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Cell death–inducing DNA fragmentation factor α–like effector A (*CIDEA*) gene V115F (G→T) polymorphism is associated with phenotypes of metabolic syndrome in Japanese men

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Abstract

Cell death–inducing DNA fragmentation factor α–like effector A (*CIDEA*) regulates energy expenditure in the adipose tissue and is implicated in the development of obesity. A single nucleotide polymorphism in the *CIDEA* gene that causes an amino acid substitution of valine 115 to c(V115F) has recently been shown to be associated with obesity in the Swedish population. Here, we determined the effects of this polymorphism on phenotypes of metabolic syndrome within the Japanese population. Two hundred seventy unrelated Japanese male workers (mean age, 44.5 years) were analyzed in a cross-sectional study. The clinical features regarding metabolic syndrome, as well as *CIDEA* V115F polymorphism, were determined for each individual. The V115F polymorphism associated with waist circumference and fasting plasma glucose. These parameters were at higher levels in the VF + FF group than in the VV group ($P < .05$). The VF + FF group compared with the VV group had a higher prevalence for abdominal obesity (odds ratio [OR] = 1.89; 95% confidence interval [CI], 1.03-3.44), high fasting plasma glucose (OR = 2.81; 95% CI, 1.03-7.67), and metabolic syndrome (OR = 3.15; 95% CI, 1.05-9.48). These results suggest that the F allele of the *CIDEA* gene may serve as a risk factor for phenotypes related to metabolic syndrome in Japanese men.

1. Introduction

Metabolic syndrome represents a specific body phenotype in conjunction with a group of metabolic abnormalities including abdominal obesity, insulin-resistant glucose metabolism, dyslipidemia, and hypertension. Metabolic syndrome independently predicts the development of both type 2 diabetes mellitus and coronary heart disease [1]. The prevalence of metabolic syndrome is high in developed countries; approximately 22% and 14.7% of the adult populations in the United States and Japan, respectively, have metabolic syndrome [2,3]. Undoubtedly, multiple and interactive effects of gene and environmental factors including diet and physical inactivity contribute to the development of this syndrome. Genetic contributions are substantially demonstrated in twin and familial studies [4], and chromosomal regions that have linkage to metabolic syndrome were identified [5-7]. Nevertheless, the genetic components are still largely unknown; and more studies are needed to clarify the susceptibility genes.

The cell death–inducing DFFA-like effector A (*CIDEA*) gene is encoded on human chromosome 18p11.21, where a susceptibility locus for type 2 diabetes mellitus and obesity resides [7]. *CIDEA* forms complex with the uncoupling protein 1 and negatively regulates the energy expenditure [8,9]. *CIDEA* also cross-talks with tumor necrosis factor α pathway and regulates lipolysis [10]. *CIDEA* is expressed in brown adipose tissue [9] in mice and white adipose tissue in human [11]. *CIDEA* expression in human adipose tissue is low in obese subjects and normalizes after weight reduction [11]. Low adipose *CIDEA* expression is associated with abdominal obesity, enlarged fat cells, insulin resistance, and increased basal lipolysis, all of which are features of metabolic syndrome [10]. These lines of evidence indicate that *CIDEA* may be a good candidate gene for obesity and metabolic syndrome susceptibility.

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Recently, polymorphisms in the *CIDEA* gene were studied in obese and nonobese Swedish subjects [12]. It was found that a single nucleotide polymorphism (SNP) that causes an amino acid substitution of valine 115 to phenylalamine (V115F) associates closely with obesity. In the current study, we aimed to determine the effects of V115F within the Japanese population with regard to phenotypes of the metabolic syndrome.

2. Subjects and methods

2.1. Subjects

The present study was undertaken as part of a larger study being carried out to investigate the association of lifestyle and genetic factors with phenotypes of metabolic syndrome in Japanese [13]. Briefly, 358 unrelated Japanese workers (age range, 20-64 years) in a company in Kanagawa Prefecture, Japan, who underwent routine health checkups in 2003 participated in the study. Because of the small number, women (n = 37) were excluded from the analysis. We also excluded those who are currently under treatment for hypertension (n = 38), diabetes (n = 8), and hyperlipidemia (n = 8). Accordingly, a total of 270 healthy Japanese men were enrolled in the genotype and phenotype association study. All subjects gave written informed consent; and the study was approved by the ethical committee of Institute of Medical Science, Tokyo Medical and Dental University.

2.2. Phenotype measurements

Body height and weight were measured, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The waist circumference (WC) was measured at the umbilicus level; and the hip circumference was measured at the widest circumference over trochanters, with the subject standing erect with the abdomen relaxed and arms at their sides and the feet together.

Blood pressure was measured twice by well-trained nurses using PWV/ABI device (Nippon Colin, Aichi, Japan) with the subjects resting in a supine position.

Blood samples were drawn after overnight fasting for 12 hours and collected in ethylenediaminetetraacetic acid–coated tubes. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), fasting plasma glucose (FPG), and glycated hemoglobin (HbA_{1c}) were measured in a routine manner. The low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald method [14].

