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The modifying effect of C-reactive protein gene polymorphisms on the association between central obesity and endometrial cancer risk

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Abstract

BACKGROUND—Obesity is a major risk factor for endometrial cancer. Obesity, particularly central obesity, is considered as a systemic inflammatory condition and strongly related to insulin resistance. C-reactive protein (CRP) is the most recognized biological marker of chronic systematic inflammation, and it is conceivable that the CRP gene may work together with obesity in the development of endometrial cancer.

METHODS—Based on a population-based case-control study in a Chinese population, we obtained obesity measurements and six *CRP* single-nucleotide polymorphisms (SNP) data from 1,046 newly diagnosed endometrial cancer cases and 1,035 age frequency matched controls. The association of the *CRP* SNPs with endometrial cancer risk and their modification on the association between obesity and endometrial cancer risk were evaluated.

RESULTS—While *CRP* SNPs alone were not associated with endometrial cancer, the associations of endometrial cancer with central obesity as measured by waist-to-hip ratio (WHR) and waist circumference seemed to be stronger in women who were homozygous for the major allele of rs1130864 (C/C) than in women whose genotypes were C/T and T/T (interaction test: $p=0.013$ for WHR and $p=0.083$ for waist circumference). When further stratified by menopausal status, we found that the observed interactions persisted mainly in premenopausal women (interaction test: $p<0.001$ for both WHR and $p=0.002$ for waist circumference).

CONCLUSIONS—Our study suggests that, in this Chinese population, obesity-related insulin resistance and proinflammatory effects may play an important role in endometrial cancer risk, and these effects were significantly modified by the *CRP* SNP rs1130864.

Keywords

c-reactive protein; obesity; endometrial cancer

Obesity has been established as a major risk factor for endometrial cancer. Previous studies have suggested that the mechanism behind this increased risk for endometrial cancer may be due to the estrogenic effects associated with obesity. However, it has recently been

hypothesized that other mechanisms, in particular, obesity-related insulin resistance and inflammation(1;2), may also contribute to the association of endometrial cancer with obesity. Obesity, particularly central obesity, is considered a systemic inflammatory condition and is strongly related to insulin resistance(3;4). Two recent studies(5;6) and several previous ones(7–10) have suggested that central obesity is a more important predictor of endometrial cancer risk than general obesity.

C-reactive protein (CRP) is the most recognized biologic marker of chronic systematic inflammation(11;12). CRP levels are correlated with obesity and insulin resistance. Polymorphisms in the *CRP* gene have been associated with CRP levels(13–17). It is of interest that the *CRP* gene is located on chromosome 1q21-q23, the same general chromosomal region as the type 2 diabetes mellitus susceptibility locus. Studies have shown that *CRP* gene variations may influence insulin sensitivity(18;19). Therefore, it is conceivable that the *CRP* gene might work in concert with obesity-related insulin resistance and inflammation in the development of endometrial cancer.

We previously reported(20) that endometrial cancer was significantly associated with central obesity as measured by waist-to-hip ratio (WHR) and waist circumference, and the positive association with general obesity as measured with body mass index (BMI) vanished after adjusting for central obesity. In this study with an expanded sample size, we evaluate the role of *CRP* gene polymorphisms in endometrial cancer risk with an emphasis on investigating the modifying effect of *CRP* gene polymorphisms on the association between central obesity and endometrial cancer risk.

