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## The genetic contribution of CIDEA polymorphisms, haplotypes and loci interaction to obesity in a Han Chinese population

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Abstract To investigate the association of tag-SNPs and haplotype structures of the *CIDEA* gene with obesity in a Han Chinese population. Five single nucleotide polymorphisms (SNPs) (rs1154588/V115F, rs4796955/SNP1, rs8092502/SNP2, rs12962340/SNP3 and rs7230480/SNP4) in the *CIDEA* gene were genotyped in a case-control study. Genotyping was performed using the sequenom matrix-assisted laser desorption/ionization time-of-flight mass spectrometry iPLEX platform. There were significant differences between the obese and control groups in genotype distributions of V115F ( $P < 0.001$ ), SNP1 ( $P = 0.006$ ) and SNP2 ( $P = 0.005$ ). Carriers of V115F-TT, SNP1-GG and SNP2-CC genotypes had a 2.84-fold (95 % CI 1.73–4.66), 2.19-fold (95 % CI 1.09–4.38) and 4.37-fold (95 % CI 1.21–15.08) increased risk for obesity, respectively. Haplotype analysis showed that GTTC (SNP1/SNP2/V115F/SNP4) had 1.41-fold (95 % CI 1.02–1.95) increased risk for obesity; whereas, haplotype TTGC had 0.48-fold (95 % CI 0.24–0.96) decreased risk for obesity. Using the multifactor dimensionality reduction method, the best model including SNP1, SNP2, V115F and SNP4 polymorphisms was identified with a maximum testing accuracy to 59.32 % and a perfect cross-validation consistency of 10/10 ( $P = 0.011$ ). Logistic analysis indicated that there was a significant interaction between SNP1 and V115F associated with obesity. Subjects having both genotypes of SNP1/GG and V115F/TT were more susceptible to obesity in the Han Chinese population (OR 2.66, 95 %: 1.22–5.80). Genotypes of V115F/TT, SNP1/GG and SNP2/CC and haplotype GTTC of *CIDEA* gene were identified as risk factors for obesity in the Han Chinese population. The interaction between SNP1 and V115F could play a joint role in the development of obesity.

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Keywords (separated by '-') Chinese - Association study - Obesity - CIDEA - Polymorphism - Haplotype

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Footnote Information

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## The genetic contribution of *CIDEA* polymorphisms, haplotypes and loci interaction to obesity in a Han Chinese population

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**Abstract** To investigate the association of tag-SNPs and haplotype structures of the *CIDEA* gene with obesity in a Han Chinese population. Five single nucleotide polymorphisms (SNPs) (rs1154588/V115F, rs4796955/SNP1, rs8092502/SNP2, rs12962340/SNP3 and rs7230480/SNP4) in the *CIDEA* gene were genotyped in a case-control study. Genotyping was performed using the sequenom matrix-assisted laser desorption/ionization time-of-flight mass spectrometry iPLEX platform. There were significant differences between the obese and control groups in genotype distributions of V115F ( $P < 0.001$ ), SNP1 ( $P = 0.006$ )

and SNP2 ( $P = 0.005$ ). Carriers of V115F-TT, SNP1-GG and SNP2-CC genotypes had a 2.84-fold (95 % CI 1.73–4.66), 2.19-fold (95 % CI 1.09–4.38) and 4.37-fold (95 % CI 1.21–15.08) increased risk for obesity, respectively. Haplotype analysis showed that GTTC (SNP1/SNP2/V115F/SNP4) had 1.41-fold (95 % CI 1.02–1.95) increased risk for obesity; whereas, haplotype TTGC had 0.48-fold (95 % CI 0.24–0.96) decreased risk for obesity. Using the multifactor dimensionality reduction method, the best model including SNP1, SNP2, V115F and SNP4 polymorphisms was identified with a maximum testing accuracy to 59.32 % and a perfect cross-validation consistency of 10/10 ( $P = 0.011$ ). Logistic analysis indicated that there was a significant interaction between SNP1 and V115F associated with obesity. Subjects having both genotypes of SNP1/GG and V115F/TT were more susceptible to obesity in the Han Chinese population (OR 2.66, 95 %: 1.22–5.80). Genotypes of V115F/TT, SNP1/GG and SNP2/CC and haplotype GTTC of *CIDEA* gene were identified as risk factors for obesity in the Han Chinese population. The interaction between SNP1 and V115F could play a joint role in the development of obesity.

**Keywords** Chinese · Association study · Obesity · *CIDEA* · Polymorphism · Haplotype

### Introduction

Obesity, largely developed from the imbalance between energy intake and expenditure, manifests as excessive total body fat. It is a result of the interaction between environmental factors and genetic loads. It has been demonstrated in twins and familial studies that genetic contributions exist [1, 2]. Linkage and association studies indicate that cell

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53 death-inducing DNA fragmentation factor alpha-like  
54 effector A (*CIDEA*) is a candidate *gene* for the develop-  
55 ment of obesity [3–5].

56 The *CIDEA* gene (18p11.12) is 23.22 kb in length with  
57 five exons and four introns. It was identified by virtue of its  
58 sequence homology to the N-terminal region of the apop-  
59 totic DNA fragmentation factor Dff40/CAD and Dff45/  
60 ICAD [6]. *CIDEA* protein is a member of the cell death-  
61 inducing DNA fragmentation factor alpha-like effector  
62 (*CIDE*) protein family. *CIDEA* is highly expressed in  
63 brown adipose tissue (BAT) of rodents and white adipose  
64 tissue (WAT) of humans, and is associated with the  
65 development of obesity in both rodents [7] and humans [8].  
66 *CIDEA*-null mice show lean phenotypes with increased  
67 metabolic rate and lipolysis in BAT, and are resistant to  
68 diet-induced obesity and diabetes mellitus [7]. In humans,  
69 *CIDEA* expression is associated with a decrease in body  
70 mass index (BMI), waist measurement, waist-to-hip ratio  
71 (WHR) and basal metabolic rate [8]. It has also been  
72 suggested that *CIDEA* expression may cross-talk with  
73 tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). TNF- $\alpha$  down-regulates  
74 *CIDEA* expression and at the same time stimulates basal  
75 lipolysis in human fat cells [9].

76 Association studies on *CIDEA* gene focused on the  
77 V115F (rs11545881) single nucleotide polymorphism  
78 (SNP), which is a non-synonymous SNP in exon 4 that  
79 results in an amino acid substitution (V115F). A study  
80 showed that the V115F polymorphism was associated with  
81 BMI both in males ( $P = 0.023$ ) and females ( $P = 0.021$ ),  
82 and G allele was a risk allele (OR 1.32, 95 % CI  
83 1.03–1.69) in a Swedish population [10]. However, our  
84 previous research in both Japanese [11] and Chinese pop-  
85 ulations [12] have shown that the T allele may serve as a  
86 risk factor for metabolic syndrome and its related  
87 phenotypes.

