Research Article

Smoking modifies the effect of two independent SNPs rs5063 and rs198358 of *NPPA* on central obesity in the Chinese Han population

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Running title: Smoking, *NPPA* SNPs and obesity

Abstract.

Obesity is the third risk factor for death in middle and high income countries. Whether DNA polymorphisms in *CORIN* and *NPPA* genes were associated with obesity, and whether these associations could be modified by smoking in the Chinese Han population were unknown. A group of 1,507 participants were recruited and genotyped for 12 tag-SNPs of *CORIN* and *NPPA* genes. Regression models were used to test the associations of SNPs with obesity. The potential SNP–smoking interactions were detected in regression models. *NPPA* SNPs rs5063 and rs198358 were associated with BMI (P = 0.0053 and 0.0037, respectively). Rs198358 was associated with obesity in both univariate and multivariable-adjusted analyses (P = 0.0138 and 0.0173, respectively). Rs5063 was associated with central obesity in both univariate and multivariable-adjusted analyses (P = 0.0454 and 0.0361, respectively). Significant interactions between cigarette smoking and rs5063 and rs198358 were detected (P = 0.0019 and 0.0006, respectively). In subgroup analyses, rs5063 and rs198358 were associated with central obesity in smokers (P = 0.0081 and 0.0037, respectively). The results of our study demonstrated that the effect of *NPPA* SNPs rs5063 and rs198358 on central obesity might be modified by smoking in the Chinese Han population. Further studies are needed to confirm the associations and elucidate the underlying mechanisms.

Key words: Corin; Atrial natriuretic peptides; Obesity; Genetic association; Smoking

Introduction

Obesity is the third risk factor for death in middle and high income countries(Narayan et al. 2010). By 2030, the respective number of overweight and obese adults was projected to be 1.35 billion and 573 million individuals(Kelly et al. 2008). In China, the total number of overweight men and women will exceed the established market economies by 2030(Kelly et al. 2008). It is known that the higher the body mass index (BMI), the greater the risk of comorbidities such as diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, obstructive sleep apnea, cancers and overall mortality(Hensrud and Klein 2006). The causes of obesity have been recognized and are largely related to a genetic predisposition and an environmental susceptibility to gain weight due to increased energy intake and reduced energy expenditures(Lifshitz and Lifshitz 2014). The genetic aspects of obesity lead to mutations in various genes responsible for controlling appetite and metabolism. Over the past two decades, several strategies, including genome-wide association studies (GWAS) have been employed for the identification of genetic determinants of obesity and have identified many loci in the human genome that link with obesity(Okada et al. 2012; Ried et al. 2016; Shungin et al. 2015; Speliotes et al. 2010; Thorleifsson et al. 2009; Wahl et al. 2017; Wen et al. 2012; Wen et al. 2014; Willer et al. 2009; Yang et al. 2012). Even so, knowledge of the underlying risk factors for obesity is still limited.

The natriuretic peptide system may have a potential role in obesity. The atrial natriuretic peptides (ANP) is a cardiac hormone with potent cardiovascular and metabolic effects(Levin et al. 1998). ANP was also reported to induce a strong lipolytic effect in cultured human adipocytes with potency similar to catecholamine activation of the β-adrenergic

receptors.(Sengenes et al. 2000) The Framingham Heart Study has shown that plasma ANP level was lower in subjects with obesity and those with metabolic risk factors(Wang et al. 2007; Wang et al. 2004). Corin , a type II transmembrane serine protease found in cardiomyocytes, converts the precursor molecules of ANP into active proteins(Chan et al. 2005). Corin acts downstream of agouti gene expression as a suppressor of the agouti pathway, indicating a potential role of corin in obesity(Enshell-Seijffers et al. 2008). Our previous study has detected the association of serum soluble corin levels with obesity(Peng et al. 2015).

Genetic variants of *NPPA* gene, which encoding the ANP precursor, were associated with hypertension, stroke, coronary artery disease, heart failure and obesity(Song et al. 2015). The human *CORIN* gene was located on chromosome 4p12-13(Yan et al. 1999). *CORIN* gene variants have been identified to alter corin protein conformation and inhibited corin zymogen activation and contribute to hypertension and heart diseases(Dong et al. 2013; Dries et al. 2005; Zhou and Wu 2014). Whether genetic variants in *NPPA*, or the convertase coding gene *CORIN*, can affect risk of obesity has not been determined in the Chinese populations.