MATERIALS AND METHODS

Data were obtained through the Shanghai Endometrial Cancer Study, a population-based case-control study. Eligible cases in the study were identified through the population-based Shanghai Cancer Registry. A total of 1,454 women aged 30–69 years with newly diagnosed endometrial cancer between 1997 and 2003 were identified. Of these, 1,204 cases (82.8%) completed an in-person interview. The median interval between diagnosis and interview for cases was 5.6 months. Controls were randomly selected from female residents of urban Shanghai through the Shanghai Resident Registry and were frequency matched to the age distribution (5-year intervals) of endometrial cancer cases. Women with a history of cancer or hysterectomy were not eligible. Of the 1,629 eligible women identified, 1,212 (74.4%) participated in the study. The study protocols were approved by the Institutional Review Boards of all institutes involved in the study. After written, informed consent was obtained, a structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive history, hormone use, prior disease history, physical activity, use of tea, tobacco, and alcohol, height and weight history during adolescence and adulthood, and family history of cancer. Usual dietary habits over the preceding 5 years were assessed using a validated, quantitative food-frequency questionnaire. The weight, height and circumferences of the waist and hips were measured by interviewers according to a standardized protocol at the time of interview.

Of the 1,204 cases and 1,212 controls who participated in the study, 857 cases and 837 controls donated a blood sample and 282 cases and 286 controls provided a buccal cell sample. Of these, DNA samples for 33 controls were used up in previous assays and a total of 93 buccal cell swab samples for cases and 55 buccal cell swab samples for controls were not genotyped because of very low DNA yield. Thus, DNA samples from 1,046 (86.9%, 857 blood and 189 buccal cell) cases and 1,035 (85.4%, 837 blood and 198 buccal cell) controls were available for genotyping.

We searched the literature and SNP databases to identify single-nucleotide polymorphisms (SNP) for the study. Seven SNPs (rs2794521, rs3093062, rs3091244, rs1800947, rs3093058, rs1130864, and rs3093059) in the *CRP* gene have been reported in the literature with functional or potential functional importance. Using HapMap Han Chinese data (last HapMap access: February 8, 2006), we selected additional tagging SNPs for the *CRP* gene using the Tagger pairwise (21)/LDSelect (22) method. We included the whole *CRP* gene plus the 5kb flanking region. The functional/potential functional SNPs were forced into a tagging SNP search. SNPs with MAF ≥ 0.05 were selected. SNPs were selected until all common SNPs in the region in question were highly correlated to one or more tagging SNPs. In order to improve coverage, we used a threshold of $r^2 \geq 0.90$ to identify htSNPs. A total of eight SNPs (rs3093059, rs2794521, rs3091244, rs1800947, rs1130864, rs2808630, rs3093075, and rs2794520) were identified for genotyping. We were not able to genotype SNPs rs3093059 and rs3091244. Thus, six SNPs were included in the analyses. Among them, rs2794521 located in the promoter region; rs1800947 is a synonymous polymorphism at codon 184; rs1130864, rs2808630, rs3093075, and rs2794520 located in the 3'UTR.

Genomic DNA was extracted from buffy coat fractions or buccal cells by using a QIAmp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. The *CRP* gene polymorphisms were genotyped with the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Inc. (ABI, Foster City, CA) using the TaqMan genotyping assay with primers and probes obtained from ABI. The TaqMan assay method has been described previously(23). Briefly, the final volume for each reaction was 5 μ L, consisting of 2.5 μ L TaqMan Universal PCR Master Mix, 0.6 μ L of each primer, 0.2 μ L of each TaqMan probe, and 5 ng genomic DNA. The PCR profile consisted of an initial denaturation step at 95°C for 10 minutes and 40 cycles of 92°C for 15 seconds and 60°C for 1 minute. The fluorescence level was measured with the ABI PRISM 7900HT sequence detector (ABI). Allele frequencies were determined by ABI SDS software.

The laboratory staff was blind to the identity of the subjects. Quality control (QC) samples were included in the genotyping assays. Each 384-well plate contained four water, eight CEPH 1347-02 DNA, eight blinded QC DNA, and eight unblinded QC DNA samples. The concordance rates for the quality control samples were 99.0% for rs2794520 and rs3093075, 100.0% for the other four SNPs. In addition, we genotyped 45 DNA samples from the Chinese participants used in HapMap and 24 DNA samples used in Perlegen as an additional quality control for all SNPs. The genotypes of the samples generated from our study were compared to data downloaded from HapMap (<http://www.hapmap.org>) and/or Perlegen (<http://genome.perlegen.com>). No data was available for rs2794521 in these databases and the concordance rates between the data generated in our lab and the data from the above databases for the other five SNPs was 100%.

