Association of the cell death-inducing DNA fragmentation factor alpha-like effector A (CIDEA) gene V115F (G/T) polymorphism with phenotypes of metabolic syndrome in a Chinese population

Ling Zhang, Ying Dai, Lili Bian, Wei Wang, Wei Wang, Masaaki Muramatsu, Qi Hua

1. Introduction

A clustering of metabolic disorders including obesity, dysglycemia, dyslipidemia, and high blood pressure is called metabolic syndrome (MetS). MetS is not only triggered by an obesigenic environment characterized by an excess intake of energy and sedentary work, but also by genetic factors which may account for the inter-individual difference in susceptibil-
ity to metabolic disorders [1,2]. The cell death-inducing DFF45-like effector A (CIDEA) protein is a member of the CIDE family, known to be important regulators of various aspects of metabolism [3,4]. CIDEA deficient mice display lean phenotypes with higher energy expenditure and are resistant to diet-induced obesity and insulin resistance [5]. The CIDEA protein is localized to lipid droplets of adipocytes and the endoplasmic reticulum of hepatocytes and controls lipid metabolism through regulating lipid droplet formation or lipogenesis [6]. Expression of the CIDEA gene positively correlates with the development of obesity and insulin sensitivity in both rodents and humans [7,8]. Thus, CIDEA is an important regulator of energy homeostasis and is a candidate gene for the development of metabolic disorders.

The CIDEA gene resides on chromosome 18p11, where positive linkage signs for obesity and type 2 diabetes have been identified [9,10]. The gene is 23.22 kb in length with 4 introns and 5 exons. One nonsynonymous single nucleotide polymorphism (SNP) is found on exon 4, codon 115, which results in an amino acid substitution of valine with phenylalanine (V115F: rs45619832). The CIDEA V115F polymorphism was initially shown to be associated with obesity in Swedish subjects, where G is the risk allele [11]. Then, the effect of the V115F polymorphism was evaluated in Japanese men and was shown to be associated with metabolic syndrome, although T was the risk allele [12].

More studies are needed to determine the association between this CIDEA gene polymorphism and metabolic disorders in different races. To our knowledge, this is the first report to study CIDEA gene polymorphisms and their association with metabolic syndrome and its related phenotypes in a Chinese population.

2. Materials and methods

The participants were recruited from subjects who attended the Cardiac Clinic at Xuanwu Hospital Capital Medical University (2005–2007) for a medical evaluation of MetS risks [13]. The inclusion criteria were one or more of the following: (1) age ≥ 50; (2) experiencing one or two components of MetS, but not diagnosed with MetS; (3) having cardiovascular diseases, non-alcoholic fatty liver, gout, polycystic ovarian syndrome, or lipatrophy; (4) having one or more of the following: obesity, type 2 diabetes, dyslipidemia, or a family history of metabolic syndrome; (5) having a family history of cardiovascular disease. Subjects with malignant tumors, severe cardiovascular diseases, and severe respiratory diseases such as chronic obstructive pulmonary disease (COPD) were excluded from the study. Those under any kind of drug treatment were also excluded. Spouses who met the inclusion criteria were also included in the study. A total of 351 participants, including 163 males and 188 females, were included in the analysis. The protocol was approved by the ethics committee of Xuanwu Hospital Capital Medical University, and all of the participants gave written informed consent. Each participant was interviewed and completed a standardized questionnaire at the Cardiac Clinic or at the subject’s home. A range of demographic factors and aspects of medical and family histories were included. The physical examinations and interview were carried out by trained nurses and physicians. Height, weight, waist circumference, and hip circumference were measured. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²). The waist circumference was measured at the umbilicus level and the hip circumference was measured at the widest circumference over the trochanters, with the subject standing erect with the abdomen relaxed, arms at their sides, and the feet together. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference. Blood pressure was measured on the right arm by well-trained nurses using a standard mercury sphygmomanometer, with the subjects resting at least 5 min in a sitting position; measurements were made three times and the average value was used in the analyses. Blood samples were collected in the morning after patients fasted overnight for at least 12 h and were used for laboratory measurements and the genomic study. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) coated tubes and stored at −80 °C for future DNA extractions. Total cholesterol (TC), triglycerides (TG), and high density lipoprotein-cholesterol (HDL-C) levels were measured with an automatic biochemistry machine (Hitachi 7170, Tokyo, Japan). Fasting plasma glucose (FPG) levels were measured by the glucose oxidase method. Low density lipoprotein-cholesterol (LDL-C) was calculated by the Friedewald method.