Cigarette smoking is one of the leading causes of preventable morbidity and mortality. The association between smoking and obesity has been extensively investigated in diverse populations(Chiolero et al. 2007; Mackay et al. 2013; Xu et al. 2007). Besides, the potential for smoking to influence genetic associations with obesity has also been explored(Fesinmeyer et al. 2013; Johnson et al. 2014). However, whether the associations between DNA polymorphisms in *CORIN* and *NPPA* genes and obesity could be modified by smoking in the Chinese population is unclear. We examined the associations between single nucleotide polymorphisms (SNPs) in *CORIN* and *NPPA* genes and obesity, and the interaction effect of the

SNPs with smoking on obesity in this study. Our results may lead to better understanding of the combined effects of *CORIN* or *NPPA* variants and smoking on obesity among Chinese Han individuals.

Methods

Study Population

A group of 1,507 adults were randomly selected from a large cohort study in community population of Jiangsu Province and judged to be free of hypertension, cardiovascular diseases, diabetes mellitus, renal or hepatic diseases. Standard questionnaire were used by trained interviewers to obtain data on demographic characteristics, lifestyle risk factors, personal medical history and family history of hypertension for all participants. Three sitting blood pressure (BP) measurements were taken and the mean of the three BP measurements was used in the analyses. Body weight and height were measured by using standard methods, and the BMI was calculated as the weight in kilograms divided by the square of the height in meters. The waist circumference (WC) was measured two times at 1 cm above the umbilicus at minimal respiration by trained observers with the subjects standing and breathing normally during the physical examination. In this study, overweight was defined as $24 \leq \text{BMI} < 28$ kg/m^2, obesity was defined as defined as BMI ≥ 28 kg/m^2 and central obesity was defined as WC ≥ 85 cm for men and as WC ≥ 80 cm for women based on the recommendations of the Working Group on Obesity in China(Zhou 2002). Cigarette smoking was defined as ever having smoked at least 100 cigarettes(He et al. 2007; Pierce et al. 1998). Alcohol consumption was defined as consuming any type of alcohol beverage at least 12 times during the past 1 year(Bazzano et al.

2007; Xiao et al. 2015). Written informed consent was obtained for all study participants. This study was approved by the ethics committee at Soochow University in China.

Biochemical measurements

Blood samples were collected in the morning after at least 8 hours of fasting. All plasma samples were frozen at -80 °C until laboratory testing was performed. Fasting plasma glucose (FPG) was measured using an oxidase enzymatic method. The concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were assessed enzymatically using an automatic biochemistry analyzer (Hitachi Inc, Tokyo, Japan) and commercial reagents. The Friedewald equation was used to calculate the low-density lipoprotein cholesterol (LDL-C), from the TC, HDL-C, and TG. All analyses were performed by the same lab.

DNA extraction, SNP selection and genotyping

Genomic DNA was isolated from white blood cells according to a standard procedure using a DNA extraction kit (Tiangen Biotech, Beijing, China). We selected tag-SNPs from these genes with pairwise r^2 thresholds of 0.8. Haploview software (version 4.2, http://www.broad.mit.edu/mpg/haploview) was used to conduct tag-SNP selection(Barrett et al. 2005). A total of 12 tag-SNPs were included in the current study. Detailed information on all tag-SNPs, including the chromosome position, minor allele frequency (MAF), and Hardy-Weinberg equilibrium (HWE) *P* values, has been searched and calculated. The selected tag-SNPs have been genotyped by using SNPscan technology, a custom-by-design 48-Plex

SNPscan™ Kit based on double ligation and multiplex fluorescence PCR (Cat#:G0104; Genesky Biotechnologies Inc., Shanghai, China)(Chen et al. 2012; Jin et al. 2015). This kit was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc., which was based on double ligation and multiplex fluorescence PCR. Briefly, 100-200 ng of DNA sample was first denatured at 98℃ for 5 min in a 10-mL reaction containing 1×DNA lysis buffer and then mixed well with a 10-mL ligation premix composed of 2 mL 103 ligase buffer, 0.5 mL ligase, 1 mL probe mix, and 7.5 mL Milli-Q water. The ligation reaction was carried out in an ABI2720 thermal cycler. Two 48-plex fluorescence PCR reactions were performed for each ligation product. PCR reactions were prepared in a 20- mL mixture containing 1×PCR master mix, 1 mL primer mix set A or set B, and 1 mL ligation product. PCR products were separated and detected by capillary electrophoresis in an ABI3730XL sequencer. Raw data were analyzed according to the information obtained for the labeling dye color and fragment size of the allele-specific ligation-PCR product.