Chi-square tests were used to evaluate case-control differences of categorical demographic factors and known risk factors. Student's t-tests were used to compare continuous variables. Chi-square tests were also used to evaluate case-control difference in the distributions of *CRP* alleles and genotypes. Hardy-Weinberg equilibrium was examined for each SNP using the Chi-square goodness-of-fit test. The frequencies of haplotypes for the *CRP* gene were derived using the software PHASE (version 2.1), and the overall association between haplotypes and endometrial cancer risk was evaluated with the permutation test(24;25). The endometrial cancer risk associated with each haplotype as compared with all other haplotype under different genetic modes (additive, dominant, and recessive) was estimated using logistic regression models using the HAPSTAT software recently developed by Lin DY and Zeng D et al(26;27). The interaction between *CRP* SNPs and obesity measurements were also evaluated using logistic regression models. For continuous obesity variables, nonlinear terms were included in the model using the restricted cubic spline function(28) to account

for their possible non-linear effects and to avoid using arbitrary cutpoints to categorize them. The adjusted model-based odds ratios (OR) and 95% confidence intervals (CI) were estimated with model parameters for the middle points of the first, second, fourth, and fifth quintiles of continuous obesity variables as compared with the middle point of the third quintile (the median) according to variable values in control group. Non-linearity and interaction were assessed with the likelihood ratio test. The potential confounding effect of major demographic factors and known endometrial cancer risk factors were evaluated by including them in the logistic models.

RESULTS

Selected demographic and risk factors for cases and controls are compared in Table 1. There were no significant ($p>0.23$) case-control differences with regard to age, education level, marital status, family income, cigarette smoking, tea consumption, or use of hormone replacement therapy. However, compared with controls, cases were less likely to drink alcohol or use oral contraceptives ($p<0.01$), and were more likely to have a positive family history of any cancer, an earlier age at menarche, a later age at menopause, and a longer cumulative time of menstruation ($p<0.01$). These factors were considered as potential confounders and were adjusted for in the logistic models.

All six *CRP* SNPs were in Hardy-Weinberg equilibrium among controls ($p>0.188$, data not shown in the table). Table 2 shows the allele and haplotype frequencies and p values for the case-control tests for the association of each individual *CRP* SNP or each haplotype with endometrial cancer under additive, dominant, and recessive genetic modes. No significant associations were found. Adjustment for potential confounders did not appreciably change the results.

Treating WHR, waist circumference, and BMI as continuous variables and using the restricted cubic spline function with 4 knots to account for non-linearity in the logistic regression models, we show in Table 3 that larger WHR and waist circumference were associated with higher endometrial cancer risk, although not in a linear manner for WHR (nonlinearity test: $p = 0.027$ for WHR and $p = 0.331$ for waist circumference). After adjusting for waist circumference, BMI was not significantly associated with endometrial cancer risk. History of diabetes mellitus was significantly associated with endometrial cancer risk. When stratified by menopausal status, we found that the association of endometrial cancer risk with WHR and waist circumference was significantly stronger in premenopausal women than in postmenopausal women (interaction test: $p < 0.001$ for WHR and $p = 0.002$ for waist circumference). Menopausal status appeared not to influence the effect of history of diabetes mellitus (interaction test: $p = 0.986$).