Overweight (28 kg/m² ≥ BMI ≥ 24 kg/m²) and overall obesity (BMI ≥ 28 kg/m²) were defined according to the criteria recommended for the Chinese population [14]. Dyslipidemia was defined as TG ≥ 150 mg/dL (1.7 mmol/L) and/or HDL-C < 40 mg/dL (1.03 mmol/L) in males, and TG ≥ 150 mg/dL (1.7 mmol/L) and/or < 50 mg/dL (1.29 mmol/L) in females. MetS was defined by the criteria of the IDF, updated in 2005. According to the new IDF definition, a person with metabolic syndrome has: central obesity (defined as waist circumference with Chinese ethnicity specific values: ≥ 90 cm in males, ≥ 80 cm in females) plus any two of the following four factors: (1) raised triglycerides: ≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality; (2) reduced HDL cholesterol: < 40 mg/dL (1.03 mmol/L) in males, < 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality; (3) raised blood pressure: systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg or treatment for previously diagnosed hypertension; (4) raised FPG: ≥ 100 mg/dL (5.6 mmol/L) or previously diagnosed with type 2 diabetes [15].

3. Genotyping

DNA was isolated by standard protocols from whole blood samples stored at −80 °C. A combined approach utilizing polymerase chain reaction (PCR) and pyrosequencing technology was used for genotyping. The CIDEA genomic sequence (NM_001279.2) was obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). The V115F (G/T) polymorphism in exon 4 was searched using the CIDEA genomic sequence. PCR primers were designed as follows: forward primer: 5'-GGTTAGAAGGCTAATGTA-3', reverse primer: 5'-GATGTCG- TAGGACACGGAGTA-3'. The antisense primer was biotinyl-
lized with streptavidin at the 5' termination. PCR amplification was carried out in a total volume of 50 μL containing 5 μL 10× PCR Buffer (50 mMol/L KCl, 20 mMol/L Tris–HiCl), 1 μL forward primer (20 μmol/L), 1 μL biotinylated reverse primer (20 μmol/L), 4 μL dNTP mix (2.5 mMol/L), 0.25 U Taq DNA polymerase, and 2 μL (10 ng) DNA. Thermocycling conditions were 94 °C initial denaturation for 3 min, followed by 40 cycles of 94 °C denaturation for 30 s, 52 °C annealing for 30 s, 72 °C extension for 1 min, and a 72 °C final extension for 10 min.

After PCR amplification, the genotyping was done using pyrosequencing technology with a PSQ™ 96MA machine (Biotage AB, Uppsala, Sweden), the detailed procedure for the pyrosequencing method is described elsewhere [16]. The sequencing primer was 5′-CAGGGCAGCCAGCAC-3′, which was designed with PSQ™ 96MA software (Gene Company Limited, HK). All the primers were synthesized by Sangon Biotech (Shanghai, Co., Ltd.).

4. Statistical analysis

For continuous variables with skewed distributions including WHR, FPG, HDL-C, and BMI, logarithmic transformations were made to enhance compliance with the normality assumption. Genotype and allele frequencies were determined with the gene counting method. Chi-square tests were used to calculate Genotype and allele frequencies were determined with the Hardy–Weinberg equilibrium and the distribution of expected, or in systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, HDL-C, and fasting plasma glucose among the three genotypes.

For all subjects, the proportions of overall obesity, central obesity, dyslipidemia, hypertension, raised FPG (RFPG), and evaluated metabolic phenotypes including overweight, overall obesity, central obesity, dyslipidemia, hypertension, high fasting plasma glucose, and MetS by multiple logistic regression analysis. The odds ratio (OR) and 95% confidence interval (95% CI) were estimated for each independent variable. Confounding factors including age (continuous variables) and gender (male = 1, female = 2) were adjusted. All probability values presented were for two-tailed tests and values of p < 0.05 were considered statistically significant. Analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

5. Results

The frequencies for the GG, GT, and TT genotypes were 29.91%, 50.71%, and 19.48%, respectively. The allele frequencies for the G and T alleles were 55.25% and 44.75%, respectively. The genotype distribution obeyed the Hardy–Weinberg’s equilibrium (p = 0.5).

