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

Jingjing Wu
Ling Zhang
Jie Zhang
Ying Dai
Lili Bian

See next page for additional authors

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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

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

Jingjing Wu · Ling Zhang · Jie Zhang · Ying Dai · Lili Bian · Manshu Song · Alyce Russell · Wei Wang

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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 = 0.005) and SNP2 (P = 0.006). 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
death-inducing DNA fragmentation factor alpha-like
effector A (CIDEA) is a candidate gene for the develop-
ment of obesity [3–5].

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

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

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

Materials and methods

Subjects

This present study was a part of the National High Tech-
ology Research and Development Program-863 of China,
a population-based cross-sectional survey on relative risk
factors of chronic non-communicable diseases (NCD) in
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-
tched 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 individ-
uals with the following: (1) physician-diagnosed dia-etes 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-
ing, 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-
urement 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 µL of sample. Paper samples were air dried over-
night, then individually placed in plastic bags with desic-
cants and stored at −20 ºC.

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Whole-genome DNA was extracted by the Chelex-100 extraction method [14]. Firstly, a piece of 3 mm × 3 mm dried blood stain was cut down and put into a 1.5 mL centrifuge tube. Then 1 mL ddH₂O was added, the tube was shaken for 10 s and placed at room temperature for half an hour. After centrifugation for 3 min at 12,500×g, the majority of the supernatant liquid was removed and 200 µL of freshly prepared 5 % (w/v) Chelex-100 was added into the tube. The sample was mixed for 10 s and followed by centrifugation for 3 min at 12,500×g again. Then the sample was then incubated at 56 °C for 30 min, followed by 100 °C for 8 min. Finally, centrifugation for 3 min at 13,000×g was performed. The supernatant liquid containing DNA was stored at 4 °C for amplification.

165 Tag-SNP selection

We downloaded Han Chinese population SNP data from the database of the international HapMap Project (HapMap Data Rel 24/phase II Nov08, on NVBI B36 assembly, dbSNP b126). Using Haploview 4.0 software, we selected five tag-SNPs of the CIDEA gene (SNP/V115F: rs11545881, SNP1: rs4796955, SNP2: rs8092502, SNP3: rs12962340, and SNP4: rs7230480) which had a minor allele frequency (MAF) ≥ 5 % in Han Beijing Chinese. Among the SNPs whose r² ≥ 0.8, we selected the one with highest MAF for genotyping. Figure 1a shows the detailed information of the selected tag-SNPs of the CIDEA gene.

177 SNP genotyping

A combined approach utilizing nested polymerase chain reaction (PCR) and pyrosequencing technology (PSQ 96MA, BIOTAGE, Sweden) was used for V115F genotyping. The nested PCR primers were designed as followed: the outside primers were 5’-CTGGCATAAGAGCA GAGTG-3’ (forward) and 5’-GAGCTGTGGGATAAG AGT-3’ (reverse), and the inner primers were 5’-GTT TAGGAAGGCTCTGA-3’ (forward) and 5’-GATGTGCG TAGGACACGGATGA-3’ (reverse). The pyrosequencing primers were 5’-CAGGGCAGCCAGCAC-3’. The first-stage PCR was executed in a 20 µL volume containing 2 µL 10× PCR buffer (including MgCl₂), 2 µL dNTPs (2.5 mM), 0.2 µL forward primer (20 µM), 0.2 µL reverse primer (20 µM), 4 µL genomic DNA (25 ng/µL), 0.08 µL Taq polymerase (5 U/µL, Takara, Japan), and 11.52 µL deionized H₂O. The second-stage PCR was executed in a 55 µL volume containing 5.5 µL 10× PCR buffer (including MgCl₂), 5.5 µL dNTPs (2.5 mM), 0.55 µL forward primer (20 µM), 0.55 µL reverse primer (20 µM), 3 µL DNA (the production of the first-stage PCR), 0.22 µL Taq polymerase (5 U/µL, Takara, Japan) and 39.68 µL deionized H₂O. PCRs were initiated by denaturation at 95 °C for 5 min, followed by 35 cycles of: 30 s at 94 °C, 30 s at 57 °C, and 60 s at 72 °C, with the PCR products prolonged for 10 min at 72 °C in the final cycle and finally held at 4 °C.