Table 4 presents the associations of endometrial cancer risk with WHR, waist circumference, and history of diabetes mellitus by genotypes (homozygous for the major allele versus heterozygous or homozygous for the minor allele) of two SNPs (rs2794520 and rs1130864). The associations of endometrial cancer risk with WHR and waist circumference seemed to be stronger in women who were homozygous for the major allele of rs1130864 (C/C) than in women who were heterozygous or homozygous for the minor allele of this SNP (C/T, T/T) (interaction test: $p = 0.013$ for WHR and $p = 0.083$ for waist circumference). When further stratifying data by menopausal status, we found that the observed interactions existed mainly in premenopausal women (interaction test: $p<0.001$ for WHR and $p=0.002$ for waist circumference) and they were not significant in postmenopausal women. A marginally significant interaction between rs1130864 and history of diabetes mellitus was observed (interaction test: $p = 0.064$) in all subjects. Similar interactions of WHR or waist circumference with rs2794520 were observed (interaction test: $p = 0.007$ for WHR and $p =$

0.002 for waist circumference) when the analysis was conducted in all subjects, but not when the data were stratified by menopausal status. No significant interaction between rs2794520 and history of diabetes mellitus was observed.

We also evaluated the interaction of the other four *CRP* SNPs (rs3093075, rs2808630, rs1800947, rs2794521) with WHR, waist circumference, and history of diabetes mellitus, and none of the interaction tests was statistically significant (These p-values are not presented in Table 4). In addition to evaluating interactions with individual SNPs, we performed haplotype analyses as well and found that the interaction between WHR and one *CRP* haplotype (C-C-T-T-G-A), which consisted of the minor alleles of rs2794520 (allele C) and rs1130864 (allele T) and the major alleles of the other four SNPs, was significant under the additive mode ($p=0.006$) and dominant mode ($p=0.008$) (results not shown in the table). However, we did not find any significant interaction of *CRP* haplotypes with waist circumference or history of diabetes mellitus.

DISCUSSION

We previously reported(29) that WHR and waist circumference but not BMI or history of diabetes mellitus were associated with endometrial cancer risk. These findings were confirmed in the current study with the expanded sample size. Both WHR and waist circumference are surrogate measures for central obesity, which is closely related to insulin resistance(30). The onset of diabetes mellitus is often preceded by many years of increasing insulin resistance. Our findings support an important role for insulin resistance in endometrial cancer risk.

Previous studies, mainly conducted in Western populations, have shown that the association between obesity and endometrial cancer risk is stronger in postmenopausal women than in premenopausal women, particularly in never-users of hormone replacement therapy(31–34). These findings support the theory that obesity may increase endometrial cancer risk through its estrogenic effects. Our study, however, found that the effect of WHR and waist circumference was significantly stronger in premenopausal women than in postmenopausal women in our study population, suggesting that a mechanism other than the estrogenic effect of obesity may be important to the etiology of endometrial cancer among Chinese women who typically have lower estrogen levels than Western women. In addition, it has been argued that in premenopausal women low levels of progesterone, rather than increased estrogen, is the predominant determinant of endometrial cancer risk(35). In premenopausal women, central obesity may increase endometrial cancer risk by increasing inflammation in the endometrium by inducing chronic anovulation and progesterone deficiency(36).

Insulin resistance and inflammation have been postulated as the major pathways linking obesity to cancer risk(37). In fact, insulin resistance is increasingly recognized as a chronic, low-level, inflammatory state, and direct correlations have been observed between inflammation markers and degree of insulin resistance(38;39). Visceral adipose tissue is an important source of three of the most important proinflammatory cytokines – CRP, interleukin (IL)-6, and tumor necrosis factor- α (TNF- α). These proinflammatory cytokines play important roles in insulin resistance(40;41).