Statistical analysis

The differences of mean levels of risk factors in individuals with and without overweight or obesity, as well as in participants with and without central obesity were compared, using a Student's *t*-test for continuous variables and χ^2 tests for categorical variables. Linear regression was used to test the association between the SNPs and BMI and WC. Logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) of obesity and central obesity with the tested SNPs. The SNP was analyzed as 0, 1 or 2 copies of the minor allele in an additive genetic model. Considering adjustment for

multiple testing corrections, a significance level of 0.004 was used. The potential covariates such as age, sex, SBP, lipid levels, and FPG were included in the multivariable models. To detect the potential SNP-smoking interactions, an extra interaction term of the genotype*smoking was included in the regression model. We also performed sub-group analyses according to the smoking status of the participants. Statistical analyses were conducted using SAS statistical software version 9.2 (SAS Institute, Cary, NC).

Results

Baseline characteristics

There were 578 (38.4%) male and 929 (61.6%) female participants in this study. The average age of these participants was 53 years old, with a range from 21 to 89 years. Among them, 342 (22.7%) and 63 (4.2%) participants were overweight and obese, respectively, and 449 (30.0%) participants were central obese. The characteristics of the participants are presented in Table 1. It seemed that obese participants were younger than the normal weight participants. Frequencies of smokers and drinkers were not different between obesity and normal weight participants. Compared with those with a normal BMI, obese participants have higher levels of SBP, DBP, TC, TG and LDL-C, and a lower level of HDL-C. Central obese individuals were more likely to be women, and have higher levels of SBP, DBP, TC, TG, LDL-C and FPG, and a lower level of HDL-C than those without central obesity.

Association between genotypes and obesity

HWE of the 12 SNPs (MAF > 10%) was tested with Fisher's exact test and no departures were

observed (Table 2). Table 2 also showed the results of association between SNPs and BMI and obesity. No *CORIN* SNP was associated with BMI. Rs17654423 seemed to be associated with obesity ($P = 0.0395$), but the association was not significant in multivariable-adjusted analysis. For *NPPA* SNPs, rs5063 and rs198358 were associated with BMI in univariate analyses ($P = 0.0053$ and 0.0037, respectively). Rs5063 was associated with obesity in univariate analyses ($P = 0.0398$). Rs198358 was associated with obesity in both univariate and multivariable-adjusted analyses ($P = 0.0138$ and 0.0173, respectively).

Association between genotypes and central obesity

We investigated the associations between the *CORIN* and *NPPA* SNPs and WC and central obesity in the 1,507 participants. The results were presented in Table 3. No *CORIN* SNP was associated with WC or central obesity. For *NPPA* SNPs, rs5063 was associated with WC in univariate ($P = 0.0231$). After adjusted for covariates, this association was not significant ($P = 0.0586$). Rs5063 was also associated with central obesity in both univariate and multivariable-adjusted analyses ($P = 0.0454$ and 0.0361, respectively).

Interaction between cigarette smoking and SNPs

Weak evidence of interaction between cigarette smoking and rs1866689 and rs10008014 on obesity was detected ($P = 0.0236$ and 0.0157, respectively) (Table 4). In subgroup analyses by separating the study population into smokers and nonsmokers, we found no significant association between the two SNPs and obesity. For central obesity, significant interactions between cigarette smoking and rs5063 and rs198358 were detected ($P = 0.0019$ and 0.0006,

respectively). In subgroup analyses, these two SNPs were associated with central obesity in smokers (P = 0.0081 and 0.0037, respectively), but not in nonsmokers (Table 4). Smokers carrying minor alleles of rs5063 and rs198358 seemed to have low risk of obesity, OR (95% confidence interval) for rs5063 and rs198358 were 0.50 (0.29, 0.83) and 0.48 (0.30, 0.79), respectively.