2.3. The criteria of metabolic syndrome

We used the criteria for metabolic syndrome as defined by the Japanese Society of Internal Medicine. The hallmark of the diagnosis is abdominal obesity (WC ≧85 cm for men and ≧90 cm for women). The other criteria, in addition to abdominal obesity, includes 2 or more of the following: (1) dyslipidemia: hypertriglyceridemia (≧150 mg/dL or 1.69 mmol/L) or low HDL-C (<40 mg/dL or 1.04 mmol/L), (2) high blood pressure (≧130/85 mm Hg), and (3) high FPG (≧110 mg/dL or 6.1 mmol/L). In this study, general obesity was defined by BMI of over 25.0 kg/m^2, according to the criteria of Japan Society for the Study of Obesity [15] and the Regional Office for the Western Pacific Region of the World Health Organization in Asians [16].

2.4. Genotype determinations

CIDEA genomic sequence (NM_001279.2) was obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The V115F (G→T) in exon 4 was searched, and polymerase chain reaction (PCR) primers and hybridization probes were designed as follows:

Sense primer: 5′-GGTTAGGAAGGCTCCTGA-3′

Antisense primer: 5′-GATGTCGTAGGACACGGAGTA-3′

Sensor probe: 5′ LC Red 640-AGCAAGTGGGGACGTG-phosphate 3′

Anchor probe: 5′-TCGCTATTCCCGACCTCTTC-GGCGGC-fluorescein 3′

The genotyping was done by melting curve analysis [17]. Briefly, PCR was performed with 0.5 μmol/L sense primer and 0.05 μmol/L antisense primer in a reaction mixture containing 0.2 μmol/L anchor probe, 0.2 μmol/L sensor probe, 10 ng of dried-down DNA, 4.0 μmol/L $MgCl_2$, 0.5 μl 10× PCR buffer, 0.2 mmol/L dNTP, and 0.5 U Faststart DNA Polymerase (Roche Diagnostics, Penzberg, Germany) in a total volume of 5 μL. The cycling program consisted of 10 minutes of initial denaturation at 94°C and 40 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 72°C for 15 seconds, followed by final extension step at 72°C for 2 minutes. After completion of PCR, denaturation and annealing of melting temperature analysis was done by heating the mixture at 94°C for 60 seconds and then keeping it at 40°C for 60 seconds. Afterward, the plate was heated from 40°C to 80°C by the gradient of 0.1°C per second. Melting curve data were collected and classified using the LightTyper instrument and software (Roche Diagnostics), and call for the genotypes were made. The DNA sequencing from selected samples were done using Applied Biosystems 3130xl Genetic Analyzer (ABI, Tokyo, Japan) to confirm the results of the genotyping.

2.5. Statistical analyses

The statistical analyses were carried out using the Statistical Package of Social Science for Windows version 11.0 (SPSS, Chicago, IL). All probability values presented were for 2-tailed tests, and the values of $P < .05$ were considered to indicate statistical significance. Genotype/allele frequencies were determined with the gene counting method. χ^2 tests were used to calculate Hardy-Weinberg equilibrium and categorical variables. Fisher exact test was used to compare the allele frequencies between the groups. General linear model analysis was applied to compare the

age-adjusted means of the features of metabolic syndrome according to *CIDEA* genotypes (VV vs VF + FF). Continuous variables that did not have a normal distribution, TG, HDL-C, FPG, and HbA_{1c} were log-transformed. Multiple logistic analysis was used to calculate the odds ratios (OR) values and 95% confidence intervals (95% CIs). Corrections for multiple testing were not taken into account.

3. Results

CIDEA V115F (G→T) polymorphism was determined in the total sample of 358. The accuracy of the genotyping was checked by sequencing 20 randomly selected samples. No discrepancies were found between the genotyping and the sequencing results. The frequencies for VV, VF, and FF genotypes were 22.6%, 52.5%, and 24.9%, respectively. The allele frequencies for V and F alleles were 48.9% and 51.1%, respectively. The genotype distribution obeyed the Hardy-Weinberg equilibrium.

The characteristics related to metabolic syndrome of the studied population (n = 270) are shown in Table 1 (left column). All of the mean values were in the reference range, indicating that this population is generally healthy working men.

Initially, we divided the subjects into genotype groups of VV (n = 62), VF (n = 147), and FF (n = 61) and found that WC tended to be larger in the VF and FF groups compared with the VV group, although there was no statistical significance. Thus, we assumed a dominant model of the F allele and combined the FF and VF groups (n = 208) and compared them with the VV group (Table 1, right column). The WC was larger in the VF + FF group compared with the VV group ($P < .05$). The FPG and HbA_{1c} were also higher in the VF + FF group compared with the VV group ($P < .05$). Other parameters of weight and serum LDL-C gave marginal P values between the 2 groups.