In the current study, *CRP* SNPs alone were not found to be significantly associated with endometrial cancer risk, but two *CRP* SNPs, rs1130864 and rs2794520, both located in the 3'UTR, modified the association of endometrial cancer risk with WHR and waist circumference. The positive association of endometrial cancer risk with increased WHR or waist circumference, although not linear for WHR, was significantly stronger in women who were homozygous for the major alleles for both SNPs. The modification of rs1130864 was

even more evident in premenopausal women. The major allele of the SNP rs1130864 has been associated with lower circulating CRP levels(42–44), while the function of rs2794520 is unknown. Marsik C et al(45) recently reported that homozygous carriers of the major allele of rs1130864 had significantly lower basal CRP concentrations and significantly higher IL-6 concentrations. However, after administering 2 ng/kg endotoxin (*Escherichia coli* bacterial lipopolysaccharide) intravenously, a much stronger inflammatory response was found among the homozygous carriers of the major allele, which included a much higher peak of TNF- α and IL-6 concentrations and increased body temperature. To explain the effect modification of rs1130864 observed in our study, we speculate that homozygous carriers of the major allele of rs1130864 may have a stronger obesity-related inflammatory response and produce more TNF- α and IL-6, leading to increased endometrial cancer risk, although the mechanism for obesity-related chronic inflammation may not be the same as endotoxin stimulation.

This is the first report to our knowledge that has investigated the interaction of *CRP* gene polymorphisms with obesity in the development of endometrial cancer. We cannot exclude the possibility that our findings are due to chance, since we analyzed multiple SNPs and environmental factors. We chose not to adjust for multiple testing in the data analyses in this exploratory study. Our findings need to be confirmed in further studies. However, the consistent pattern observed in the study and the interaction with SNP rs1130864, which was found by an independent study to be associated with decreased circulating CRP levels but increased inflammatory response (higher levels of IL-6 and TNF- α), appears to argue against a chance finding as the sole explanation. In summary, our study suggests that in the Chinese population obesity-related insulin resistance and proinflammatory effects may play an important role in addition to the estrogenic effect of obesity in endometrial cancer risk, and these effects may be significantly modified by the *CRP* SNP rs1130864.

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Table 1

Comparison of basic characteristics between cases and controls

	Cases (n=1046)		Controls (n=1035)		p
	%		%		
Education					
Elementary education or less	21.5		22.5		
Middle school	38.1		37.8		
High School	26.0		26.8		
More than high school	14.5		12.9		0.717
Marital status					
Single	1.5		1.2		
Married	87.0		87.8		
Separated/divorced/widowed	11.5		11.0		0.716
Family income in previous year					
<10,000 yuan	12.1		10.9		
10,000–19,999 yuan	41.6		39.4		
20,000–29,999 yuan	21.4		25.1		
30,000 yuan	25.0		24.7		0.230
Ever smoked	3.2		3.5		0.680
Ever drank alcohol	3.1		5.5		0.006
Ever drank tea	30.1		31.2		0.409
Cancer among first-degree relatives	35.5		29.3		0.003
Postmenopausal	56.5		61.8		0.015
Oral contraceptive use	18.3		25.1		<0.001
Hormone replacement therapy	4.7		4.3		0.633
	mean (sd)		mean (sd)		
Age at interview	54.3(8.5)		54.5(8.5)		0.636
Age at menarche	14.5(1.7)		14.8(1.8)		<0.001
Age at menopause ^a	50.3(3.6)		49.1(3.6)		<0.001
Years of menstruation	32.8(4.9)		30.7(5.3)		<0.001

^aIn postmenopausal women.

Table 2
Case-control tests for the association of six *CRP* SNPs with endometrial cancer risk under different genetic modes.