The genotyping of the other four tag-SNPs (SNP1: rs4796955, SNP2: rs8092502, SNP3: rs12962340 and SNP4: rs7230480) was performed using the sequenom matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) iPLEX platform [15]. This technique is a high-throughput MS method for detecting SNPs. According to the manufacturers’ instructions, the whole process includes: multiplex PCR amplification, shrimp alkaline phosphatase treatment, iPLEX primer extension, clean resin, MALDI-TOF MS analysis and data analysis [16, 17].

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

Statistical analysis

Each polymorphism was evaluated for Hardy–Weinberg equilibrium by online software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). P ≥ 0.01 was considered to obey the Hardy–Weinberg equilibrium. The distributions of allelic and genotypic frequencies were analyzed using χ² test. The single locus association between a polymorphism and obesity was estimated by multiple logistic regression analysis, with age and gender adjusted. For continuous variables with normal distribution, we used ANOVA to detect the difference of distribution between the different genotypes. The variables which were non-normal distributions were analyzed via rank sum test. The statistical analyses were carried out...
using SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA). The frequencies of the haplotypes and association analyses were completed by Haploview software (version 4.0; Mark Daly’s Laboratory, Broad Institute; http://sourceforge.net/projects/haploview/) [18]. We analyzed the presence of interactions associated with obesity susceptibility between the tag-SNPs by multifactor dimensionality reduction method (MDR) (version 1.1.0; Computational Genetics Laboratory, Dartmouth Medical School, Lebanon, NH; www.epistasis.org) and logistic regression. The MDR method is nonparametric and model-free, which is directly applicable to case–control studies to detect the interaction between gene–gene and gene–environment. The best MDR model is determined to have a P value < 0.05, a maximum testing accuracy and a high cross-validation consistency (CVC) [19]. Probability values presented were for two-tailed tests and P < 0.05 was considered statistically significant.

250 Results

251 V115F polymorphism of CIDEA gene and obesity

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

253 V115F genotypic frequencies for the GG, GT and TT were 19.54, 59.70, and 20.75 %, respectively. The allelic frequencies of G and T alleles were 49.39 and 50.61 %, respectively. The genotypic distribution of the V115F followed Hardy–Weinberg equilibrium in the controls (P = 0.011). The frequency of the TT genotype was significantly higher in the obese group compared to the control group (23.62 vs. 18.71 %, P < 0.001) (Table 2).

254 Multiple logistic regression analysis (age and gender adjusted) identified that participants with the TT genotype were 2.84-fold at risk (95 % CI 1.73–4.66, P < 0.001) and those with the GT genotype were 2.63-fold at risk (95 % CI 1.72–4.01, P < 0.001) for obesity when compared to those with the GG genotype. Meanwhile, \( \chi^2 \) analysis results showed that participants with the T allele were 1.46-fold (95 % CI 1.19–1.80, P < 0.001) at risk for obesity when compared to those with the G allele.

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

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<td>Age (years)</td>
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<td>49.97 ( \pm ) 4.92</td>
<td>49.18 ( \pm ) 4.88</td>
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<td>0.007</td>
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<td>26.20 ( \pm ) 4.92</td>
<td>26.44 ( \pm ) 4.98</td>
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<td>0.334</td>
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<td>SBP (mmHg)</td>
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<td>85.10 ( \pm ) 14.02</td>
<td>84.64 ( \pm ) 14.02</td>
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<td>HC (cm)</td>
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<tr>
<td>WHR</td>
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Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio.
higher in TT group than those in GG + GT group (BMI, P < 0.001; SBP, P < 0.001; DBP, P < 0.001; WC, P = 0.012; HC, P = 0.042; WHR, P < 0.001, respectively) (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)
Association between the other four tag-SNPs and obesity

We genotyped another four selected CIDEA tag-SNPs (SNP1 G/T, SNP2 T/C, SNP3 T/A, SNP4 C/T) in 330 participants (obese/controls = 157/173). Distributions of the genotypes and alleles of the four SNPs are listed in Table 2. Analysis showed that the controls were in Hardy–Weinberg equilibrium at SNP1 (P = 0.039), SNP2 (P = 0.076) and SNP4 (P = 0.740), while the genotypic distribution of SNP3 did not follow Hardy–Weinberg equilibrium (P < 0.001), so SNP3 was excluded from further analysis. The MAF of these SNPs (SNP1T, SNP2C and SNP4T) were 44.80, 19.65 and 14.17 % in controls, respectively (Table 2). These were consistent with the MAF of Han Chinese in Beijing, China (http://www.ncbi.nlm.nih.gov/pubmed).