88 In this study, we genotyped V115F (rs11545881) in  
89 another Chinese sample to validate this risk allele for obesity,  
90 and further selected another four tag-SNPs in the *CIDEA*  
91 gene (rs4796955/SNP1, rs8092502/SNP2, rs12962340/  
92 SNP3, and rs7230480/SNP4). This was done to investigate a  
93 possible interaction between the effects of SNPs and hap-  
94 lotypes of the *CIDEA* gene on obesity in Han Chinese.

## 95 Materials and methods

### 96 Subjects

97 This present study was a part of the National High Tech-  
98 nology Research and Development Program-863 of China,  
99 a population-based cross-sectional survey on relative risk  
100 factors of chronic non-communicable diseases (NCD) in  
101 the Chinese population during a 2-year period of

2007–2008. We selected 309 obese and 433 controls from  
the 3,000 participants of this nation-wide study and mat-  
ched on age, gender and residence. An individual was  
defined as being obese if they had a BMI of 28 kg/m<sup>2</sup> or  
more, according to the recommended standard by the  
Cooperative Meta-analysis Group of Working Group on  
Obesity in China [13]. We excluded from this study indi-  
viduals with the following: (1) physician-diagnosed dia-  
betes mellitus, coronary heart disease, myocardial  
infarction, stroke, cancer, severe kidney or liver diseases;  
(2) infectious diseases; (3) secondary obesity caused by  
other reasons; and (4) Cushing Syndrome.

All of the participants signed informed consents before  
participating in this study, with approval been granted by  
the Ethical Committee, Capital Medical University, Bei-  
jing, China.

### Measurement of anthropometric parameters

Following an interview by questionnaire, which covered  
demographic characteristics, residential history, socioeco-  
nomic status, personal behavior and medical history, all  
participants were asked to fast overnight before having a  
physical examination. Body weight, height, waist circum-  
ference (WC), hip circumference (HC), systolic blood  
pressure (SBP) and diastolic blood pressure (DBP) were  
measured by well-trained community doctors. Each mea-  
surement was performed three times and the average value  
was calculated as a final reading. Height and weight were  
measured to the nearest 0.1 kg and 0.1 cm respectively,  
with participants wearing light indoor clothing without  
shoes. BMI was calculated as weight in kilograms divided  
by height in meters squared (kg/m<sup>2</sup>). After inhalation and  
exhalation, WC was obtained at the midpoint between the  
lowest rib and the iliac crest to the nearest 0.1 cm, while  
the subject stood upright, with arms hanging freely and feet  
together. HC was measured over nonrestrictive underwear  
or light-weight shorts at the level of the maximum exten-  
sion of the buttocks in a horizontal level, without com-  
pressing the skin. WHR was calculated as WC divided by  
HC. Blood pressure was measured by mercury sphygmo-  
manometer on the right arm of the participant in a com-  
fortable sitting position after at least a 15 min rest.

### Finger capillary blood collection and DNA preparation

Finger capillary blood was collected in the morning after  
an overnight fasting, and stored on 903 specimen collection  
paper (Kent, UK). The saver card has a sample collection  
area of five 1.3 cm circles with each circle holding  
75–80  $\mu$ L of sample. Paper samples were air dried over-  
night, then individually placed in plastic bags with desic-  
cants and stored at  $-20^{\circ}\text{C}$ .



151 Whole-genome DNA was extracted by the Chelex-100  
 152 extraction method [14]. Firstly, a piece of 3 mm × 3 mm  
 153 dried blood stain was cut down and put into a 1.5 mL  
 154 centrifuge tube. Then 1 mL ddH<sub>2</sub>O was added, the tube  
 155 was shaken for 10 s and placed at room temperature for  
 156 half an hour. After centrifugation for 3 min at 12,500×g,  
 157 the majority of the supernatant liquid was removed and  
 158 200 μL of freshly prepared 5 % (w/v) Chelex-100 was  
 159 added into the tube. The sample was mixed for 10 s and  
 160 followed by centrifugation for 3 min at 12,500×g again.  
 161 The sample was then incubated at 56 °C for 30 min, fol-  
 162 lowed by 100 °C for 8 min. Finally, centrifugation for  
 163 3 min at 13,000×g was performed. The supernatant liquid  
 164 containing DNA was stored at 4 °C for amplification.

### 165 Tag-SNP selection

166 We downloaded Han Chinese population SNP data from  
 167 the database of the international HapMap Project (HapMap  
 168 Data Rel 24/phase II Nov08, on NVBI B36 assembly,  
 169 dbSNP b126). Using Haploview 4.0 software, we selected  
 170 five tag-SNPs of the *CIDEA* gene (SNP/V115F:  
 171 rs11545881, SNP1: rs4796955, SNP2: rs8092502, SNP3:  
 172 rs12962340, and SNP4: rs7230480) which had a minor  
 173 allele frequency (MAF)  $\geq 5\%$  in Han Beijing Chinese.  
 174 Among the SNPs whose  $r^2 \geq 0.8$ , we selected the one with  
 175 highest MAF for genotyping. Figure 1a shows the detailed  
 176 information of the selected tag-SNPs of the *CIDEA* gene.

### 177 SNP genotyping

178 A combined approach utilizing nested polymerase chain  
 179 reaction (PCR) and pyrosequencing technology (PSQ  
 180 96MA, BIOTAGE, Sweden) was used for V115F

