Cross-talk between ANGPTL4 gene SNP rs1044250 and weight management is a risk factor of Mets

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Original investigation

Keywords: Metabolic Syndrome, Single Nucleotide Polymorphism, ANGPTL4, Weight Management

Posted Date: August 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-54841/v1

Abstract

Background

The prevalence of metabolic syndrome (Mets) is closely related to the increased incidence of cardiovascular events. Angiopoietin-like protein 4 (ANGPTL4) is contributory to the regulation of lipid metabolism, herein, may provide a target for gene-aimed therapy of Mets. This case-control study was designed to elucidate the relationship between *Angiopoietin-like protein 4* (*ANGPTL4*) gene single nucleotide polymorphism (SNP) rs1044250 and the onset of Mets, and to explore the effect of interaction between SNP rs1044250 and weight management on Mets.

Methods

We have recruited 1018 Mets cases and 1029 controls in this study. The SNP rs1044250 was detected, base-line information and Mets-related indicators were collected. A 5-year follow-up survey was carried out to track the lifestyle changes, drug treatments and changes in Mets-related indicators.

Results

ANGPTL4 gene SNP rs1044250 is an independent risk factor for increased waist circumference (OR 1.618, 95% CI [1.119–2.340]; $p = 0.011$) and elevated blood pressure (OR 1.323, 95% CI [1.002–1.747]; $p = 0.048$), the prevalence of Mets (OR 1.875, 95% CI [1.363–2.580]; $p < 0.001$) is increased. The follow-up survey shows that rs1044250 CC genotype patients with weight gain have an increased number of Mets components (M [Q1, Q3]: CC 1 (0, 1), CT + TT 0 [-1, 1]; $p = 0.021$); The interaction between SNP rs1044250 and weight management is a risk factor for increased SBP ($\beta = 0.075$, $p < 0.001$) and increased DBP ($\beta = 0.097$, $p < 0.001$), the synergistic effect is negative ($S < 1$).

Conclusion

ANGPTL4 gene SNP rs1044250 is an independent risk factor for increased waist circumference and elevated blood pressure, therefore, for Mets. Weight management that interacts negatively with *ANGPTL4* polymorphism is an essential lifestyle intervention approach for elevated blood pressure.

Introduction

With the global economy thriving, the Western diet and the sedentary living habits have been disseminated, and physical labor has been largely reduced. As a result, the prevalence of Mets has increased drastically in recent years[1]. The 2010–2012 Chinese National Nutrition and Health Survey suggested that the general prevalence of Mets has reached 24.2%, including 24.6% for men and 23.8% for women[2]. Mets is highly concerned as it doubles the risk of cardiovascular disease, while the all-cause mortality rate for Mets patients increases by 1.5 times[3]. Controlling the incidence of Mets and reducing complications is a world-wide urgent. However, current Mets guidelines recommend treatments mainly based on lifestyle interventions, including smoking cessation, Mediterranean diet, 30–60 minutes of physical exercise per day, and a minimum of 5% weight-loss goal for obese patients[4]. Though the molecular-targeted drug therapy has been implemented for a variety of diseases, there is no specific drug for Mets treatment yet[4]. New options are urgently required for Mets, especially in the field of gene-targeted therapy.

Mets is a heterogenic and multifactorial diagnosis. The whole-gene linkage analysis is failed to identify loci that correspond to functional gene[5]. The Genome-Wilde Association Study (GWAS) has inherent limitations on the minimum frequency of SNP, and the total effect of identified loci only explains a small proportion of Mets prevalence[5]. Under these circumstances, the SNP study is still the most important method for Mets gene research. It has been found that over 870 SNPs are associated with obesity[6], 477 SNPs with lipid metabolism[7], more than 200 SNPs with blood pressure[8], and around 250 SNPs with glucose tolerance[9]. However, most of these gene loci were related to single Mets components, among which the lipid metabolism-associated SNPs showed the strongest relevance to Mets[10]. Furthermore, current researches have been inadequate in gene-environmental interaction study. We expect to find a locus that participates in multiple components of Mets, and to carry out a research on the interaction of gene polymorphism and environments.

As universally acknowledged, lipid metabolism, especially triglyceride (TG) metabolism plays a central role in Mets pathogenesis[11]. TG elevation is one of the components of Mets and a risk factor for abdominal obesity[12], and the synthesis of TG is associated with glucose metabolism through the tricarboxylic acid cycle[13]. Consequently, the gene locus featured by TG regulation is expected to become the target of Mets gene-directed therapy. Lipoprotein lipase (LPL) regulates TG hydrolysis in circulation and in adipose tissue. Angiopoietin-like protein 4 (ANGPTL4) is characterized by a reversible inhibitor LPL[14]. It has been reported that ANGPTL4 is not only involved in the regulation of blood lipids[14], but also blood pressure[15, 16], glucose tolerance[17], and that the circulating ANGPTL4 content is closely related to fasting and physical exercise[18]. Therefore, the *ANGPTL4* gene is considered feasible for the gene-environment interaction study.