SNP1/GG and SNP2/CC genotypes were more frequent in obesity (adjusted by age and gender) indicated that both SNP1 and SNP2 polymorphisms were significantly associated with obesity (P = 0.006 and 0.005, respectively) (Table 2). SNP1/GG and SNP2/CC genotypes were more frequent in the obese group compared to the control group (P = 0.027, 0.025, respectively).

The analysis results of SNP1 showed overall that WC and BMI levels in the GG genotype (WC = 90.31 ± 10.91 cm; BMI = 27.08 ± 4.24 kg/m²) were significantly higher compared to any other two genotypes (GT: WC = 86.06 ± 12.00 cm, P = 0.030; BMI = 25.45 ± 4.45 kg/m²; P = 0.030; TT: WC = 85.94 ± 11.36 cm, P = 0.002; BMI = 25.41 ± 4.43 kg/m², P = 0.028), suggesting that participants with the GG genotype were more susceptible to obesity. Multiple logistic regression analysis (adjusted for age and gender) analysis revealed that when compared with the TT genotype, participants carrying the GG genotype had a 1.55-fold (95 % CI 1.13–2.12, P = 0.006) risk of obesity. All of these results indicated that the variant G allele of SNP1 was the risk allele of obesity.

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

Haplotypes analysis of the selected tag-SNPs of CIDEA gene

When we combined the four tag-SNPs and inferred haplotypes using Haplovie 4.0 software, ten possible haplotypes were derived from the observed genotypes (SNP1/SNP2/V115F/SNP4) (Fig. 1b). Six haplotypes with frequencies above 5 % were haplotype 1 (H1-G/T-T-G/T-T-C) (33.5 %), H2-G/T-G/T-T-C) (20.7 %), H3-T-G/T-T-C) (9.4 %), H4-T-C) (9.3 %), H5-T-G/T-T-C) (6.7 %) and H6-T-G/T-C) (6.0 %) (Table 3). H1 was more common in the obese participants (37.55 %) compared to the controls (29.91 %, P = 0.039), while H6 was common in the controls (7.92 %) compared to the obese (3.98 %, P = 0.034). The risk of obesity was significantly increased among the participants carrying haplotype H1 (OR 1.41, 95 % CI 1.02–1.95), and decreased among participants with haplotype H6 (OR 0.48, 95 % CI 0.24–0.96).

Interaction analysis of CIDEA gene tag-SNPs on obesity

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

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

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Genotype</th>
<th>Freq.</th>
<th>Obesity</th>
<th>Control</th>
<th>χ² value</th>
<th>P value</th>
<th>OR 95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP1</td>
<td>SNP2</td>
<td>V115F</td>
<td>SNP4</td>
<td>n (%)</td>
<td>n (%)</td>
<td>χ²</td>
<td>P</td>
</tr>
<tr>
<td>H1</td>
<td>G</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>0.335</td>
<td>117.9 (37.55)</td>
<td>103.5 (29.91)</td>
</tr>
<tr>
<td>H2</td>
<td>G</td>
<td>T</td>
<td>G</td>
<td>C</td>
<td>0.207</td>
<td>65.9 (20.99)</td>
<td>70.8 (20.46)</td>
</tr>
<tr>
<td>H3</td>
<td>T</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>0.094</td>
<td>26.4 (8.41)</td>
<td>35.4 (10.23)</td>
</tr>
<tr>
<td>H4</td>
<td>T</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>0.089</td>
<td>31.1 (9.90)</td>
<td>27.8 (8.03)</td>
</tr>
<tr>
<td>H5</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>0.067</td>
<td>15.5 (4.94)</td>
<td>28.5 (8.24)</td>
</tr>
<tr>
<td>H6</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>C</td>
<td>0.060</td>
<td>12.5 (3.98)</td>
<td>27.4 (7.92)</td>
</tr>
</tbody>
</table>

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 of 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 a 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/CC (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.