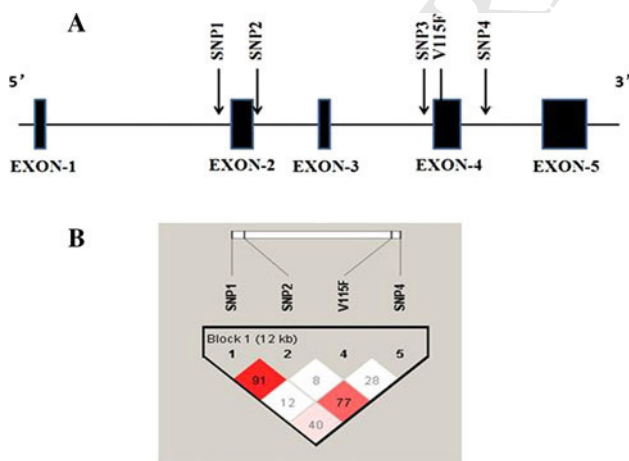
181 genotyping. The nested PCR primers were designed as fol-  
 182 lowed: the outside primers were 5'-CTGGCATAAGAGCA  
 183 GAGTG-3' (forward) and 5'-GAGCCTGTGGGATAAG  
 184 AGT-3' (reverse), and the inner primers were 5'-GGT  
 185 TAGGAAGGCTCCTGA-3' (forward) and 5'-GATGTCG  
 186 TAGGACACGGAGTA-3' (reverse). The pyrosequencing  
 187 primers were 5'-CAGGGCAGCCAGCAC-3'. The first-  
 188 stage PCR was executed in a 20 μL volume containing 2 μL  
 189 10× PCR buffer (including MgCl<sub>2</sub>), 2 μL dNTPs (2.5 mM),  
 190 0.2 μL forward primer(20 μM), 0.2 μL reverse pri-  
 191 mer(20 μM), 4 μL genomic DNA (25 ng/μL), 0.08 μL *Taq*  
 192 polymerase (5 U/μL, Takara, Japan), and 11.52 μL deion-  
 193 ized H<sub>2</sub>O. The second-stage PCR was executed in a 55 μL  
 194 volume containing 5.5 μL 10× PCR buffer (including  
 195 MgCl<sub>2</sub>), 5.5 μL dNTPs (2.5 mM), 0.55 μL forward primer  
 196 (20 μM), 0.55 μL reverse primer (20 μM), 3 μL DNA (the  
 197 production of the first-stage PCR), 0.22 μL *Taq* polymerase  
 198 (5 U/μL, Takara, Japan) and 39.68 μL deionized H<sub>2</sub>O. PCRs  
 199 were initiated by denaturation at 95 °C for 5 min, followed  
 200 by 35 cycles of: 30 s at 94 °C, 30 s at 57 °C, and 60 s at  
 201 72 °C, with the PCR products prolonged for 10 min at 72 °C  
 202 in the final cycle and finally held at 4 °C.

203 The genotyping of the other four tag-SNPs (SNP1:  
 204 rs4796955, SNP2: rs8092502, SNP3: rs12962340 and  
 205 SNP4: rs7230480) was performed using the sequenom  
 206 matrix-assisted laser desorption/ionization time-of-flight  
 207 (MALDI-TOF) mass spectrometry (MS) iPLEX platform  
 208 [15]. This technique is a high-throughput MS method for  
 209 detecting SNPs. According to the manufacturers' instruc-  
 210 tions, the whole process includes: multiplex PCR amplifi-  
 211 cation, shrimp alkaline phosphatase treatment, iPLEX  
 212 primer extension, clean resin, MALDI-TOF MS analysis  
 213 and data analysis [16, 17].

214 We randomly selected 30 samples from the participants  
 215 to validate the genotyping results of all the five SNPs using  
 216 another genotyping method, i.e., Sanger dideoxy method to  
 217 confirm the identity.

### 218 Statistical analysis

219 Each polymorphism was evaluated for Hardy–Weinberg  
 220 equilibrium by online software ([http://ihg2.helmholtz-  
 221 muenchen.de/cgi-bin/hw/hwa1.pl](http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl)).  $P \geq 0.01$  was consid-  
 222 ered to obey the Hardy–Weinberg equilibrium. The distri-  
 223 butions of allelic and genotypic frequencies were  
 224 analyzed using  $\chi^2$  test. The single locus association  
 225 between a polymorphism and obesity was estimated by  
 226 multiple logistic regression analysis, with age and gender  
 227 adjusted. For continuous variables with normal distribu-  
 228 tion, we used ANOVA to detect the difference of distri-  
 229 bution between the different genotypes. The variables  
 230 which were non-normal distributions were analyzed via  
 231 rank sum test. The statistical analyses were carried out



**Fig. 1** a The location of the tag-SNPs in the *CIDEA* gene. The exons were indicated by black boxes. b LD plot among five tag-SNPs of *CIDEA* gene

232 using SPSS version 19.0 for Windows (SPSS Inc., Chi-  
 233 cago, IL, USA). The frequencies of the haplotypes and  
 234 association analyses were completed by Haploview soft-  
 235 ware (version 4.0; Mark Daly's Laboratory, Broad Insti-  
 236 tute; <http://sourceforge.net/projects/haploview/>) [18]. We  
 237 analyzed the presence of interactions associated with  
 238 obesity susceptibility between the tag-SNPs by multifactor  
 239 dimensionality reduction method (MDR) (version 1.1.0;  
 240 Computational Genetics Laboratory, Dartmouth Medical  
 241 School, Lebanon, NH; [www.epistasis.org](http://www.epistasis.org)) and logistic  
 242 regression. The MDR method is nonparametric and model-  
 243 free, which is directly applicable to case-control studies to  
 244 detect the interaction between gene-gene and gene-envi-  
 245 ronment. The best MDR model is determined to have a  
 246  $P$  value  $<0.05$ , a maximum testing accuracy and a high  
 247 cross-validation consistency (CVC) [19]. Probability val-  
 248 ues presented were for two-tailed tests and  $P < 0.05$  was  
 249 considered statistically significant.

## 250 Results

### 251 V115F polymorphism of *CIDEA* gene and obesity

252 V115F (G/T) was genotyped in 742 participants (309 obese  
 253 vs. 433 controls), with the basal demographic and clinical  
 254 characteristics of these participants summarized in  
 255 Table 1. The obese group had significantly higher levels of  
 256 BMI, SBP, DBP, WC, HC and WHR compared to the  
 257 control group. No significant differences were found in age  
 258 and gender among the two groups (Table 1).

259 V115F genotypic frequencies for the GG, GT and TT  
 260 were 19.54, 59.70, and 20.75 %, respectively. The allelic  
 261 frequencies of G and T alleles were 49.39 and 50.61 %,  
 262 respectively. The genotypic distribution of the V115F  
 263 followed Hardy-Weinberg equilibrium in the controls  
 264 ( $P = 0.011$ ). The frequency of the TT genotype was sig-  
 265 nificantly higher in the obese group compared to the con-  
 266 trol group (23.62 vs. 18.71 %,  $P < 0.001$ ) (Table 2).  
 267 Multiple logistic regression analysis (age and gender  
 268 adjusted) identified that participants with the TT genotype  
 269 were 2.84-fold at risk (95 % CI 1.73-4.66,  $P < 0.001$ ) and  
 270 those with the GT genotype were 2.63-fold at risk  
 271 (95 % CI 1.72-4.01,  $P < 0.001$ ) for obesity when com-  
 272 pared to those with the GG genotype. Meanwhile,  $\chi^2$   
 273 analysis results showed that participants with the T allele  
 274 were 1.46-fold (95 % CI 1.19-1.80,  $P < 0.001$ ) at risk for  
 275 obesity when compared to those with the G allele.