Discussion

This is the first study to evaluate the associations of *CORIN* and *NPPA* gene SNPs with obesity and central obesity, and the interaction effect of the SNPs with smoking on obesity and central obesity in the Chinese Han population. We found that *NPPA* gene SNPs rs5063 and rs198358 were associated with obesity and central obesity. Significant interactions between these two SNPs and smoking on central obesity were detected. Our study suggested that the effect of the two independent SNPs rs5063 and rs198358 of *NPPA* on central obesity might be modified by smoking in the Chinese Han population.

Human ANP exerts an important role in lipolysis(Sengenes et al. 2000). The Framingham Heart Study has shown that plasma levels of ANPs were lower in subjects with obesity and those with metabolic risk factors(Wang et al. 2007; Wang et al. 2004). *NPPA* gene encodes the ANP precursor. The potential relationships between *NPPA* genetic polymorphism and essential hypertension have been widely explored(Wang et al. 2016). However, no study has reported the relationship between DNA polymorphisms in *NPPA* genes and obesity, including GWAS. We first showed that *NPPA* SNPs s5063 and rs198358 were associated with obesity in the Chinese Han population. Our data also suggested that smokers carrying one or two minor alleles of

rs5063 or rs198358 might have lower risk of central obesity. Conversely, major allele homozygote smokers might have higher risk. This means that these two SNPs are protective for smokers with central obesity. Rs5063 is a missense mutation in exon 1 of the *NPPA* gene, while rs198358 located at the downstream region. The underlying mechanisms of these two SNPs on obesity need to elucidate in future studies.

It is known that nicotine has a direct effect on adipose tissue metabolism(Carney and Goldberg 1984). Besides, it has been shown that the mechanism by which smoking regulates adiposity likely involves appetite suppression via neural pathways(Mineur et al. 2011). Although genetic associations with obesity may differ by smoking status(Fesinmeyer et al. 2013), the precise mechanisms by which the interaction of genetic variants with smoking affects obesity were still largely unknown. In the present study, we observed that the effect of rs5063 or rs198358 on central obesity might be modified by smoking, which has not been reported in previous studies. Smokers carrying the major alleles of rs5063 or rs198358 might have higher risk of central obesity. This effect could not be seen in nonsmokers. We still cannot determine if smoking affects the function of rs5063 or rs198358, or if the SNPs could change smoking behavior. In view of the effect of ANP and smoking on adipose metabolism, future studies are needed to determine if smoking could influence the circulating ANP levels, and whether this change is associated with the food intake and energy expenditure.

Corin is a converting enzyme in the natriuretic peptide system. Recent studies have shown the association of circulating soluble corin and heart failure(Dong et al. 2010), acute coronary syndrome(Peleg et al. 2013), osteoporosis(Zhou et al. 2013), pregnant hypertension(Zaki et al. 2012) and obesity(Peng et al. 2015). *CORIN* gene variants have been identified to alter corin

protein conformation and inhibited corin zymogen activation and contribute to hypertension and heart disease(Dong et al. 2013; Dries et al. 2005; Zhou and Wu 2014). The present study first evaluated the associations between *CORIN* gene SNPs and obesity in Chinese individuals. Unfortunately, we did not detect a significant association. As an ANP convertase, the function of corin on lipolysis should not as direct as ANP. The effect sizes of genetic variants in *CORIN* gene on obesity may be much smaller than those of *NPPA* gene, or the mutations may not have an effect on obesity risk.

This study has some potential limitations. First, the sample size of this study is relatively small. This means that some SNPs especially with weaker genetic effects would not be detected in our study. Moreover, the associations between the small effect SNPs and obesity was not significant when adjusted for age, BP and lipid levels, which have much larger effect sizes. Second, no association between the *CORIN* polymorphism and obesity was likely due to the small sample size. Besides, only several tag-SNPs of *CORIN* have tested in the present studies. So it cannot conclude that *CORIN* gene variants were not associated with obesity. Finally, there were 21 (4.8%) men and 42 (6.3%) women with central obesity in BMI normal group (435 men and 667 women), which could not be considered as normal for comparison against obese and overweight individuals. However, the proportion was very small. When excluded these people in the analyses about obesity, the results didn't vary.