SNP	Allele frequency		p for case-control test under different genetic modes		
	Case(%)	Control(%)	Additive	Dominant	Recessive
rs2794520					
T	59.0	58.4			
C	41.0	41.6	0.674	0.769	0.685
rs3093075					
C	83.4	82.6			
A	16.6	17.4	0.515	0.224	0.197
rs2808630					
T	83.6	84.7			
C	16.4	15.3	0.345	0.184	0.401
rs1130864					
C	95.0	93.9			
T	5.0	6.1	0.140	0.196	0.123
rs1800947					
G	94.7	94.7			
C	5.3	5.3	0.967	0.968	0.986
rs2794521					
A	83.6	84.7			
G	16.4	15.3	0.353	0.191	0.410
Haplotype ^b					
TCTCGA	53.9	53.1	0.649	0.239	0.633
CATCGA	16.2	16.7	0.648	0.315	0.257
CCCCGG	15.9	14.8	0.350	0.152	0.329
CCTTGA	5.1	6.3	0.189	0.357	0.999
TCCTCA	5.3	5.2	0.871	0.783	0.648
CCTCGA	3.3	3.5	0.672	0.442	0.086
P = 0.900 ^d					

^dThe p value was derived from the permutation test for the overall haplotype difference.

b In the order of rs2794520, rs3093075, rs2808630, rs1130864, rs1800947, and rs2794521.

Model-based odds ratios and 95% confidence intervals^a of endometrial cancer risk associated with obesity-related measures and history of diabetes mellitus

Table 3

Category ^a	Middle point ^b	All women		Premenopausal women		Postmenopausal women	
		Number ^c	OR(95% CI) ^d	Number ^c	OR(95% CI) ^d	Number ^c	OR(95% CI) ^d
WHR							
0.774	0.750	92/206	0.7(0.6–0.9)	31/103	0.4(0.3–0.6)	61/103	1.0(0.8–1.3)
0.775–0.803	0.791	144/207	0.8(0.8–0.9)	78/97	0.7(0.6–0.8)	66/110	0.9(0.8–1.0)
0.804–0.830	0.814	200/207	1.0	88/75	1.0	112/132	1.0
0.831–0.864	0.845	270/207	1.4(1.2–1.6)	125/72	1.4(1.1–1.8)	145/135	1.3(1.1–1.6)
>0.864	0.886	338/206	1.6(1.3–2.0)	132/48	1.8(1.3–2.6)	206/158	1.5(1.1–2.0)
Interaction test		p<0.001					
Waist circumference(cm)							
71	67	71/208	0.5(0.3–0.6)	33/114	0.2(0.1–0.4)	38/94	0.8(0.5–1.2)
72–76	73	141/232	0.7(0.6–0.8)	69/112	0.5(0.4–0.6)	72/120	0.8(0.7–1.0)
77–80	78	168/180	1.0	91/71	1.0	77/109	1.0
81–87	83	282/234	1.5(1.3–1.7)	128/64	1.8(1.4–2.4)	154/170	1.4(1.1–1.6)
>87	91	382/179	2.3(1.7–3.1)	133/34	4.1(2.4–7.0)	249/145	2.0(1.4–2.8)
Interaction test		p=0.002					
BMI							
20.92	19.64	104/207	1.1(0.9–1.5)	50/99	1.3(0.9–1.9)	54/108	1.1(0.8–1.6)
20.93–22.68	21.85	128/205	1.0(0.9–1.1)	64/92	1.1(0.9–1.3)	64/113	1.0(0.9–1.1)
22.69–24.32	23.44	190/208	1.0	92/87	1.0	98/121	1.0
24.33–26.47	25.33	214/206	1.0(0.9–1.2)	92/63	0.9(0.7–1.2)	122/143	1.1(0.9–1.3)
>26.47	28.44	408/207	1.1(0.8–1.5)	156/54	0.7(0.4–1.2)	252/153	1.3(0.9–1.9)
Interaction test		p=0.184					
History of diabetes mellitus							
No		880/957	1.0	408/381	1.0	472/576	1.0
Yes		156/71	1.9(1.4–2.6)	45/12	1.6(0.8–3.3)	111/59	2.0(1.4–2.8)
Interaction test		p=0.986					

^aWHR, waist circumference, and BMI were categorized by quintiles according to the values in control group.

^b The middle values in each quintile according to the distributions in control group.

^c Number of cases/controls in each quintile.