ANGPTL4 gene that encodes the expression of ANGPTL4 locates on human chromosome 19. The N-terminal oligomerized ANGPTL4 plays an important role in the regulation of circulating TG levels by mediating LPL inhibition[19]. It has been reported that the *ANGPTL4* gene is related to obesity and weight management[18]. ANGPTL4 involves in the regulation of body weight by white adipose tissue (WAT) decomposition[20], and it participates in the up-regulation of circulating free fatty acids (FFA) during fasting and exercise[21]. In addition, the C-terminus of ANGPTL4 interacts with extracellular matrix receptors through an N-linked glycan chains, which selectively prevents the activation of the Raf/MEK/ERK1/2MAP cascade in endothelial cells, therefore, inhibits the process of neovascularization[15]. In regard to the multifunctional characteristic of ANGPTL4, we speculate that the *ANGPTL4* gene may become a potential target for Mets gene-directed study.

We selected a missense mutation rs1044250 (6959C > T, T266M) located on the highly conserved sequence encoding the C-terminus of ANGPTL4. Using case-control and follow-up studies, we investigated the impact of SNP rs1044250 on the risks for Mets and its components, afterward, we analyzed the effect of interactions between lifestyle intervention and SNP rs1044250 on Mets. We speculate that the study of *ANGPTL4* gene polymorphism and lifestyle management is potentially helpful to elucidate the susceptibility of Mets, and may provide a feasible way for Mets intervention.

Methods

Participants in this study were Han population from Shandong Province, China, surveyed from January to December 2007. Blood samples were collected and Mets-related body indicators were measured by qualified investigators. Medical history and basic information of the study population were collected in the form of questionnaires. The diagnostic criteria of Mets referred to the jointly established diagnose of IDF and AHA/NHLBI in 2009. Participants were divided into control group and Mets group according to the diagnostic criteria. Exclusion criteria included:

heart valve disease, and malignant tumors. Patients with

missing key information in questionnaire, invalid blood test indicators or genotype results were excluded. Eventually, a total of 1029 subjects in the control group and 1018 in the Mets group were included in this study. Of all the participants, 202 controls and 831 Mets cases participated in a 5-year follow-up survey.

Basic data collection and laboratory examination: The questionnaire, physical examination and blood sample collection in the cross-section and follow-up surveys were all conducted using standard protocols. The questionnaire included detailed information on previously diagnosed diseases and medication history. Height, weight and waist circumference (WC) were measured in person by qualified surveyors. Omron HEM-7011 electronic sphygmomanometer (Omron, Dalian, China) was used to measure the blood pressure on the right arm after a 5-minute rest in sitting position, three consecutive readings of each individual were recorded and the averages were calculated. Blood samples of participants were collected after overnight-fasting, Mets related indicators were detected and the DNA extraction were performed in a standard laboratory. Beckman Coulter LX20 chemical analyzer (Beckman Coulter, Brea, CA) was used to determine the blood glucose. Genomic DNA was extracted using the blood DNA extraction kit D3133-03 (Magen, Guangzhou, China) according to the instructions. The genotype of rs1044250 was detected by the Sequenom MassArray genotyping system (Sequenom, San Diego, CA). Agarose gel electrophoresis was used to determine the quality of DNA extraction when the genotyping was failed.

Statistical analysis: χ^2 goodness-of-fit test is used to test the Hardy-Weinberg equilibrium at rs1044250 locus. Continuous variables are presented in $\bar{x} \pm S$ and compared by independent-samples t test or one-way analysis of variances. Categorical variables are presented in proportions and compared by χ^2 test, χ^2 is calibrated by Bonferroni when the minimum sample size is lower than 5. Counting variables are presented in Median [Quartile1, Quartile3] (M [Q1, Q3]) and analyzed by Nonparametric test. Multi-factor Logistic regression is used to analyze the risk factors for Mets and its components. The differentials in laboratory indicators after 5-year follow-up are calculated by subtracting the cross-section value from the follow-up value, differentials are recorded in "Δ". Multiple stepwise linear regression is used to analyze the effects of gene polymorphism on the number of Mets component and on changes of systolic blood pressure (SBP), diastolic blood pressure (DBP) and high-density lipid-c (HDL-c). Ordinal Logistic regression is used to elucidate the impact of gene polymorphism on the number changes of Mets components. Crossover analysis is applied to analyze the interaction between two independent variables on Mets. All statistical analysis is operated using SPSS 26.0 (Chicago, Illinois SPSS). A two-tailed *p* value of less than 0.05 is considered to be statistically significant.