In summary, the results of our study demonstrated that *NPPA* SNPs rs5063 and rs198358 were associated with obesity, and the effect of these SNPs on central obesity might be modified by smoking in the Chinese Han population. Smokers carrying the major alleles of rs5063 or rs198358 might have higher risk of central obesity. Further studies are needed to confirm the

associations and elucidate the underlying mechanisms.

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Conflict of Interest Statement

The authors wish to declare they have no conflicts of interest.

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Table1 Characteristics of study participants

Characteristics	**BMI**				**WC**		
	Obesity (n = 63)	**Overweight** (n = 342)	**Normal weight** (n = 1102)	***P* value**	**Central obesity** (n = 449)	**Normal WC** (n = 1058)	***P* value**
Age, year	49.05±11.69	50.23±11.79	53.90±12.90	<0.0001	52.03±12.40	53.22±12.84	0.0968
Male, %	30.16	36.26	39.47	0.2220	34.08	40.17	0.0261
Smokers, %	23.81	26.02	30.13	0.2258	26.95	29.77	0.2688
Drinkers, %	14.29	22.51	21.23	0.3406	21.16	21.27	0.9625
SBP, mmHg	125.0±9.13	124.4±9.17	121.6±10.65	<0.0001	125.1±8.96	121.2±10.69	<0.0001
DBP, mmHg	79.59±6.79	78.35±6.40	74.42±7.58	<0.0001	78.04±6.58	74.46±7.65	<0.0001
TC, mmol/L	4.78±1.01	4.67±0.96	4.48±0.96	0.0003	4.72±1.00	4.44±0.94	<0.0001
TG, mmol/L	1.78±0.89	1.88±1.45	1.33±0.80	<0.0001	1.86±1.32	1.31±0.80	<0.0001
HDL-C, mmol/L	1.16±0.34	1.20±0.42	1.37±0.48	<0.0001	1.20±0.41	1.37±0.49	<0.0001
LDL-C, mmol/L	2.68±0.89	2.56±0.81	2.46±0.78	0.0227	2.62±0.85	2.44±0.77	0.0002
FPG, mmol/L	5.21±0.99	5.07±0.88	5.07±1.00	0.5660	5.19±1.10	5.03±0.91	0.0032

Table 2 Association between *CORIN/NPPA* gene polymorphisms and BMI and obesity

SNP	Position	MAF	HWE*	BMI		Obesity			
		%		*P*	$P_{\_adj}$#	OR（95%CI）	*P*	$OR_{\_adj}$#（95%CI）	$P_{\_adj}$
CORIN									
rs2289433	exon 1	34.3	0.075	0.0836	0.4147	0.97(0.82,1.16)	0.7624	0.98(0.82,1.17)	0.8089
rs1866689	intron 1	43.5	0.870	0.2376	0.6872	1.01(0.85,1.18)	0.9501	1.03(0.87,1.22)	0.7308
rs10008014	intron 6	24.6	0.289	0.1080	0.6504	1.03(0.85,1.24)	0.7833	1.02(0.84,1.25)	0.8208
rs17654423	intron 6	26.6	0.670	0.1710	0.1009	1.21(1.01,1.46)	**0.0395**	1.18(0.99,1.43)	0.0825
rs10517195	exon 9	15.7	0.167	0.5588	0.8438	1.02(0.82,1.28)	0.8448	1.10(0.87,1.39)	0.4155
rs2271037	intron 9	38.7	0.551	0.5192	0.9437	1.06(0.90,1.25)	0.5174	1.12(0.95,1.33)	0.1860
rs2351784	intron 11	23.0	0.335	0.5168	0.5187	1.03(0.85,1.25)	0.7680	1.05(0.85,1.28)	0.6722
rs12509275	intron 17	13.8	0.064	0.5377	0.2594	1.13(0.89,1.43)	0.3140	1.20(0.94,1.53)	0.1498
rs3749585	exon 22	48.0	0.774	0.4685	0.5912	0.99(0.85,1.16)	0.9084	0.96(0.82,1.14)	0.6646
NPPA									
rs632793	upstream	14.4	0.604	0.8337	0.9924	0.94(0.75,1.17)	0.5583	0.93(0.74,1.17)	0.5408
rs5063	exon 1	11.6	0.981	**0.0053**	0.1128	0.77(0.60,0.99)	**0.0398**	0.78(0.60,1.01)	0.0565
rs198358	downstream	10.0	0.583	**0.0037**	0.1336	0.74(0.58,0.94)	**0.0138**	0.74(0.58,0.95)	**0.0173**