### Table 4 Summary of the MDR interaction models

<table>
<thead>
<tr>
<th>Model</th>
<th>Training bal. acc. (%)</th>
<th>Testing bal. acc. (%)</th>
<th>Sign test ( (P) )</th>
<th>CV consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP2</td>
<td>57.49</td>
<td>54.58</td>
<td>6 (0.377)</td>
<td>9/10</td>
</tr>
<tr>
<td>SNP1SNP2</td>
<td>60.23</td>
<td>53.56</td>
<td>7 (0.172)</td>
<td>7/10</td>
</tr>
<tr>
<td>SNP1SNP2V115</td>
<td>63.23</td>
<td>54.75</td>
<td>9 (0.011*)</td>
<td>7/10</td>
</tr>
<tr>
<td>SNP1SNP2V115SNP4</td>
<td>65.91</td>
<td>59.32</td>
<td>9 (0.011*)</td>
<td>10/10</td>
</tr>
</tbody>
</table>

\* \( P < 0.05 \)

## 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 expression in humans is inversely associated with BMI, WC, WHR and basal metabolic rate. Some studies have reported that CIDEA protein...
expression was decreased two-fold in obese humans and normalized after weight reduction [9]. A study in 40 obese women showed that CIDEA gene expression is significantly up-regulated as a result of the energy-restricted diets intervention [21]. In contrast to the mechanism in mice, a study found that there is a cross-talk between CIDEA and TNF-α in human adipose tissue [9], and this has consequences for lipolysis. CIDEA decreases the availability of TNF-α by inhibiting cytokine secretion predominately through post-transcriptional mechanisms, which in turn counteracts the ability of TNF-α to stimulate lipolysis. TNF-α down-regulates the expression of CIDEA through signaling via c-Jun NH2-terminal kinase (JNK), which in turn increases the availability of TNF-α and thereby lipolytic stimulation [9].

In a recent energy restriction intervention study [8], a significant inverse correlation has been found between UCP1 and CIDEA expression levels, indicating a possible interaction between CIDEA and UCP1 in humans. CIDEA is also associated with insulin sensitivity in humans [22]. Recently, a study found that starvation-induced apoptosis in adipocytes is significantly inhibited when insulin decreased CIDEA mRNA expression levels, suggesting that CIDEA is a novel gene regulated by insulin in human adipocytes and that it may play a key role in obesity [23].

CIDEA polymorphisms have been reported to be associated with human obesity in Swedish, Japanese and Chinese populations. In this study, the G allele frequency of V115F was 49.39%, which was lower than that previously reported in the Chinese (55.25%) population and higher than reported in the Japanese (48.90%) population [11, 12]. Multiple logistical regression results showed that participants with the TT genotype had a 2.84-fold (95% CI 1.73–4.66, P < 0.001) risk for obesity compared to those with the GG genotype. There was a trend that all the index levels of obesity related phenotypes in the participants were higher in the TT genotype group compared to the GG genotype groups (TT > GT > GG). Both genetic and continuous variable analyses indicated that the T allele of V115F SNP was a risk factor for obesity in Chinese. This result is consistent with our previous studies in both Japanese [11] and Chinese studies [12], but conflicts with the Swedish study [10]. This result could be due to the so-called “flip-flop” phenomenon, where, within differing ethnic groups, disease marker associations with reversed risk alleles are found [24, 25].

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

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

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

There is significant evidence showing that complex diseases are induced by gene–gene, gene–environmental and gene–environmental–behavior interactions. It is conceivable that obesity is the result of interactions between multiple genetic variations. In this study, the combined results of the nonparametric MDR approach and the parametric logistic analysis (adjusted by age and gender) indicated that the interaction between SNP1/GG and V115F/TT could increase the susceptibility of obesity occurring. Although our data cannot explain the biological mechanism, the result suggests that an interaction model could provide guidance to experimental studies on the metabolic pathway of obesity.

For this population screening study, 903 specimen collection paper was used to collect finger blood, which causes less discomfort to the subjects. The dried blood spots needed minimal storage space, caused little biohazard risk and were convenient for transportation [14]. The method also had the disadvantage of not having fresh blood samples for blood biochemical analyses such as triglyceride, total cholesterol, high density lipoprotein, which are associated with lipolysis. There were other limitations in our study. Firstly, the confounding factors such as diet, physical activity and environment were not considered. Secondly, all the associations offered in this study were a population-genetics based approach supported by statistical analyses, and therefore the explanation of the biological mechanism of obesity needs further investigation. Furthermore, recent interesting findings collectively highlight the complicated metabolite profiles in...
obesity at omics level, inspiring that post-genomics complementary approaches in obesity research are needed [29].

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

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