276 In genotypic model (GG vs. GT vs. TT), we found that  
 277 the average BMI, WC, HC and WHR measurements were  
 278 highest in patients with the TT genotype followed by GT  
 279 and GG. In the dominant model (TT vs. TG + GG), we  
 280 found that these obesity related levels were significantly

**Table 1** Characteristics of 742 participants based on the V115F genotype

Variable	Total	Control	Obesity	$P_1$	GG	GT	TT	GG + GT	GT + TT	$P_2$	$P_3$	$P_4$
Number (%)	742 (100.00)	433 (58.36)	309 (41.64)	-	145 (19.54)	443 (59.70)	154 (20.75)	588 (79.25)	597 (80.46)	-	-	-
Gender (male, %)	316 (42.6)	183 (42.26)	133 (43.04)	0.832 <sup>Δ</sup>	54 (37.24)	189 (42.66)	73 (47.40)	243 (41.33)	263 (43.89)	0.206 <sup>Δ</sup>	0.175 <sup>Δ</sup>	0.147 <sup>Δ</sup>
Age (years)	49.65 ± 12.12	49.42 ± 12.41	49.97 ± 11.71	0.539 <sup>§</sup>	50.24 ± 0.85	49.26 ± 10.96	50.24 ± 12.76	49.50 ± 11.95	49.51 ± 11.45	0.056 <sup>§</sup>	0.723 <sup>#</sup>	0.515 <sup>§</sup>
BMI (kg/m <sup>2</sup> )	26.20 ± 4.82	23.02 ± 2.32	30.65 ± 3.76	<0.001 <sup>#*</sup>	24.68 ± 3.90	26.44 ± 4.94	26.94 ± 4.97	26.00 ± 4.76	26.57 ± 4.95	<0.001 <sup>#*</sup>	<0.001 <sup>#*</sup>	<0.001 <sup>#*</sup>
SBP (mmHg)	132.84 ± 21.45	129.10 ± 21.11	138.08 ± 20.85	<0.001 <sup>#*</sup>	131.73 ± 23.13	132.57 ± 20.91	134.66 ± 21.40	132.36 ± 21.45	133.11 ± 21.04	0.585 <sup>#</sup>	<0.001 <sup>#*</sup>	<0.001 <sup>#*</sup>
DBP (mmHg)	84.93 ± 12.65	82.36 ± 12.48	88.54 ± 12.02	<0.001 <sup>#*</sup>	82.85 ± 14.02	85.10 ± 11.90	86.43 ± 13.23	84.54 ± 12.47	85.44 ± 12.26	0.101 <sup>#</sup>	<0.001 <sup>#*</sup>	<0.001 <sup>#*</sup>
WC (cm)	88.61 ± 12.15	82.18 ± 9.90	97.66 ± 8.79	<0.001 <sup>#*</sup>	84.64 ± 11.77	89.16 ± 12.21	90.80 ± 11.51	88.04 ± 12.25	89.58 ± 12.05	<0.001 <sup>#*</sup>	0.012 <sup>§</sup>	<0.001 <sup>#*</sup>
HC (cm)	101.27 ± 10.11	96.28 ± 8.03	108.28 ± 8.43	<0.001 <sup>#*</sup>	99.18 ± 10.83	101.44 ± 10.19	102.75 ± 8.82	100.88 ± 10.39	101.78 ± 9.86	0.008 <sup>§*</sup>	0.042 <sup>§</sup>	0.005 <sup>§*</sup>
WHR	0.88 ± 0.10	0.85 ± 0.92	0.91 ± 0.11	<0.001 <sup>#*</sup>	0.85 ± 0.07	0.88 ± 0.12	0.88 ± 0.07	0.87 ± 0.11	0.88 ± 0.11	0.001 <sup>#*</sup>	<0.001 <sup>#*</sup>	<0.001 <sup>#*</sup>
Obesity, N (%)	309 (41.64)	-	-	-	35 (24.14)	201 (45.37)	73 (47.40)	236 (40.14)	274 (45.00)	<0.001 <sup>#*</sup>	0.103 <sup>Δ</sup>	<0.001 <sup>#*</sup>

Values are mean ± SD or number and percentage.  $P_1$  values are calculated by  $\chi^2$  (<sup>Δ</sup>) or one-ANOVA/T test (<sup>§</sup>) or rank sum test (<sup>#</sup>) or  $\chi^2$  logistic regression (age and sex adjusted). \*  $P < 0.05$ .  $P_1$  value: obesity group versus control group;  $P_2$  value: GG versus TT;  $P_3$  value: GG + GT versus TT;  $P_4$  value: GT + TT versus GG

Abbreviations: BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, WC waist circumference, HC hip circumference, WHR waist-hip rate

**Table 2** Multiple logistic regression analysis of associations between the *CIDEA* genotypes and obesity

SNPs	Polymorphism	Control <sup>a</sup>	Obesity <sup>a</sup>	<i>P</i> value <sup>b</sup>	OR	95 % CI
SNP1	Genotype			0.006*		
	GG	46 (26.59)	68 (43.31)	0.027*	2.19	1.09–4.38
	GT	99 (57.23)	70 (44.59)	0.903	1.04	0.54–2.02
	TT	28 (16.18)	19 (12.10)	–	1.00	–
	Allele					
	G	191 (55.20)	206 (65.61)	0.006 <sup>Δ</sup> *	1.55	1.13–2.12
SNP2	Genotype			0.005*		
	CC	3 (1.73)	13 (8.39)	0.025*	4.37	1.21–15.80
	TC	62 (35.84)	36 (23.23)	0.035*	0.59	0.04–0.96
	TT	108 (62.43)	106 (68.39)	–	1.00	–
	Allele					
	C	68 (19.65)	62 (20.00)	0.911 <sup>Δ</sup>	1.02	0.70–1.50
SNP3	Genotype			0.367		
	TT	91 (81.98)	76 (88.37)	0.811	1.16	0.35–3.80
	TA	13 (11.71)	5 (5.81)	0.424	0.53	0.11–2.50
	AA	7 (6.31)	5 (5.81)	–	1.00	–
	Allele					
	T	195 (87.84)	157 (91.28)	0.272 <sup>Δ</sup>	1.45	0.75–2.82
SNP4	Genotype			0.968		
	CC	128 (73.99)	117 (74.52)	0.799	1.22	0.26–5.67
	CT	41 (23.7)	37 (23.57)	0.810	1.21	0.25–5.86
	TT	4 (2.31)	3 (1.91)	–	1.00	–
	Allele					
	C	297 (85.84)	271 (86.31)	0.862 <sup>Δ</sup>	1.04	0.670–1.62
V115F	Genotype			<0.001*		
	TT	81 (18.71)	73 (23.62)	<0.001*	2.84	1.73–4.66
	GT	242 (55.89)	201 (65.05)	<0.001*	2.63	1.72–4.01
	GG	110 (25.40)	35 (11.33)	–	1.00	–
	Allele					
	T	404 (46.65)	347 (56.15)	<0.001 <sup>Δ</sup> *	1.46	1.19–1.80
Combined genotypes	Genotype			<0.001*		
	0-risk	106 (61.27)	61 (38.85)	–	1.00	–
	1-risk	56 (32.37)	72 (45.68)	0.001*	2.23	1.40–3.58
	2-risk	11 (63.58)	24 (15.29)	0.001*	3.79	1.74–8.28