Table 1 describes the clinical characteristics and parameters according to the CIDEA genotypes. There were significantly higher levels of weight, waist circumference, hip circumference, WHR, WHR, BMI, and triglycerides in the TT and GT genotypes than in the GG genotype (p < 0.05). No significant differences were found in age and gender, as expected, or in systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, HDL-C, and fasting plasma glucose among the three genotypes.

For all subjects, the proportions of overall obesity, central obesity, dyslipidemia, hypertension, raised FPG (RFPG), and

<table>
<thead>
<tr>
<th>Parameter Total</th>
<th>GG</th>
<th>GT</th>
<th>TT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>351 (100)</td>
<td>105 (29.91)</td>
<td>178 (50.71)</td>
<td>68 (19.38)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.86 ± 16.14</td>
<td>51.00 ± 15.95</td>
<td>50.25 ± 16.39</td>
<td>52.24 ± 15.90</td>
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<tr>
<td>Gender (male, N %)</td>
<td>163 (46.44)</td>
<td>42 (40.00)</td>
<td>84 (47.19)</td>
<td>37 (54.41)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.89 ± 73.47</td>
<td>66.76 ± 12.39</td>
<td>73.47 ± 71.27^A</td>
<td>75.67 ± 18.28^B</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>88.57 ± 12.33</td>
<td>84.64 ± 12.08</td>
<td>89.62 ± 12.67^A</td>
<td>91.97 ± 9.92^B</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>102.01 ± 10.44</td>
<td>99.55 ± 11.83</td>
<td>102.46 ± 9.91^A</td>
<td>104.70 ± 8.61^B</td>
</tr>
<tr>
<td>WHR*</td>
<td>0.87 ± 0.97</td>
<td>0.85 ± 0.69</td>
<td>0.88 ± 0.12^A</td>
<td>0.88 ± 0.07^A</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>25.98 ± 5.05</td>
<td>24.45 ± 3.78</td>
<td>26.49 ± 5.36^A</td>
<td>26.83 ± 5.51^B</td>
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<tr>
<td>Abdomen circumference (cm)</td>
<td>97.29 ± 12.46</td>
<td>95.39 ± 13.83</td>
<td>98.09 ± 11.41</td>
<td>98.16 ± 2.73</td>
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<td>SBP (mm Hg)</td>
<td>135.96 ± 22.77</td>
<td>133.85 ± 23.91</td>
<td>136.01 ± 22.63</td>
<td>139.08 ± 21.25</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>86.30 ± 13.69</td>
<td>84.17 ± 1.53</td>
<td>86.85 ± 13.06</td>
<td>88.13 ± 13.74</td>
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<td>TG (mg/dL)</td>
<td>165.29 ± 119.55</td>
<td>138.81 ± 96.96</td>
<td>174.71 ± 127.30^A</td>
<td>181.65 ± 125.32^B</td>
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<tr>
<td>TC*</td>
<td>195.4 ± 444.18</td>
<td>190.39 ± 37.95</td>
<td>200.38 ± 46.84</td>
<td>190.49 ± 45.19</td>
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<tr>
<td>HDL-C* (mg/dL)</td>
<td>51.53 ± 41.45</td>
<td>53.58 ± 27.72</td>
<td>51.91 ± 33.99</td>
<td>47.36 ± 10.44</td>
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<tr>
<td>LDL-C* (mg/dL)</td>
<td>68.60 ± 73.39</td>
<td>73.71 ± 52.76</td>
<td>69.65 ± 90.08</td>
<td>66.50 ± 48.92</td>
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<tr>
<td>FPG* (mg/dL)</td>
<td>102.88 ± 31.11</td>
<td>98.06 ± 22.01</td>
<td>104.14 ± 31.71</td>
<td>107.02 ± 39.81</td>
</tr>
<tr>
<td>Overall obesity, N (%)</td>
<td>99 (28.21)</td>
<td>21 (20.00)</td>
<td>56 (31.46)</td>
<td>22 (47.83)</td>
</tr>
<tr>
<td>Central obesity, N (%)</td>
<td>231 (65.81)</td>
<td>56 (53.33)</td>
<td>123 (69.10)</td>
<td>52 (76.47)</td>
</tr>
<tr>
<td>Dyslipidemia, N (%)</td>
<td>146 (41.30)</td>
<td>31 (29.52)</td>
<td>80 (44.94)</td>
<td>35 (51.47)</td>
</tr>
<tr>
<td>Hypertension, N (%)</td>
<td>245 (69.80)</td>
<td>70 (66.67)</td>
<td>124 (69.66)</td>
<td>51 (75.00)</td>
</tr>
<tr>
<td>RFPG, N (%)</td>
<td>123 (35.04)</td>
<td>32 (30.48)</td>
<td>69 (38.76)</td>
<td>22 (32.35)</td>
</tr>
<tr>
<td>MetS, N (%)</td>
<td>163 (46.64)</td>
<td>35 (33.33)</td>
<td>89 (50.00)</td>
<td>39 (57.35)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or percentage. p values are calculated from ANOVA or $\chi^2$ test. N, number. Abbreviation: WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein; FPG, fasting plasma glucose; MetS, metabolic syndrome; RFPG, raised fasting plasma glucose. ^A^Significant inter-group difference compared to GG genotype.

