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

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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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death-inducing DNA fragmentation factor alpha-like effector A (*CIDEA*) is a candidate *gene* for the development 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 apoptotic 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 populations [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 haplotypes of the *CIDEA* gene on obesity in Han Chinese.

Materials and methods

Subjects

This present study was a part of the National High Technology 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 matched on age, gender and residence. An individual was defined as being obese if they had a BMI of 28 kg/m^2 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 individuals with the following: (1) physician-diagnosed diabetes 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, Beijing, China.

Measurement of anthropometric parameters

Following an interview by questionnaire, which covered demographic characteristics, residential history, socioeconomic status, personal behavior and medical history, all participants were asked to fast overnight before having a physical examination. Body weight, height, waist circumference (WC), hip circumference (HC), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by well-trained community doctors. Each measurement 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^2). 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 extension of the buttocks in a horizontal level, without compressing the skin. WHR was calculated as WC divided by HC. Blood pressure was measured by mercury sphygmomanometer on the right arm of the participant in a comfortable 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 overnight, then individually placed in plastic bags with desiccants and stored at −20 °C.

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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 ddH_2O 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. 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.

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 r^2 ≥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.

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′-GAGCCTGTGGGATAAG AGT-3′ (reverse), and the inner primers were 5′-GGT TAGGAAGGCTCCTGA-3′ (forward) and 5′-GATGTCG TAGGACACGGAGTA-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 $MgCl_2$), 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 H_2O. The second-stage PCR was executed in a 55 μL volume containing 5.5 μL 10× PCR buffer (including $MgCl_2$), 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 H_2O. 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). $P \geq 0.01$ was considered to obey the Hardy–Weinberg equilibrium. The distributions of allelic and genotypic frequencies were analyzed using χ^2 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

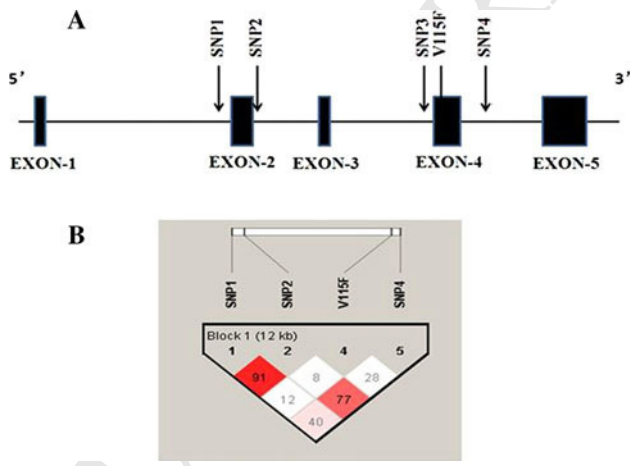

Fig. 1 **a** The location of the tag-SNPs in the *CIDEA* gene. The exons were indicated by *black boxes*. **b** LD plot among five tag-SNPs of *CIDEA* gene

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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.

Results

V115F polymorphism of *CIDEA* gene and obesity

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).

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). 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, χ^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.

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

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 ± 0.92	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)	$<0.001^{\wedge*}$	0.103^{Δ}	$<0.001^{\Delta*}$

Values are mean ± SD or number and percentage. P values are calculated by χ^2 ($^{\Delta}$) or one-ANOVE/T test ($^{\$}$) or rank sum test ($^{\#}$) or ^ logistic regression (age and sex adjusted). * $P < 0.05$. P_1 value: obesity group versus control group; P_2 value: GG versus GT 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

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

SNPs	Polymorphism	Control[a]	Obesity[a]	*P* 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
	T	155 (44.80)	108 (34.39)			
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
	T	278 (80.35)	248 (80.00)			
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
	A	27 (12.16)	15 (8.72)			
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
	T	49 (14.16)	43 (13.69)			
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)	$<0.001^{\Delta}$*	1.46	1.19–1.80
	G	462 (53.35)	271 (43.85)			
Combined genotypes				<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 ($^{\Delta}$) or multiple logistic regression (age and sex adjusted)

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)

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

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). Multiple logistic regression analysis (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/m^2) 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/m^2, $P = 0.030$; TT: WC = 85.94 ± 11.36 cm, $P = 0.002$; BMI = 25.41 ± 4.43 kg/m^2, $P = 0.028$), suggesting that participants with the GG genotype were more susceptible to obesity. Multiple logistic regression (adjusted for age and gender) analysis revealed that when compared with the TT genotype, participants carrying the GG genotype had a 2.19-fold (95 % CI 1.09–4.38, $P = 0.027$) risk of obesity, and when compared with the T allele, participants with the G allele 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 Haploview 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)-GTTC (33.5 %), H2-GTGC (20.7 %), H3-TCGC (9.4 %), H4-TCTC (8.9 %), H5-TTTC (6.7 %) and H6-TTGC (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

Haplotypes	Genotype				Freq.	Obesity	Control	χ^2 value	*P* value	OR	95 % CI
	SNP1	SNP2	V115F	SNP4		*n* (%)	*n* (%)				
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

* $P < 0.05$

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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

* $P < 0.05$

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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 NH_2-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

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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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