OR odd ratio, 95 % CI 95 % confidence interval

\*  $P < 0.05$

<sup>a</sup> Numbers are frequencies and percentage

<sup>b</sup>  $P$  value was calculated by  $\chi^2$  test (<sup>Δ</sup>) or multiple logistic regression (age and sex adjusted)

281 higher in TT group than those in GG + GT group (BMI,  
282  $P < 0.001$ ; SBP,  $P < 0.001$ ; DBP,  $P < 0.001$ ; WC,  
283  $P = 0.012$ ; HC,  $P = 0.042$ ; WHR,  $P < 0.001$ , respectively)  
284 (Table 1). In the recessive model (GG vs. GT + TT), the

differences of these levels were significantly higher in  
GT + TT group compared to the GG group as expected  
(BMI,  $P < 0.001$ ; SBP,  $P < 0.001$ ; DBP,  $P < 0.001$ ; WC,  
 $P < 0.001$ ; HC,  $P = 0.005$ , WHR,  $P < 0.001$ , respectively)

285  
286  
287  
288

289 (Table 1). The distribution of the two genotype frequencies  
290 were significantly different between the obese and controls  
291 ( $P < 0.001$ ).

#### 292 Association between the other four tag-SNPs 293 and obesity

294 We genotyped another four selected *CIDEA* tag-SNPs  
295 (SNP1 G/T, SNP2 T/C, SNP3 T/A, SNP4 C/T) in 330  
296 participants (obese/controls = 157/173). Distributions of  
297 the genotypes and alleles of the four SNPs are listed in  
298 Table 2. Analysis showed that the controls were in Hardy–  
299 Weinberg equilibrium at SNP1 ( $P = 0.039$ ), SNP2  
300 ( $P = 0.076$ ) and SNP4 ( $P = 0.740$ ), while the genotypic  
301 distribution of SNP3 did not follow Hardy–Weinberg  
302 equilibrium ( $P < 0.001$ ), so SNP3 was excluded from  
303 further analysis. The MAF of these SNPs (SNP1T, SNP2C  
304 and SNP4T) were 44.80, 19.65 and 14.17 % in controls,  
305 respectively (Table 2). These were consistent with the  
306 MAF of Han Chinese in Beijing, China (<http://www.ncbi.nlm.nih.gov/pubmed>). Multiple logistic regression analysis  
307 (adjusted by age and gender) indicated that both SNP1 and  
308 SNP2 polymorphisms were significantly associated with  
309 obesity ( $P = 0.006$  and  $0.005$ , respectively) (Table 2).  
310 SNP1/GG and SNP2/CC genotypes were more frequent in  
311 the obese group compared to the control group ( $P = 0.027$ ,  
312  $0.025$ , respectively).

314 The analysis results of SNP1 showed overall that WC and  
315 BMI levels in the GG genotype (WC =  $90.31 \pm 10.91$  cm;  
316 BMI =  $27.08 \pm 4.24$  kg/m<sup>2</sup>) were significantly higher  
317 compared to any other two genotypes (GT: WC =  $86.06 \pm$   
318  $12.00$  cm,  $P = 0.030$ ; BMI =  $25.45 \pm 4.45$  kg/m<sup>2</sup>,  $P =$   
319  $0.030$ ; TT: WC =  $85.94 \pm 11.36$  cm,  $P = 0.002$ ; BMI =  
320  $25.41 \pm 4.43$  kg/m<sup>2</sup>,  $P = 0.028$ ), suggesting that partici-  
321 pants with the GG genotype were more susceptible to  
322 obesity. Multiple logistic regression (adjusted for age and  
323 gender) analysis revealed that when compared with the TT  
324 genotype, participants carrying the GG genotype had a 2.19-  
325 fold (95 % CI 1.09–4.38,  $P = 0.027$ ) risk of obesity, and

when compared with the T allele, participants with the G 326  
allele had a 1.55-fold (95 % CI 1.13–2.12,  $P = 0.006$ ) risk 327  
of obesity. All of these results indicated that the variant G 328  
allele of SNP1 was the risk allele of obesity. 329

The analysis results of SNP2 showed that WC levels were 330  
higher based on genotypes of CT ( $84.55 \pm 11.62$  cm) < TT 331  
( $88.31 \pm 11.32$  cm) < CC ( $92.75 \pm 11.70$  cm), and there 332  
was a statistically significant difference between the three 333  
genotypes ( $P = 0.005$ ). Logistic regression analysis of 334  
SNP2 showed that when compared with the TT genotype, 335  
participants with the CC genotype had a 4.37-fold (95 % CI 336  
1.21–15.80,  $P = 0.025$ ) risk, while the CT genotype was 337  
lower with a 0.59-fold (95 % CI 0.04–0.96,  $P = 0.035$ ) risk 338  
of obesity. No significant difference was detected in the BMI 339  
according to the genotypes. 340

#### 341 Haplotypes analysis of the selected tag-SNPs of *CIDEA* 342 gene

When we combined the four tag-SNPs and inferred haplo- 343  
types using Haploview 4.0 software, ten possible haplotypes 344  
were derived from the observed genotypes (SNP1/SNP2/  
345 V115F/SNP4) (Fig. 1b). Six haplotypes with frequencies 346  
above 5 % were haplotype 1 (H1)-GTTC (33.5 %), H2- 347  
GTGC (20.7 %), H3-TCGC (9.4 %), H4-TCTC (8.9 %), 348  
H5-TTTC (6.7 %) and H6-TTGC (6.0 %) (Table 3). H1 was 349  
more common in the obese participants (37.55 %) compared 350  
to the controls (29.91 %,  $P = 0.039$ ), while H6 was common 351  
in the controls (7.92 %) compared to the obese (3.98 %, 352  
 $P = 0.034$ ). The risk of obesity was significantly increased 353  
among the participants carrying haplotype H1 (OR 1.41, 354  
95 % CI 1.02–1.95), and decreased among participants with 355  
haplotype H6 (OR 0.48, 95 % CI 0.24–0.96). 356

#### 357 Interaction analysis of *CIDEA* gene tag-SNPs 358 on obesity

Assuming a combined model (i.e. homozygous risk geno- 359  
types vs. the combining group of the other two genotypes), 360

