene to determine their association with metabolic syndrome, which is actually a disorder of energy utilization and storage. So, metabolic syndrome could be associated with different risk factors and conditions. Therefore, further studies must be conducted to elucidate the mechanism through which metabolic syndrome develops in human beings. However, more functional annotation for these SNPs such as eQTL, if such data become available, and investigation of the gene pathway for MetS will be excavated in future studies. These studies will further confirm our current data and therefore the need to develop a genetic test for individuals with a high risk of MetS.

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Author Disclosure Statement

No competing financial interests exist.

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