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency

*: *P* value for HWE using Fisher's exact test.

#: Adjusted for age, SBP, lipid levels.

Table 3 Association between *CORIN/NPPA* gene polymorphisms and waist circumference and central obesity

SNP	WC		Central obesity			
	P	$P_{\_adj}^{\#}$	**OR（95%CI）**	***P***	$OR_{\_adj}^{\#}$（95%CI）	$P_{\_adj}$
CORIN						
rs2289433	0.2576	0.6018	0.97(0.82,1.15)	0.7199	0.98(0.82,1.17)	0.7884
rs1866689	0.6918	0.8837	0.97(0.83,1.14)	0.6913	0.99(0.84,1.17)	0.8912
rs10008014	0.2064	0.3150	1.10(0.91,1.33)	0.9310	1.12(0.92,1.36)	0.2763
rs17654423	0.3752	0.2500	1.09(0.91,1.30)	0.3657	1.07(0.89,1.28)	0.5031
rs10517195	0.6952	0.9942	0.92(0.74,1.14)	0.4463	0.97(0.77,1.21)	0.7686
rs2271037	0.2535	0.5789	0.95(0.81,1.12)	0.5370	1.00(0.84,1.18)	0.9777
rs2351784	0.5216	0.4345	1.00(0.83,1.21)	0.9915	1.02(0.83,1.24)	0.8849
rs12509275	0.3663	0.6342	1.04(0.83,1.30)	0.7486	1.07(0.84,1.36)	0.5916
rs3749585	0.2840	0.8347	1.00(0.85,1.16)	0.9616	0.97(0.82,1.14)	0.6756
NPPA						
rs632793	0.9980	0.6666	0.91(0.74,1.14)	0.4209	0.91(0.72,1.14)	0.3995
rs5063	**0.0231**	0.0586	0.78(0.61,1.00)	**0.0454**	0.76(0.58,0.98)	**0.0361**
rs198358	0.0642	0.1063	0.82(0.65,1.04)	0.1011	0.81(0.63,1.04)	0.0965

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency

*: *P* value for HWE using Fisher's exact test.

#: Adjusted for age, sex, SBP, lipid levels, FPG.

Table 4 Association between *CORIN/NPPA* gene polymorphisms and obesity or central obesity in smokers and nonsmokers

	Smokers (n=436)				**Nonsmokers** (n=1071)				
	OR（95%CI）	***P***	**$OR_{\_adj}$[#]（95%CI）**	**$P_{\_adj}$**	**OR（95%CI）**	***P***	**$OR_{\_adj}$[#]（95%CI）**	**$P_{\_adj}$**	**$P_{\_interaction}$**
Obesity									
rs1866689	1.29(0.92,1.79)	0.1351	1.35(0.94,1.95)	0.1060	0.93(0.77,1.12)	0.4519	0.94(0.78,1.14)	0.5346	**0.0236**
rs10008014	1.46(0.98,2.19)	**0.0645**	1.44(0.93,2.23)	0.1040	0.92(0.74,1.15)	0.4584	0.92(0.74,1.15)	0.4569	**0.0157**
Central obesity									
rs5063	0.52(0.33,0.81)	**0.0041**	0.50(0.29,0.83)	**0.0081**	0.92(0.68,1.24)	0.5820	0.91(0.67,1.24)	0.5557	**0.0019**
rs198358	0.49(0.32,0.75)	**0.0010**	0.48(0.30,0.79)	**0.0037**	1.03(0.77,1.37)	0.8617	1.03(0.76,1.40)	0.8269	**0.0006**

#: Adjusted for age, sex, SBP, lipid levels, FPG.