**Table 3** Frequencies of the haplotypes based on the tag-SNPs in obese and controls

Haplotypes	Genotype				Freq.	Obesity <i>n</i> (%)	Control <i>n</i> (%)	$\chi^2$ value	<i>P</i> value	OR	95 % CI
	SNP1	SNP2	V115F	SNP4							
H1	G	T	T	C	0.335	117.9 (37.55)	103.5 (29.91)	4.28	0.039*	1.41	1.02–1.95
H2	G	T	G	C	0.207	65.9 (20.99)	70.8 (20.46)	0.03	0.869	1.03	0.71–1.51
H3	T	C	G	C	0.094	26.4 (8.41)	35.4 (10.23)	0.64	0.425	0.81	0.48–1.37
H4	T	C	T	C	0.089	31.1 (9.90)	27.8 (8.03)	0.71	0.401	1.26	0.74–2.15
H5	T	T	T	C	0.067	15.5 (4.94)	28.5 (8.24)	2.9	0.088	0.58	0.31–1.10
H6	T	T	G	C	0.060	12.5 (3.98)	27.4 (7.92)	4.51	0.034*	0.48	0.24–0.96

OR odd ratio, 95 % CI 95 % confidence interval

\*  $P < 0.05$

we did combined analyses for the three SNPs which were significantly associated with obesity in the previous single locus analysis; SNP1 (GG vs. GT + TT); SNP2 (CC vs. CT + TT); V115F (TT vs. GT + GG). Compared with those carrying genotypes of SNP1/GT + TT, SNP2/CT + TT and V115F/GT + GG, participants carrying only one of the three homozygous risk genotypes (SNP1/GG or SNP2/CC or V115F/TT) were associated with a 2.23-fold (95 % CI 1.40–3.58,  $P = 0.001$ ) increased risk to obesity, while the risk was statistically increased to 3.79-fold (95 % CI 1.74–8.28,  $P = 0.001$ ) among individuals carrying two of the three homozygous risk genotypes (SNP1/GG\*SNP2/CC, SNP2/CC\*V115F/TT, SNP1/GG\*V115F/TT) (Table 2). Furthermore, we found that among the participants with two homozygous risk genotypes, 91.43 % of them were carrying both genotypes of SNP1/GG and V115F/TT. The other 8.57 % were carrying both genotypes of SNP2/CC and V115F/TT.

In logistic regression models (adjusted by age and gender), the interaction between SNP1 and V115F was significantly associated with the susceptibility of obesity ( $P = 0.012$ ). The interaction showed that individuals with both genotypes of SNP1/GG and V115F/TT were associated with 2.66-fold (95 % CI 1.22–5.80,  $P = 0.012$ ) risk of obesity, compared with the others. The risk was increased to 3.21-fold when compared to participants with both genotypes of SNP1/TT and V115F/GG (95 % CI 1.33–7.73,  $P = 0.009$ ).

MDR analysis was also used to detect the interaction between the four tag-SNPs (V115F, SNP1, SNP2 and SNP4). Table 4 summarizes the best interaction models. In one-factor model, SNP2 was the best attribute for predicting obesity (testing accuracy = 54.58 %; CVC = 9/10,  $P = 0.377$ ). SNP1 and SNP2 was the best two-factor model (testing accuracy = 53.56 %, CVC = 7/10,  $P = 0.172$ ). However, by following the best model selected principle, the best model was determined to be a four-loci site model, which includes the polymorphisms of SNP1, SNP2, V115F and SNP4, with a maximum testing accuracy to 59.32 % and a perfect CVC of 10/10 ( $P = 0.011$ ). Thus, the interaction dendrogram (Fig. 2) showed that these four SNPs linked by green lines were on the same branch, suggesting a synergistic interaction effect on modulating the risk of obesity.



**Fig. 2** Interaction dendrogram. The different color connections show the degree of interaction from synergy (red) to redundancy (blue)

## Discussion

In this study, we genotyped five tag-SNPs in the *CIDEA* gene and investigated their associations with the risk of obesity in a Han Chinese population. We found that SNP1-rs4796955/GG genotype, SNP2-rs8092502/CC genotype, V115F-rs11545881/TT genotype and haplotype GTTC were associated with an increased risk of obesity ( $P < 0.05$ ). The MDR analysis identified a significant four-factor interaction model including SNP1, SNP2, V115F and SNP4, suggesting that there was an interaction between the four SNPs. The logistic regression analysis (adjusted by age and gender) showed the interaction between SNP1 and V115F was significantly associated with the susceptibility of obesity.

Both human and mouse models show that *CIDEA* protein is emerging as an important regulator of the lipid metabolic pathway, and it plays important roles in lipid storage, lipid droplet format, lipolysis and the development of metabolic disorders such as obesity, diabetes mellitus, hepatic steatosis and cardiovascular diseases [7–9]. Mice with a deficiency in *CIDEA* were resistant to high-fat diet-induced obesity and diabetes mellitus with an increased metabolic rate, lipolysis in BAT and core body temperature when subjected to cold treatment, suggesting that *CIDEA* is important in energy expenditure in adipose tissues [7]. Their lean phenotype seems to be due to a loss of *CIDEA* protein direct suppression of uncoupling protein 1 (UCP1) activity in BAT [20]. However, there are some striking discrepancies between human and rodent *CIDEA* protein expression patterns. *CIDEA* protein is highly expression in BAT of rodent but in WAT of humans [8]. In contrast with the mouse model, *CIDEA* protein expression in humans is inversely associated with BMI, WC, WHR and basal metabolic rate. Some studies have reported that *CIDEA* protein

**Table 4** Summary of the MDR interaction models

Model	Training bal. acc. (%)	Testing bal. acc. (%)	Sign test ( $P$ )	CV consistency
SNP2	57.49	54.58	6 (0.377)	9/10
SNP1SNP2	60.23	53.56	7 (0.172)	7/10
SNP1SNP2V115	63.23	54.75	9 (0.011*)	7/10
SNP1SNP2V115SNP4	65.91	59.32	9 (0.011*)	10/10

\*  $P < 0.05$

439 expression was decreased two-fold in obese humans and  
 440 normalized after weight reduction [9]. A study in 40 obese  
 441 women showed that *CIDEA* gene expression is significantly  
 442 up-regulated as a result of the energy-restricted diets inter-  
 443 vention [21]. In contrast to the mechanism in mice, a study  
 444 found that there is a cross-talk between *CIDEA* and TNF- $\alpha$  in  
 445 human adipose tissue [9], and this has consequences for  
 446 lipolysis. *CIDEA* decreases the availability of TNF- $\alpha$  by  
 447 inhibiting cytokine secretion predominately through post-  
 448 transcriptional mechanisms, which in turn counteracts the  
 449 ability of TNF- $\alpha$  to stimulate lipolysis. TNF- $\alpha$  down-regu-  
 450 lates the expression of *CIDEA* through signaling via c-Jun  
 451 NH<sub>2</sub>-terminal kinase (JNK), which in turn increases the  
 452 availability of TNF- $\alpha$  and thereby lipolytic stimulation [9].  
 453 In a recent energy restriction intervention study [8], a sig-  
 454 nificant inverse correlation has been found between UCP1  
 455 and *CIDEA* expression levels, indicating a possible interac-  
 456 tion between *CIDEA* and UCP1 in humans. *CIDEA* is also  
 457 associated with insulin sensitivity in humans [22]. Recently,  
 458 a study found that starvation-induced apoptosis in adipocytes  
 459 is significantly inhibited when insulin decreased *CIDEA*  
 460 mRNA expression levels, suggesting that *CIDEA* is a novel  
 461 gene regulated by insulin in human adipocytes and that it  
 462 may play a key role in obesity [23].