Results

Basic characteristics of the control group and the Mets group

Participants in the control group (n = 1029) and the Mets group (n = 1018) are matched by gender and age, differences of the weight, WC, body mass index (BMI), SBP, DBP, triglyceride (TG), total cholesterol (TC), low-density lipid-c (LDL-c), HDL-c, fasting plasma glucose (FPG) levels between the Control group and the Mets group are statistically significant ($p < 0.001$, Table 1). Frequencies of the C allele and the T allele on rs1044250 locus account for 92.1% and 7.9% respectively in the study population. Distribution of CC, CT, TT genotypes in the control group ($\chi^2 = 0.02$; $p = 0.99$), the Mets group ($\chi^2 = 1.48$; $p = 0.48$), and the study population ($\chi^2 = 1.58$; $p = 0.45$) are followed with Hardy -Weinberg genetic balance.

Table 1
Baseline characteristics of Control group and Mets group

	Controls (n = 1029)	Mets (n = 1018)	*p*
Male %	48.5	46	0.253
Age (years)	51.29 ± 9.78	51.64 ± 9.80	0.425
Weight (kg)	58.33 ± 8.30	72.60 ± 11.08	< 0.001
WC (cm)	75.68 ± 6.10	91.11 ± 8.46	< 0.001
BMI (kg/m^2)	22.13 ± 3.46	27.32 ± 3.46	< 0.001
SBP (mmHg)	119.69 ± 9.90	148.50 ± 19.95	< 0.001
DBP (mmHg)	74.66 ± 6.44	90.53 ± 10.45	< 0.001
TC (mmol/L)	4.17 ± 0.53	4.68 ± 1.18	< 0.001
TG (mmol/L)	0.93 ± 0.31	2.32 ± 1.57	< 0.001
HDL-c (mmol/L)	1.59 ± 0.34	1.49 ± 0.52	< 0.001
LDL-c (mmol/L)	2.58 ± 0.52	3.19 ± 0.82	< 0.001
FPG (mmol/L)	4.59 ± 0.51	5.96 ± 2.11	< 0.001

Continuous variables are recorded by $\bar{x} \pm S$; categorical variables are recorded in proportions; Mets, metabolic syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-c; LDL-c, low-density lipoprotein-c; FPG, fasting plasma glucose.

Risks of the SNP rs1044250 on Mets and the components of Mets

The laboratory indicators among patients with CC, CT and TT genotypes at ANGPTL4 gene rs1044250 locus are compared respectively, the result indicates that the baseline data among genotypes in the control group do not show difference ($p > 0.05$, Supplemental Table S1), while in the Mets group, HDL-c is significantly higher in patients with TT genotype than patients with CC or CT genotypes ($p < 0.05$, Supplemental Table S1).

The frequencies of rs1044250 genotypes are significantly different between the control group and the Mets group ($\chi^2 = 25.556$; $p < 0.001$, Supplemental Table S2), and the frequency of T alleles in the control group is significantly lower than that in the Mets group ($\chi^2 = 25.991$; $p < 0.001$, Supplemental Table S2). The study population is subdivided into groups respectively according to the presence or absence of each of the five components of Mets. The frequencies of CC, CT, TT genotypes as well as the frequencies of C allele and T allele are significantly different between the normal WC group and the increased WC group, statistical significances were also found between the normal TG group and the elevated TG group, the normal blood pressure group and the elevated blood pressure group, the normal FPG group and the elevated FPG group ($p < 0.05$, Supplemental Table S2).

Multi-factor stepwise Logistic regression analysis shows the SNP rs1044250 is an independent risk factor for metabolic syndrome (OR 1.875 [95% CI 1.363–2.580]; $p < 0.001$, Table 2). Accordingly, the five components of Mets are studied respectively, the SNP rs1044250 is an independent risk factor for increased WC (OR 1.618 [95% CI 1.119–

2.340]; $p = 0.011$, Table 2) and increased blood pressure (OR 1.323 [95% CI 1.002–1.747]; $p = 0.048$, Table 2). In addition, patients with various rs1044250 genotypes are significantly different in the numbers of Mets components (M [Q1, Q3]: CC 2 [0, 3], CT 3 [0, 3], TT 3 [3, 3]; $p = 0.001$). Multivariate linear regression analysis confirms that the SNP rs1044250 (β = 0.044; $p = 0.007$), age (β = 0.068; $p < 0.001$), weight (β = 0.221; $p < 0.001$), BMI (β = 0.387; $p < 0.001$) and LDL-c (β = 0.200; $p < 0.001$) are independent risk factors for the increased number of Mets components carried by patients.