^d The odds ratios (OR) and 95% confidence intervals (CI) were estimated for the middle points of the first, second, fourth, and fifth quintiles of continuous obesity variables as compared with the middle point of the third quintile (the median) with model parameters derived from logistic regression models which included the restricted cubic spline function with 4 knots to account for non-linearity and were adjusted for age at menarche, menopausal status, total years of menstruation, oral contraceptive use, cancer history in first degree relatives, and BMI (for WHR and waist circumference) or waist circumference (for BMI and history of diabetes mellitus).

Table 4

The effect of WHR, waist circumference, and a history of diabetes mellitus on endometrial cancer risk by genotypes of two *CRP* SNPs (rs1130864 and rs2794520)

Category ^a	rs1130864				rs2794520			
	C/C		C/T & T/T		T/T		C/T & C/C	
	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c
All women								
WHR								
0.774	76/184	0.7(0.5–0.9)	14/19	0.9(0.5–1.6)	30/80	0.8(0.6–1.2)	60/122	0.7(0.5–0.9)
0.775–0.803	129/182	0.8(0.7–0.9)	13/23	0.9(0.7–1.2)	41/80	0.8(0.7–0.9)	100/125	0.9(0.8–1.0)
0.804–0.830	169/177	1.0	28/26	1.0	61/62	1.0	133/143	1.0
0.831–0.864	240/182	1.4(1.2–1.7)	25/24	1.0(0.7–1.7)	105/70	1.8(1.4–2.3)	159/135	1.2(1.0–1.5)
>0.864	313/173	1.8(1.4–2.3)	24/29	0.7(0.3–1.3)	115/52	2.6(1.8–3.8)	219/149	1.4(1.0–1.8)
Interaction test		p=0.013				p=0.007		
Waist circumference (cm)								
71	63/181	0.5(0.3–0.7)	8/22	0.4(0.2–1.3)	20/72	0.5(0.3–0.9)	50/131	0.4(0.3–0.6)
72–76	127/207	0.7(0.6–0.8)	12/24	0.7(0.5–1.1)	47/87	0.7(0.5–0.8)	92/145	0.7(0.6–0.8)
77–80	146/161	1.0	16/18	1.0	48/67	1.0	112/110	1.0
81–87	248/201	1.5(1.3–1.8)	32/30	1.1(0.7–1.8)	94/74	1.9(1.4–2.5)	184/158	1.3(1.1–1.6)
>87	343/148	2.8(2.0–3.8)	36/27	0.9(0.4–2.3)	143/44	4.6(2.7–8.0)	233/130	1.8(1.3–2.6)
Interaction test		p=0.083				p=0.002		
History of diabetes mellitus								
No	779/840	1.0	88/104	1.0	299/325	1.0	564/620	1.0
Yes	140/54	2.2(1.5–3.1)	16/16	0.9(0.4–2.0)	49/18	2.1(1.1–3.9)	103/50	1.8(1.2–2.7)
Interaction test		p=0.064				p=0.387		
Premenopausal women								
WHR								
0.774	26/90	0.4(0.3–0.7)	3/12	0.2(0.1–1.1)	10/44	0.4(0.2–0.8)	20/57	0.4(0.2–0.7)
0.775–0.803	69/86	0.7(0.6–0.8)	8/9	0.6(0.4–1.0)	20/34	0.7(0.5–0.8)	56/62	0.8(0.7–0.9)
0.804–0.830	73/64	1.0	13/10	1.0	31/22	1.0	56/52	1.0
0.831–0.864	108/63	1.5(1.2–2.0)	13/8	0.9(0.3–2.2)	45/27	2.0(1.3–3.1)	75/45	1.2(0.9–1.6)