463 *CIDEA* polymorphisms have been reported to be asso-  
 464 ciated with human obesity in Swedish, Japanese and Chi-  
 465 nese populations. In this study, the G allele frequency of  
 466 V115F was 49.39 %, which was lower than that previously  
 467 reported in the Chinese (55.25 %) population and higher  
 468 than reported in the Japanese (48.90 %) population [11,  
 469 12]. Multiple logistical regression results showed that  
 470 participants with the TT genotype had a 2.84-fold  
 471 (95 % CI 1.73–4.66,  $P < 0.001$ ) risk for obesity compared  
 472 to those with the GG genotype. There was a trend that all  
 473 the index levels of obesity related phenotypes in the par-  
 474 ticipants were higher in the TT genotype group compared  
 475 to the GG genotype groups (TT > GT > GG). Both  
 476 genetic and continuous variable analyses indicated that the  
 477 T allele of V115F SNP was a risk factor for obesity in  
 478 Chinese. This result is consistent with our previous studies  
 479 in both Japanese [11] and Chinese studies [12], but con-  
 480 flicts with the Swedish study [10]. This result could be due  
 481 to the so-called “flip-flop” phenomenon, where, within  
 482 differing ethnic groups, disease marker associations with  
 483 reversed risk alleles are found [24, 25].

484 The possible impact of amino acid substitution of V115F  
 485 on the structure and function of *CIDEA* protein would be  
 486 benign based on the POLYPHEN analysis [12]. We con-  
 487 sidered that there might be some other causal variants at this  
 488 locus, whose polymorphism, interaction or linkage disequi-  
 489 librium could contribute to obesity; therefore, we further  
 490 genotyped another four tag-SNPs of the *CIDEA* gene to test  
 491 our hypotheses.

492 In single locus analysis, we found that two other new  
 493 SNPs (SNP1 and SNP2) were associated with obesity.  
 494 Subjects with SNP1/GG and SNP2/CC genotypes had  
 495 higher levels of WC, and were associated with 2.19-fold  
 496 and 4.37-fold increased susceptibility to obesity when  
 497 compared with other genotype groups. Both SNP1 and  
 498 SNP2 were intronic polymorphisms whose functions were  
 499 not known. However, there have been reports about the  
 500 association between intronic polymorphisms and different  
 501 diseases [26–28]. For example, it was reported that up to  
 502 40 % of transcription factor binding sites are located within  
 503 introns. The exact molecular mechanisms of how the SNP1  
 504 and SNP2 variants affect obesity are unknown and require  
 505 further investigation.

506 In haplotype analysis, we found that haplotype GTTC had  
 507 1.41-fold risk, while haplotype TTGC was a protective factor  
 508 for obesity. Not surprisingly, the differences between hap-  
 509 lotype GTTC and TTGC were associated with SNP1 G/T and  
 510 V115F G/T alleles. Both of these risk alleles (SNP1/G and  
 511 V115F/T) contributed to the risk haplotype of GTTC, while  
 512 the protective alleles (SNP1/T and V115F/G) contributed  
 513 to the haplotype TTGC. Logistic regression analysis found that  
 514 there was a statistically significant interaction between these  
 515 two SNPs, and participants with both SNP1/GG and V115F/  
 516 TT genotypes had 2.66-fold risk of developing obesity.

517 There is significant evidence showing that complex dis-  
 518 eases are induced by gene–gene, gene–environmental and  
 519 gene–environmental–behavior interactions. It is conceivable  
 520 that obesity is the result of interactions between multiple  
 521 genetic variations. In this study, the combined results of the  
 522 nonparametric MDR approach and the parametric logistic  
 523 analysis (adjusted by age and gender) indicated that the  
 524 interaction between SNP1/GG and V115F/TT could increase  
 525 the susceptibility of obesity occurring. Although our data  
 526 cannot explain the biological mechanism, the result suggests  
 527 that an interaction model could provide guidance to experi-  
 528 mental studies on the metabolic pathway of obesity.

529 For this population screening study, 903 specimen col-  
 530 lection paper was used to collect finger blood, which causes  
 531 less discomfort to the subjects. The dried blood spots needed  
 532 minimal storage space, caused little biohazard risk and were  
 533 convenient for transportation [14]. The method also had the  
 534 disadvantage of not having fresh blood samples for blood  
 535 biochemical analyses such as triglyceride, total cholesterol,  
 536 high density lipoprotein, which are associated with lipolysis.  
 537 There were other limitations in our study. Firstly, the con-  
 538 founding factors such as diet, physical activity and environ-  
 539 ment were not considered. Secondly, all the associations  
 540 offered in this study were a population-genetics based  
 541 approach supported by statistical analyses, and therefore the  
 542 explanation of the biological mechanism of obesity needs  
 543 further investigation. Furthermore, recent interesting findings  
 544 collectively highlight the complicated metabolite profiles in

545 obesity at omics level, inspiring that post-genomics comple-  
546 mentary approaches in obesity research are needed [29].

547 In conclusion, this is the first attempt to haplotype four  
548 SNPs in the *CIDEA* gene in a Han Chinese population, and  
549 we found that SNP1-rs4796955, SNP2-rs8092502, V115F-  
550 rs11545881, haplotype GTTC and haplotype TTGC were  
551 associated with the susceptibility of obesity. The strong  
552 interaction between SNP1 and V115F could play a joint  
553 role in the development of obesity. Further studies with  
554 ethnically diverse populations and functional evaluation  
555 are warranted to confirm our findings.