Table 2
Logistic regression analysis of Mets and Mets components

	Independent variables	β	*p*	OR	95% CI	
					Lower	Upper
Mets						
	Sex (male vs female)	-0.812	< 0.001	0.444	0.313	0.630
	Age (years)	0.019	0.009	1.019	1.005	1.034
	Weight (kg)	0.081	< 0.001	1.085	1.055	1.115
	BMI (kg/m^2)	0.351	< 0.001	1.420	1.308	1.541
	LDL-c (mmol/L)	1.210	< 0.001	3.355	2.699	4.171
	rs1044250	0.629	< 0.001	1.875	1.363	2.580
Increased WC						
	Sex (male vs female)	-2.367	< 0.001	0.094	0.059	0.149
	Age (years)	0.033	< 0.001	1.034	1.015	1.053
	Weight (kg)	0.104	< 0.001	1.110	1.073	1.148
	BMI (kg/m^2)	0.265	< 0.001	1.303	1.185	1.434
	TG (mmol/L)	0.328	< 0.001	1.388	1.225	1.573
	HDL-c (mmol/L)	-0.372	0.029	0.689	0.493	0.963
	SBP (mmHg)	0.046	< 0.001	1.047	1.033	1.060
	DBP (mmHg)	0.068	< 0.001	1.070	1.047	1.094
	rs1044250	0.481	0.011	1.618	1.119	2.340
Elevated BP						
	Age (years)	0.035	< 0.001	1.036	1.024	1.048
	BMI (kg/m^2)	0.327	< 0.001	1.387	1.338	1.438
	FPG (mmol/L)	0.308	< 0.001	1.361	1.237	1.497
	LDL-c (mmol/L)	0.535	< 0.001	1.708	1.439	2.028
	rs1044250	0.280	0.048	1.323	1.002	1.747
Increased TG						
	BMI (kg/m^2)	0.095	< 0.001	1.099	1.051	1.150
	WC (cm)	0.065	< 0.001	1.067	1.048	1.086

Mets, metabolic syndrome; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-c; LDL-c, low-density lipoprotein-c; WC, waist circumference; FPG, fasting plasma glucose; rs1044250, (CC = 0, TC = 1, TT = 2); OR, odds ratio; 95% CI, 95%

	Independent variables	β	*p*	OR	95% CI	
					Lower	Upper
	TC (mmol/L)	0.708	< 0.001	2.030	1.778	2.319
	FPG (mmol/L)	0.146	< 0.001	1.158	1.079	1.242
	DBP (mmHg)	0.049	< 0.001	1.050	1.038	1.062
	rs1044250	0.046	0.752	1.047	0.786	1.395
Decreased HDL-c						
	Sex (male vs female)	0.491	< 0.001	1.634	1.332	2.004
	WC (cm)	0.028	< 0.001	1.028	1.016	1.040
	DBP (mmHg)	0.022	< 0.001	1.022	1.012	1.032
	rs1044250	-0.078	0.532	0.925	0.723	1.182
Increased FPG						
	WC (cm)	0.046	< 0.001	1.048	1.034	1.062
	TC (mmol/L)	0.647	< 0.001	1.911	1.601	2.281
	TG (mmol/L)	0.140	0.002	1.151	1.054	1.256
	HDL-c (mmol/L)	-0.447	0.016	0.640	0.444	0.921
	SBP (mmHg)	0.013	0.001	1.013	1.005	1.021
	DBP (mmHg)	0.018	0.011	1.018	1.004	1.032
	rs1044250	0.126	0.361	1.135	0.866	1.487

Mets, metabolic syndrome; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-c; LDL-c, low-density lipoprotein-c; WC, waist circumference; FPG, fasting plasma glucose; rs1044250, (CC = 0, TC = 1, TT = 2); OR, odds ratio; 95% CI, 95% confidence interval.

Baseline Characteristic Changes Of The Study Population

Compared with the control group, the weight, WC, BMI, SBP, DBP, TG, TC, LDL-c and FPG of the Mets group are significantly reduced in 5 years ($p < 0.05$, Table 3). In the Mets group, HDL-c level is significantly elevated in patients with TT genotype than that in CC genotype or CT genotype patients ($p < 0.05$, Table 3). Linear regression analysis shows that the SNP rs1044250 (β = -0.065; $p = 0.017$), Sex (β: = -0.069; $p = 0.012$) and TG (β = -0.611; $p < 0.001$) are independent risk factors for HDL-c reduction in patients with Mets in the 5-year follow-up survey.