rs1130864										rs2794520									
		C/C		C/T & T/T								T/T							
Category ^a		Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c
>0.864		125/35	2.4(1.6–3.6)	6/11	0.4(0.1–1.8)	47/14	3.2(1.7–6.0)	84/34	1.3(0.9–2.1)										
Interaction test			p<0.001																
Waist circumference (cm)																			
71		30/99	0.2(0.1–0.4)	3/13	0.2(0.0–1.4)	9/39	0.2(0.1–0.4)	23/72	0.3(0.2–0.5)										
72–76		61/100	0.5(0.4–0.6)	6/11	0.7(0.3–1.3)	22/39	0.4(0.3–0.5)	45/73	0.6(0.5–0.7)										
77–80		77/64	1.0	10/7	1.0	24/30	1.0	64/41	1.0										
81–87		111/51	2.1(1.6–2.9)	15/11	0.7(0.3–1.9)	47/20	3.1(1.9–5.0)	79/43	1.4(1.0–2.0)										
>87		123/24	6.9(3.8–12.6)	9/8	0.4(0.1–2.3)	51/13	12.1(4.5–32.6)	80/21	2.6(1.4–4.9)										
Interaction test			p=0.002																
History of diabetes mellitus																			
No		360/328	1.0	39/46	1.0	141/134	1.0	258/240	1.0										
Yes		41/8	2.0(0.9–4.6)	4/4	0.7(0.1–3.3)	12/4	1.7(0.3–4.1)	32/8	1.8(0.8–4.3)										
Interaction test			p=0.218																
Postmenopausal women																			
WHR																			
0.774		49/94	0.9(0.7–1.2)	11/7	1.9(0.7–4.9)	20/36	1.2(0.7–2.1)	40/65	0.9(0.7–1.3)										
0.775–0.803		60/96	0.9(0.8–1.0)	5/14	1.1(0.8–1.5)	21/46	0.9(0.8–1.1)	44/63	0.9(0.8–1.0)										
0.804–0.830		96/113	1.0	15/16	1.0	30/40	1.0	77/91	1.0										
0.831–0.864		132/119	1.3(1.1–1.6)	12/16	1.1(0.6–2.2)	60/43	1.5(1.1–2.1)	84/90	1.3(1.0–1.6)										
>0.864		188/138	1.5(1.1–2.0)	18/18	1.2(0.5–2.9)	68/38	2.1(1.3–3.5)	135/115	1.3(1.0–1.9)										
Interaction test			p=0.360																
Waist circumference (cm)																			
71		33/82	0.8(0.5–1.2)	5/9	1.0(0.2–4.4)	11/33	1.2(0.6–2.7)	27/59	0.7(0.4–1.1)										
72–76		66/107	0.8(0.7–1.0)	6/13	0.9(0.5–1.4)	25/48	0.9(0.7–1.2)	47/72	0.8(0.7–1.0)										
77–80		69/97	1.0	6/11	1.0	24/37	1.0	48/69	1.0										
81–87		137/150	1.3(1.1–1.6)	17/19	1.5(0.8–2.8)	47/54	1.6(1.1–2.4)	105/115	1.2(1.0–1.5)										
>87		220/124	2.0(1.3–2.9)	27/19	1.9(0.6–6.2)	92/31	3.3(1.7–6.7)	153/109	1.6(1.0–2.5)										
Interaction test			p=0.823																
History of diabetes mellitus																			

Category ^a	rs1130864				rs2794520			
	C/C		C/T & T/T		T/T		C/T & C/C	
	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c	Number ^b	OR (95% CI) ^c
No	419/512	1.0	49/58	1.0	158/188	1.0	306/380	1.0
Yes	99/46	2.2(1.5–3.3)	12/12	1.1(0.4–2.9)	37/14	2.9(1.4–6.1)	71/42	1.8(1.1–2.7)
Interaction test	p=0.144						p=0.198	

^a WHR, waist circumference, and BMI were categorized in the same way as presented in Table 3.

^b Number of cases/controls in each quintile.

^c The odds ratios (OR) and 95% confidence intervals (CI) were estimated with the same methods as described in the footnotes of Table 3.