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

Footnote Information

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

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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- α (TNF- α). TNF- α 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/m² 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/m²). 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 μ L of sample. Paper samples were air dried over-
night, then individually placed in plastic bags with desic-
cants and stored at -20° 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 ddH₂O 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 MgCl₂), 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 H₂O. The second-stage PCR was executed in a 55 μL
 194 volume containing 5.5 μL 10× PCR buffer (including
 195 MgCl₂), 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 H₂O. 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 χ^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

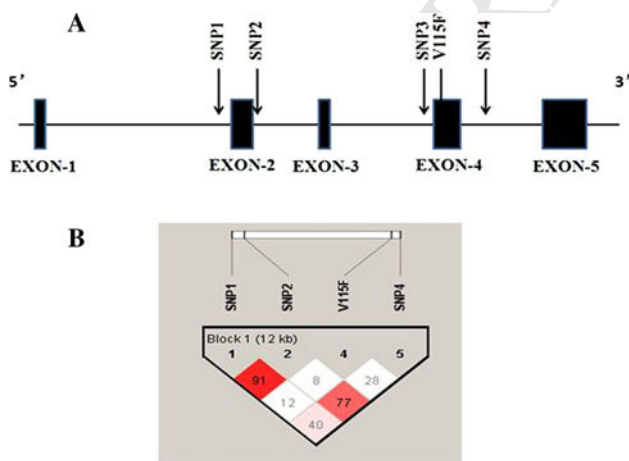


Fig. 1 a The location of the tag-SNPs in the *CIDEA* gene. The exons were indicated by black boxes. b LD plot among five tag-SNPs of *CIDEA* gene

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) 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, χ^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 ^Δ	54 (37.24)	189 (42.66)	73 (47.40)	243 (41.33)	263 (43.89)	0.206 ^Δ	0.175 ^Δ	0.147 ^Δ
Age (years)	49.65 ± 12.12	49.42 ± 12.41	49.97 ± 11.71	0.539 [§]	50.24 ± 0.85	49.26 ± 10.96	50.24 ± 12.76	49.50 ± 11.95	49.51 ± 11.45	0.056 [§]	0.723 [#]	0.515 [§]
BMI (kg/m ²)	26.20 ± 4.82	23.02 ± 2.32	30.65 ± 3.76	<0.001 [#]	24.68 ± 3.90	26.44 ± 4.94	26.94 ± 4.97	26.00 ± 4.76	26.57 ± 4.95	<0.001 [#]	<0.001 [#]	<0.001 [#]
SBP (mmHg)	132.84 ± 21.45	129.10 ± 21.11	138.08 ± 20.85	<0.001 [#]	131.73 ± 23.13	132.57 ± 20.91	134.66 ± 21.40	132.36 ± 21.45	133.11 ± 21.04	0.585 [#]	<0.001 [#]	<0.001 [#]
DBP (mmHg)	84.93 ± 12.65	82.36 ± 12.48	88.54 ± 12.02	<0.001 [#]	82.85 ± 14.02	85.10 ± 11.90	86.43 ± 13.23	84.54 ± 12.47	85.44 ± 12.26	0.101 [#]	<0.001 [#]	<0.001 [#]
WC (cm)	88.61 ± 12.15	82.18 ± 9.90	97.66 ± 8.79	<0.001 [#]	84.64 ± 11.77	89.16 ± 12.21	90.80 ± 11.51	88.04 ± 12.25	89.58 ± 12.05	<0.001 [#]	0.012 [§]	<0.001 [#]
HC (cm)	101.27 ± 10.11	96.28 ± 8.03	108.28 ± 8.43	<0.001 [#]	99.18 ± 10.83	101.44 ± 10.19	102.75 ± 8.82	100.88 ± 10.39	101.78 ± 9.86	0.008 [§]	0.042 [§]	0.005 [§]
WHR	0.88 ± 0.10	0.85 ± 0.092	0.91 ± 0.11	<0.001 [#]	0.85 ± 0.07	0.88 ± 0.12	0.88 ± 0.07	0.87 ± 0.11	0.88 ± 0.11	0.001 [#]	<0.001 [#]	<0.001 [#]
Obesity, N (%)	309 (41.64)	-	-	-	35 (24.14)	201 (45.37)	73 (47.40)	236 (40.14)	274 (45.00)	<0.001 [#]	0.103 ^Δ	<0.001 [#]

Values are mean ± SD or number and percentage. P values are calculated by χ^2 (^Δ) or one-ANOVA/T test ([§]) or rank sum test ([#]) or χ^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 ^a	Obesity ^a	<i>P</i> value ^b	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 ^Δ *	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 ^Δ	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 ^Δ	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 ^Δ	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 ^Δ *	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

* $P < 0.05$

^a Numbers are frequencies and percentage

^b P value was calculated by χ^2 test (^Δ) 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)

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 ± 10.91 cm;
316 BMI = 27.08 ± 4.24 kg/m²) 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 ± 4.45 kg/m², $P =$
319 0.030 ; TT: WC = 85.94 ± 11.36 cm, $P = 0.002$; BMI =
320 25.41 ± 4.43 kg/m², $P = 0.028$), 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 ± 11.62 cm) < TT 331
(88.31 ± 11.32 cm) < CC (92.75 ± 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> (%)	χ^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- α in
 445 human adipose tissue [9], and this has consequences for
 446 lipolysis. *CIDEA* decreases the availability of TNF- α by
 447 inhibiting cytokine secretion predominately through post-
 448 transcriptional mechanisms, which in turn counteracts the
 449 ability of TNF- α to stimulate lipolysis. TNF- α down-regu-
 450 lates the expression of *CIDEA* through signaling via c-Jun
 451 NH₂-terminal kinase (JNK), which in turn increases the
 452 availability of TNF- α 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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