Table 3
Baseline characteristics changes in 5-year follow-up survey of various rs1044250 genotypes in the Control group and the Mets group

	Control			Mets			
	CC (n = 174)	CT + TT (n = 28)	Total (n = 202)	CC (n = 667)	CT (n = 151)	TT (n = 13)	Total (n = 202)
Sex (male%)	47.7%	57.1%	49.0%	47.5%	39.7%	30.8%	45.9%
Age (years)	51.64 ± 9.30	51.07 ± 10.51	51.56 ± 9.45	51.42 ± 9.68	52.47 ± 10.20	54.00 ± 10.57	51.65 ± 9.79
ΔWeight (kg)	-0.31 ± 1.64	-0.52 ± 1.66	-0.37 ± 1.64	-2.11 ± 3.11	-1.98 ± 2.78	-1.90 ± 1.50	-2.08 ± 3.03‡
ΔBMI (kg/m^2)	0.09 ± 0.86	-0.02 ± 0.65	0.07 ± 0.84	-0.55 ± 1.08	-0.51 ± 1.00	-0.46 ± 0.63	-0.54 ± 1.06‡
ΔWC (cm)	-0.54 ± 3.02	-0.98 ± 2.24	-0.60 ± 2.92	-4.37 ± 3.36	-3.91 ± 3.08	-4.27 ± 3.45	-4.28 ± 3.31‡
ΔSBP (mmHg)	1.07 ± 6.79	0.17 ± 5.53	0.95 ± 6.62	-7.16 ± 13.39	-6.90 ± 11.75	-4.13 ± 8.09	-7.06 ± 13.04‡
ΔDBP (mmHg)	-0.27 ± 2.53	0.38 ± 1.59	-0.18 ± 2.43	-5.46 ± 6.85	-5.17 ± 6.69	-4.97 ± 6.96	-5.40 ± 6.82‡
ΔTG (mmol/L)	0.32 ± 0.50	0.23 ± 0.58	0.31 ± 0.51	-0.84 ± 1.81	-0.70 ± 1.43	-1.02 ± 1.90	-0.82 ± 1.75‡
ΔTC (mmol/L)	0.13 ± 1.08	-0.08 ± 1.15	0.10 ± 1.09	-0.33 ± 1.31	-0.21 ± 1.33	-0.75 ± 1.61	-0.32 ± 1.32‡
ΔHDL-c (mmol/L)	-0.24 ± 0.48	-0.09 ± 0.36	-0.22 ± 0.46	-0.25 ± 0.66	-0.32 ± 0.64	-0.83 ± 1.12*†	-0.27 ± 0.67
ΔFPG (mmol/L)	0.43 ± 1.42	0.59 ± 1.34	0.45 ± 1.41	-0.12 ± 2.43	-0.38 ± 2.53	-0.10 ± 1.82	-0.17 ± 2.44‡

Continuous variables are recorded by $\bar{x} \pm S$; Categorical variables are recorded in proportions; * statistically significant compared with CC genotype in the Mets group; †statistically significant compared with CT genotype in the Mets group; ‡ statistically significant compared with the Mets group; Δ, follow-up values minus baseline values; Mets, metabolic syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-c; FPG, fasting plasma glucose.

Changes In The Number Of Mets Components

The study population is subdivided into Weight loss group (ΔWeight < 0) and Weight gain group (ΔWeight ≥ 0), the distribution of rs1044250 genotypes (fisher = 1.162; $p = 0.532$) and T alleles ($\chi^2 = 0.878$; $p = 0.349$) in these groups do not show significant difference. Among patients with CC, CT and TT genotypes at rs1044250 locus, changes of the number of Mets components do not show any difference (M [Q1, Q3]: CC 0 [-1, 1], CT 0 [-1, 1], TT 0 [-1, 1]; $p = 0.529$, Supplemental Table S2). However subgroup analysis based on weight management indicates that the number

changes of Mets components between CC genotype and CT or TT genotype in the weight gain group are significantly different (M [Q1, Q3]: CC 1 (0, 1), CT + TT 0 [-1, 1]; $p = 0.021$, Supplemental Table S3), while there is no difference showed in the weight loss group (M [Q1, Q3]: CC 0 [-1, 1], CT + TT -0 [-1, 1], $p = 0.732$, Supplemental Table S3). Indeed, under an ordinal regression model, the SNP rs1044250 (β = -0.703; $p = 0.024$), TG (β = -0.337; $p < 0.001$) and FPG (β = -0.242; $p = 0.003$) are independent protective factors for the number of Mets components when the body weight is increased, which suggests that people with CC genotype are more likely to catch-up with the number of Mets components.

Interaction Of SNP rs1044250 And Weight Management

In the weight loss group, the HDL-c level is significantly decreased in patients with TT genotype at rs1044250 locus compared with CC ($p = 0.002$, Table 4) and CT ($p = 0.008$, Table 4) genotype. In the weight gain group, the SBP ($p = 0.002$, Table 4) and DBP (Table 4; $p = 0.004$) of CC genotype are significantly higher than that of CT genotype. Accordingly, the interaction of SNP rs1044250 and weight management on SBP (F = 3.291; $p = 0.038$, Table 4), DBP (F = 3.026; $p = 0.049$, Table 4) and HDL-c (F = 6.269; $p = 0.002$, Table 4) are statistically significant.