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## 571 References

- 572 1. Stunkard AJ, Sørensen TIA, Hanis C et al (1986) An adoption  
573 study of human obesity. *N Engl J Med* 314:193–198
- 574 2. Bouchard C, Tremblay A, Després JP et al (1990) The response to  
575 long-term overfeeding in identical twins. *N Engl J Med*  
576 322:1477–1482
- 577 3. Chagnon YC, Rice T, Pérusse L et al (2001) Genomic scan for  
578 genes affecting body composition before and after training in  
579 Caucasians from HERITAGE. *J Appl Physiol* 90:1777–1787
- 580 4. Chen W, Li S, Cook NR et al (2004) An autosomal genome scan  
581 for loci influencing longitudinal burden of body mass index from  
582 childhood to young adulthood in white sibships: the Bogalusa  
583 heart study. *Int J Obes Relat Metab Disord* 28:462–469
- 584 5. Parker A, Meyer J, Lewitzky S et al (2001) A gene conferring  
585 susceptibility to type 2 diabetes in conjunction with obesity is  
586 located on chromosome 18p11. *Diabetes* 50:675–680
- 587 6. Inohara N, Koseki T, Chen S et al (1998) *CIDE*, a novel family of  
588 cell death activators with homology to the 45 kDa subunit of the  
589 DNA fragmentation factor. *EMBO J* 17:2526–2533
- 590 7. Zhou Z, Yon Toh S, Chen Z et al (2003) *Cidea*-deficient mice have  
591 lean phenotype and are resistant to obesity. *Nat Genet* 35:49–56
- 592 8. Gummesson A, Jernås M, Svensson PA et al (2007) Relations of  
593 adipose tissue *CIDEA* gene expression to basal metabolic rate,  
594 energy restriction, and obesity: population-based and dietary  
595 intervention studies. *J Clin Endocrinol Metab* 92:4759–4765
- 596 9. Nordström EA, Rydén M, Backlund EC et al (2005) A human-  
597 specific role of cell death-inducing DFFA (DNA fragmentation  
598 factor- $\alpha$ )-like effector A (*CIDEA*) in adipocyte lipolysis and  
599 obesity. *Diabetes* 54:1726–1734
- 600 10. Dahlman I, Kaaman M, Jiao H et al (2005) The *CIDEA* gene  
601 V115F polymorphism is associated with obesity in Swedish  
602 subjects. *Diabetes* 54:3032–3034

- 603 11. Zhang L, Miyaki K, Nakayama T et al (2008) Cell death-  
604 inducing DNA fragmentation factor [alpha]-like effector A (*CIDEA*)  
605 gene V115F (G→T) polymorphism is associated with  
606 phenotypes of metabolic syndrome in Japanese men. *Metabolism*  
607 57:502–505
- 608 12. Zhang L, Dai Y, Bian L et al (2011) Association of the cell death-  
609 inducing DNA fragmentation factor alpha-like effector A (*CIDEA*)  
610 gene V115F (G/T) polymorphism with phenotypes of  
611 metabolic syndrome in a Chinese population. *Diabetes Res Clin*  
612 Pract 91:233–238
- 613 13. Zhapu BF, Cooperative Meta-Analysis Group of Working Group  
614 on Obesity in China (2002) Predictive values of body mass index  
615 and waist circumference for risk factors of certain related dis-  
616 eases in Chinese adults: study on optimal cut-off points of body  
617 mass index and waist circumference in Chinese adults. *Biomed*  
618 *Environ Sci* 15:83–96
- 619 14. Zhang J, Zhang L, Song MS et al (2010) Detection of HBV-DNA  
620 in dried bloodstains on filter paper by nested polymerase chain  
621 reaction. *Lab Med* 41:535–539
- 622 15. Jurinke C, van den Boom D, Cantor CR et al (2001) Automated  
623 genotyping using the DNA MassArray™ technology. *Methods*  
624 *Mol Biol* 170:103–116
- 625 16. Söderlund-Strand A, Dillner J, Carlson J (2008) High-throughput  
626 genotyping of oncogenic human papilloma viruses with MALDI-  
627 TOF mass spectrometry. *Clin Chem* 54:86–92
- 628 17. Schaeffeler E, Zanger UM, Eichelbaum M et al (2008) Highly  
629 multiplexed genotyping of thiopurine *S*-methyltransferase vari-  
630 ants using MALDI-TOF mass spectrometry: reliable genotyping  
631 in different ethnic groups. *Clin Chem* 54:1637–1647
- 632 18. Barrett JC, Fry B, Maller J et al (2005) Haploview: analysis and  
633 visualization of LD and haplotype maps. *Bioinformatics* 21:263–265
- 634 19. Ritchie MD, Hahn LW, Roodi N et al (2001) Multifactor-  
635 dimensionality reduction reveals high-order interactions among  
636 estrogen-metabolism genes in sporadic breast cancer. *Am J Hum*  
637 *Genet* 69:138–1347
- 638 20. Lin SC, Li P (2004) *CIDEA*, a novel link between brown adipose  
639 tissue and obesity. *Trends Mol Med* 10:434–439
- 640 21. Dahlman I, Linder K, Nordström EA et al (2005) Changes in  
641 adipose tissue gene expression with energy-restricted diets in  
642 obese women. *Am J Clin Nutr* 81:1275–1285
- 643 22. Puri V, Ranjit S, Konda S et al (2008) *Cidea* is associated with  
644 lipid droplets and insulin sensitivity in humans. *Proc Natl Acad*  
645 *Sci USA* 105:7833–7888
- 646 23. Ito M, Nagasawa M, Hara T et al (2010) Differential roles of *CIDEA*  
647 and *CIDE*C in insulin-induced anti-apoptosis and lipid  
648 droplet formation in human adipocytes. *J Lipid Res* 51:1676–1684
- 649 24. Tan EK, Tan C, Shen H et al (2003) Alpha synuclein promoter  
650 and risk of Parkinson’s disease: microsatellite and allelic size  
651 variability. *Neurosci Lett* 336:70–72
- 652 25. Chartier-Harlin MC, Kachergus J, Roumier C et al (2004) Alpha-  
653 synuclein locus duplication as a cause of familial Parkinson’s  
654 disease. *Lancet* 364:1167–1169
- 655 26. Zhang Z, Wang S, Wang M et al (2008) Genetic variants in  
656 *RUNX3* and risk of bladder cancer: a haplotype-based analysis.  
657 *Carcinogenesis* 29:1973–1978
- 658 27. Sano M, Kuroi N, Nakayama T et al (2005) Association study of  
659 calcitonin-receptor-like receptor gene in essential hypertension.  
660 *Am J Hypertens* 18:403–408
- 661 28. Lehman DM, Fu DJ, Freeman AB et al (2005) A single nucleo-  
662 tide polymorphism in *MGEA5* encoding *O*-GlcNAc-selective *N*-  
663 acetyl- $\beta$ -*d* glucosaminidase is associated with type 2 diabetes in  
664 Mexican Americans. *Diabetes* 54:1214–1221
- 665 29. Szymańska E, Bouwman J, Strassburg K et al (2012) Gender-  
666 dependent associations of metabolite profiles and body fat dis-  
667 tribution in a healthy population with central obesity: towards  
668 metabolomics diagnostics. *OMICS* 16:652–667

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