Table 1
Characteristics of 270 study subjects according to the *CIDEA* V115F genotype

Parameters	Total	VV genotype	VF + FF genotype	*P*
n	270	62	208	–
Age (y)	44.5 ± 12.0	44.1 ± 12.7	44.7 ± 11.8	.729
Weight (kg)	65.9 ± 11.4	64.3 ± 9.4	67.5 ± 11.8	.050
WC (cm)	82.8 ± 9.6	81.2 ± 8.8	84.3 ± 9.8	.025
Hip circumference (cm)	94.6 ± 6.9	93.6 ± 6.4	95.7 ± 7.0	.035
BMI (kg/m^2)	23.2 ± 3.5	22.9 ± 2.8	23.4 ± 3.7	.331
TC (mg/dL)	203.2 ± 37.6	199.9 ± 37.5	206.5 ± 37.5	.200
TG (mg/dL)[a]	129.5 ± 81.4	134.4 ± 89.0	128.0 ± 79.1	.629
HDL-C (mg/dL)[a]	55.6 ± 14.2	57.2 ± 14.3	55.1 ± 14.2	.258
LDL-C (mg/dL)	120.2 ± 34.7	115.6 ± 34.9	124.9 ± 33.4	.058
FPG (mg/dL)[a]	97.3 ± 27.8	91.9 ± 22.2	99.0 ± 29.1	.042
HbA_{1c} (%)[a]	4.95 ± 0.57	4.80 ± 0.53	4.99 ± 0.57	.038
Systolic blood pressure (mm Hg)	132.0 ± 17.0	131.5 ± 15.2	132.5 ± 17.6	.655
Diastolic blood pressure (mm Hg)	80.1 ± 12.4	80.1 ± 11.8	80.2 ± 12.6	.945

TC indicates total cholesterol.
[a] These values were analyzed in log-transformed values.

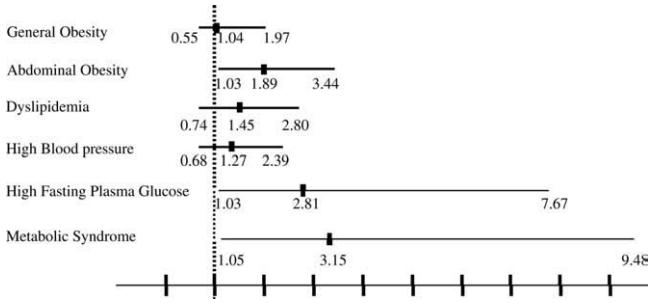

Fig. 1. The prevalence of metabolic syndrome–related phenotypes according to *CIDEA* V115F (G→T) polymorphism. The OR and 95% CI were calculated by multiple logistic analysis adjusting for age using VV genotype as a reference.

According to the criteria of metabolic syndrome, the prevalence of metabolic syndrome in this population was 14.9% (n = 39). We determined the prevalence of the phenotypes of general and abdominal obesity, dyslipidemia, high FPG, and high blood pressure; and multiple logistic analysis was performed (Fig. 1). All of the features of metabolic syndrome tended to have higher OR in the VF + FF groups than in the VV groups, although abdominal obesity and high FPG gave a significant association ($P < .05$).

We also performed the same analysis using the new International Diabetes Foundation criteria for metabolic syndrome [18]. The prevalence of metabolic syndrome was 18.7% (n = 49) when these criteria were applied. Essentially the same association was found under this criteria (OR = 4.21; 95% CI, 1.42-12.41; $P = .009$), indicating that our results are robust to different criteria.

4. Discussion

Using an occupational cohort, we determined the *CIDEA* gene V115F (G→T) polymorphism and found that there is an association between the F allele and the phenotypes of metabolic syndrome.

One interesting aspect is that we found positive association in WC but not in BMI (Table 1). This is also reflected in that the association was found for abdominal obesity (WC ≧85 cm) but not general obesity (BMI ≧25 kg/m^2) as shown in Fig. 1. Recent studies have shown that abdominal obesity rather than general obesity faithfully predicts the risk for coronary heart disease and type 2 diabetes mellitus, at least in Asian populations [19,20]. Thus, this polymorphism might be more useful in predicting these conditions.

When the subjects with higher obesity (BMI ≧30.0 kg/m^2) in this population were sought, there were 11 subjects; and all of them were VF + FF subjects. Whether V115F associates

with general obesity under the criteria of BMI needs to be studied in a larger sample size.

The association between the V115F polymorphism was also found for FPG and HbA_{1c}. This appears to be in accordance with the notion that *CIDEA* acts in energy expenditure and that the *CIDEA*-deficient mice have lower glucose levels and are resistant to diet-induced diabetes [8].

The initial study of the V115F polymorphism reported in the Swedish population showed VV genotype groups having a higher BMI than VF or FF groups [12]. Apparently, the risk allele in the Swedish and Japanese populations is the exact opposite. The difference might be explained as follows. The V115F may not be associated with functional differences and merely be a genetic marker that is in linkage disequilibrium with other causative SNP(s). It can be further explained if V115F and the causative SNP are on different haplotypes between the Swedish and Japanese. Alternatively, there might be a different environmental exposure for developing obesity and metabolic syndrome among different races that reverses the effects of the V115F.

The role of *CIDEA* on obesity and metabolic syndrome warrants more study, and it is of particular importance to study this in different populations and to determine the functional differences of this nonsynonymous SNP.

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