Table 4
Factorial analysis SNP rs1044250 and weight management on clinical characteristic changes

	Weight loss			Weight gain			*p*		
	CC (n = 709)	CT (n = 146)	TT (n = 11)	CC (n = 132)	CT (n = 32)	TT (n = 3)	rs1044250	ΔWeight	Rs1044250 *ΔWeight
ΔWeight (kg)	-2.23 ± 2.81	-2.27 ± 2.68	-2.25 ± 1.35	0.94 ± 2.24	0.53 ± 0.81	0.67 ± 1.15			
ΔBMI (kg/m^2)	-0.61 ± 0.94	-0.61 ± 0.97	-0.62 ± 0.51	0.61 ± 1.16	0.36 ± 0.38	0.55 ± 0.54			
ΔWC (cm)	-4.46 ± 3.11	-4.26 ± 2.76	-5.45 ± 2.05	1.10 ± 2.33	0.20 ± 2.11	0.67 ± 2.75			
ΔSBP (mmHg)	-6.48 ± 13.12	-5.79 ± 10.81	-4.55 ± 8.55	0.04 ± 8.77	-6.12 ± 13.51‡	0.33 ± 5.86	0.113	0.191	0.038
ΔDBP (mmHg)	-5.06 ± 6.70	-4.51 ± 6.40	-5.97 ± 7.13	-0.78 ± 4.14	-3.51 ± 6.99 ‡	0.56 ± 0.84	0.285	0.007	0.049
ΔTG (mmol/L)	-0.69 ± 1.69	-0.58 ± 1.39	-1.33 ± 1.91	-0.14 ± 1.64	-0.48 ± 1.33	0.79 ± 0.31			
ΔTC (mmol/L)	-0.30 ± 1.28	-0.21 ± 1.36	-0.80 ± 1.74	0.07 ± 1.22	-0.17 ± 0.89	0.59 ± 1.97			
ΔHDL-c (mmol/L)	-0.25 ± 0.64	-0.31 ± 0.59	-1.07 ± 0.97*†	-0.22 ± 0.53	-0.19 ± 0.67	0.41 ± 0.83	0.889	< 0.001	0.002
ΔFPG (mmol/L)	-0.04 ± 2.32	-0.24 ± 2.47	-0.12 ± 1.98	0.16 ± 1.96	-0.21 ± 2.16	0.34 ± 0.75			

Continuous variables are recorded by $\bar{x} \pm S$; * statistically significant compared with CC genotype in the Weight loss group; †statistically significant compared with CT genotype in the Weight loss group; ‡ statistically significant compared with CC genotype in the Weight gain group; Δ, follow-up values minus baseline values; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-c; FPG, fasting plasma glucose.

The independent variable ΔWeight*rs1044250 is included into the multi-factor stepwise linear regression equations, of which the dependent variable is ΔSBP and ΔDBP respectively. Therefore, the interaction of SNP rs1044250 and weight management is an independent risk factor for elevated SBP (β = 0.075; $p < 0.001$, Table 5) and elevated DBP (β = 0.097; $p < 0.001$, Table 5). The independent variable ΔWeight*rs1044250 is excluded from the linear regression equations of ΔHDL-c (β = 0.004, $p = 0.851$, Table 5).

Table 5
Linear regressions analysis of ΔSBP, ΔDBP and ΔHDL-c

		β	*p*	R^2
ΔSBP (mmHg)				
	Antihypertensive	-0.215	0.000	0.719
	Antidiabetic	0.058	0.001	
	WC (cm)	0.135	0.000	
	BMI (kg/m^2)	0.052	0.032	
	TG (mmol/L)	0.047	0.006	
	SBP (mmHg)	-0.835	0.000	
	DBP (mmHg)	0.112	0.000	
	ΔWeight*rs1044250	0.075	0.000	
ΔDBP (mmHg)				
	Antihypertensive	-0.373	0.000	0.696
	Antidiabetic	0.048	0.007	
	WC (cm)	0.148	0.000	
	HDL-c (mmol/L)	-0.035	0.045	
	DBP (mmHg)	-0.621	0.000	
	ΔWeight*rs1044250	0.097	0.000	
ΔHDL-c (mmol/L)				
	Antidiabetic	-0.060	0.003	0.580
	WC (cm)	-0.072	0.003	
	TG (mmol/L)	-0.052	0.013	
	HDL-c (mmol/L)	-0.741	0.000	
	ΔWeight*rs1044250	0.004	0.851	

Δ, follow-up values minus baseline values; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-c, high-density lipoprotein-c; WC, waist circumference; FPG, fasting plasma glucose; rs1044250, (CC = 0, TC = 1, TT = 2).

A crossover analysis is conducted to study the interaction of ANGPTL4 gene SNP rs1044250 and weight management on ΔSBP, ΔDBP, ΔHDL-c under linear regression model. In the linear regression model with ΔSBP as the dependent variable, the independent variables SNP rs1044250 (β = 0.193; $p < 0.001$, Table 6) and rs1044250*Weight Management (β = -0.093; $p = 0.013$, Table 6) are included in the regression equation, meanwhile the interaction between SNP rs1044250 and weight management on ΔSBP is negative (synergy index = 0.558); in the linear regression model with ΔDBP as dependent variable, the SNP rs1044250 (β = 0.241; $p < 0.001$, Table 6) and

rs1044250* Weight Management (β = -0.078; p = 0.035, Table 6) are included in the regression equation, and the synergistic effect between SNP rs1044250 and weight management are negative (synergy index = 0.696).

Table 6
Crossover analysis of SNP rs1044250 and WM on ΔSBP, ΔDBP and ΔHDL-c

		β	OR	p	S
ΔSBP					
	WM	0.024	1.024	0.477	0.558
	Dom	0.193	1.212	0.000	
	Dom*WM	-0.093	0.911	0.013	
ΔDBP					
	WM	0.027	1.027	0.429	0.696
	Dom	0.241	1.273	0.000	
	Dom*WM	-0.078	0.925	0.035	
ΔHDL-c					
	WM	-0.066	0.936	0.053	0.106*
	Dom	0.017	1.017	0.620	
	Dom*WM	0.054	1.056	0.154	

SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-c, high-density lipoprotein-c; WM, Weight loss = 0, Weight gain = 1; Dom, CC = 0, CT + TT = 1, OR, odds ratio; S, synergy index; S = [EXP(β_{WM} + β_{Dom} + β_{Dom*WM}) -1] / [EXP(β_{WM}) + EXP(β_{Dom}) − 2], * the denominator is calibrated from negative to positive

Discussion

This is the first study that comprehensively identifies the *ANGPTL4* gene SNP rs1044250 as an independent risk factor for Mets by increasing the carriers' WC and blood pressure. The number of Mets components in CC genotype individuals increase when body weight is poorly managed. Consistently the SNP rs1044250 and weight management are negatively correlated on the effect of blood pressure.

In 2007, a GWAS study found that fat mass and obesity-associated protein (FTO) polymorphism was associated with weight gain and increased BMI[22]. It was the first GWAS study which ushered in the era of gene SNP research on Mets. Since then, the effects of gene polymorphisms have been related to obesity[6], lipid metabolism[7], glucose tolerance and hypertension[8, 9]. Because of the heterogeneity and multifactorial nature of Mets, current SNP studies have been limited on single Mets components, the effects of multifunctional gene loci on Mets are seldom reported. Under this circumstance, various studies have suggested that ANGPTL4 is involved in the regulation of multiple components of Mets, including lipid metabolism[14], obesity[20], blood pressure and glucose tolerance[15–17].

Regarding the multifunctional feature, the major role of ANGPTL4 is regulating the TG content in circulation and maintaining the balance of adipopexis in WAT[23]. The N-terminal oligomerized ANGPTL4 in circulation inhibits LPL activity, therefore, increases the circulating TG level[2], while the SNP rs1044250 mainly alters the activity of

ANGPTL4 C-terminus[24]. Previous study has reported that the SNP rs1044250 only accounts for 0.8% of patients with decreased serum TG, and the TG reducing effect of SNP rs1044250 will not be significant after the impact of ANGPTL4 N-terminus related SNP rs110843064 (E40K) is excluded[2]. Accordingly, our result suggests that the rs1044250 polymorphism do not have independent effect on circulating TG.

This study proves that the SNP rs1044250 is an independent risk factor for increased WC. Preciously, it has been reported that overexpression of purified ANGPTL4 C-terminus in mouse accelerates the decomposition of WAT lipid, suggesting a lipolytic activity of ANGPTL4 C-terminal domain in fat cells independent from LPL[25]. Therefore, the SNP rs1044250 is likely to induce an increase in WC by lowering the level of WAT lipolysis. In addition, the WC and waist-to-hip ratio is increased in adipocyte ANGPTL4 knockout mice[18]. Another research has found that the ANGPTL4 knockout mice fed with high-fat diet show granuloma lesions in intestine and WAT, as well as lymphangitis and mesenteric lymphadenitis[26, 27], which suggests that ANGPTL4 is essential to the lymphatic drainage of lipids from WAT to the liver. A study has also reported that ANGPTL4 reduces appetite by inhibiting hypothalamic AMPK activity, accordingly, the ANGPTL4 knockout mice show increased appetite after fasting[28]. Regarding features of ANGPTL4 discussed above, we speculate that SNP rs1044250 causes abdominal fat accumulation and increased WC by promoting the WAT lipid recruitment, destroying the integrity of WAT-hepatic lymphoid tissue as well as reducing appetite.

The ANGPTL4 gene SNP rs1044250 is an independent risk factor for elevated blood pressure. It has been reported that the C-terminal domain of ANGPTL4 protein inhibits VEGF and bFGF-mediated angiogenesis[15], and SNP rs1044250 is capable of altering the activity of ANGPTL4 C-terminus, therefore, the SNP rs1044250 may lead to a decreased angiogenesis and dysfunctional endothelial repairment. ANGPTL4 gene knockout mice are prone to have coronary arteritis and mesenteric vasculitis when fed with a high-fat diet[29]. The dysfunction of endothelial repairment can increase vascular resistance and lead to an increased blood pressure in both direct and indirect manners. Consistently, a Finnish clinical study also has confirmed that circulating ANGPTL4 protein levels are significantly up-regulated in patients with hypertension[20]. Based on the risk of the SNP rs1044250 on WC and blood pressure, the ANGPTL4 gene polymorphism is considered a risk factor for Mets. A step forward, we attempt to cast light on the interaction between ANGPTL4 polymorphisms and lifestyle interventions, and to find a feasible way for rs1044250-targeted Mets therapy.

At present, the management of Mets is mainly based on lifestyle interventions. The body weight reflects a time superposition effect of lifestyle. Therefore, weight management has become a recommended indicator of lifestyle interventions to Mets. In 2017, the international panel recommended a minimum 5% weight loss target for obese patients[4]. Our result shows that patients with rs1044250 CC genotype are more likely to have an increased number of Mets components as body weight increased. The elevated blood pressure is considered the major cause of increased number of Mets components. Accordingly, the synergistic effect of weight management and SNP rs1044250 on blood pressure is negative, in other words, the superimposed effect of these two independent variables is less than the sum of their effects alone. Studies have shown that muscle-derived ANGPTL4 levels is increased during exercise or fasting[21], and that WAT lipolysis is positively correlated to the circulating ANGPTL4 levels[18]. We speculate the lack of exercise leads to a decrease in myogenic ANGPTL4, which in turn results in the accumulation of WAT and weight gain. Therefore, the transient decrease of ANGPTL4 level in circulation during weight gain is negatively correlated to the risk of SNP rs1044250 on blood pressure. Under this circumstance, the blood pressure of rs1044250 wild-type patients shows a catch-up effect, which may relate to a disorder of lipid metabolism.

This study elaborated on the interaction between ANGPTL4 gene SNP rs1044250 and weight management on Mets. Future studies need to clarify the effect of SNP rs1044250 on the structure of ANGPTL4 C-terminus, and to detect the effect of gene polymorphism on Raf/MEK/ERK1/2 MAP pathway. The effect of SNP rs1044250 on circulating lipid metabolism under fasting and exercise conditions also requires experimental verification.

Summary And Conclusions

In conclusion, we have found that the ANGPTL4 gene SNP rs1044250 increases the incidence of Mets in Shandong Han population by increasing blood pressure and WC. The number of Mets components in patients with CC genotype at rs1044250 locus shows a catch-up effect when the body weight is increased, while weight-loss could significantly inhibit the increase of SBP and DBP caused by SNP rs1044250.

Abbreviations

Mets, metabolic syndrome; SNP: single nucleotide polymorphism; ANGPTL4: Angiopoietin-like protein 4; WAT: white adipose tissue; LPL: lipoprotein lipase; GWAS: genome-wilde association study; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglyceride; TC: total cholesterol; HDL-c: high-density lipoprotein-c; LDL-c: low-density lipoprotein-c; WC: waist circumference; FPG: fasting plasma glucose; FTO: fat mass and obesity-associated protein

Declarations

- Ethics approval and consent to participate: this study was approved by the Ethics Committee of Cheeloo College of Medicine. All participants are aware of the usage of their data and blood samples.
- Consent for publication: not applicable.
- Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request Zhihao Wang.
- Competing interests: The authors declare that they have no competing interests.
- Funding: This work was supported by the research grants from the National Natural Science Foundation of China (81900332, 81873534, 81702194, 81600633, 81670411, 81570400, 81801953, 81470560, 81471036, 81800761), Key research and development program of Shandong Province (2019GSF108041, 2018GSF118002, 2018GSF118017, 2017GSF18156), the Natural Science Foundation of Shandong Province (ZR2019QH010, ZR2014HQ037, ZR2017BH023).
- Authors' contributions: ZT and JP wrote this manuscript, ZT, JP and HL participated in the data integration and analysis, WS, YL, JX and YT provided ideas and critical thoughts to this manuscript, WZ, MZ and ZW were the designer and reviewer of this work. ZW is responsible for the integrity of this study and has exclusive access to all the data.
- Acknowledgements: We thank all the participants for providing their information and blood samples, and the excellent work of all the staff during the questionnaire survey and sample collection is highly appreciated.

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