* Skewed distributed and analyzed by log-transformed values.
MetS were 28.21%, 65.81%, 41.10%, 69.80%, 35.04%, and 46.64%, respectively (Table 1). The proportions of central obesity, dyslipidemia, and MetS were higher in the TT and GT genotypes than in the GG genotype ($p < 0.05$). The proportion of overall obesity, hypertension, and RFFG were not different among the three genotypes.

**Fig. 1** shows the results of the multiple logistic regression model adjusted for age and gender, showing the odds ratio and 95% CI for the GT, TT, and the combined GT + TT genotypes over the GG genotype. Central obesity, dyslipidemia, and MetS displayed a significant association ($p < 0.05$); while, there were no associations with RFFG and hypertension ($p > 0.05$).

When male ($n = 163$, mean age = 48.93 $\pm$ 15.56 years) and female subjects ($n = 188$, mean age = 52.54 $\pm$ 16.47 years) were analyzed separately, the proportion of overall obesity was significantly higher in males (32.5%) than in females (22.9%). Multiple logistic regression adjusted for age in each gender indicated that the odds ratio for MetS in the TT + GG genotype compared to the GG genotype in males was OR = 2.36, 95% CI: 1.11–5.01, and in females was OR = 2.22, 95% CI: 1.09–4.51. Thus, the effect of the genotype did not significantly differ between genders.

**6. Discussion**

The present study demonstrated a positive association between the CIDEA gene V115F (G/T) genotype and metabolic phenotypes of central obesity, dyslipidemia, and MetS in a Chinese population. The T allele was the risk allele for these disorders. The CIDEA gene is a novel candidate for obesity and metabolic disorders [17]. CIDEA has been shown to reside in the inner membrane of mitochondria and regulates thermogenesis [18], together with the uncoupling protein-1 (UCP1) [5]. Thus, our results suggest the functional variant of CIDEA might alter energy homeostasis and affect the risk of MetS.

The CIDEA polymorphism was also associated with the triglyceride level. This is reminiscent of the function of CIDEA in free fatty acid metabolism [19]. CIDEA esterifies free fatty acid into triglycerides and sequesters it in adipocytes [19]. This activity is also related to the lipolytic function of TNF-alpha. Thus, it is tempting to speculate that altered CIDEA function may induce a cascade of free fatty acid overload into the circulation.

Previous studies in Swedish and Japanese populations showed the CIDEA gene V115F (G/T) polymorphism is associated with obesity, but the risk alleles differed [11,12]. The results of our study were similar to the Japanese study, in which the T allele was the risk factor for metabolic disorders [12]. In Japanese men, the odds ratio for MetS was OR = 3.15; 95% CI: 1.05–9.48 (GG vs GT + TT); with the same model, the effect on the Chinese population was OR = 2.36; 95% CI: 1.42–3.92. Since both have a high CI due to small sample size, we cannot compare the size of the effect between the Japanese and Chinese subjects, but can say that the risk appears similar. We also demonstrated that the genetic effect was seen equally in both genders.

In the Swedish study, subjects with the GG genotype had higher BMIs than those with GT or TT genotypes, indicating the G allele is the risk allele [11]. The reason for this contradiction is currently not clear, but heterogeneous effects of the CIDEA
V115F variant may vary in different populations due to differences in genetic backgrounds or environmental factors. It may also be due to the “flip-flop phenomenon”, in which the CIDEA V115F polymorphism may have an inter-locus correlation with a causal variant at another locus through linkage disequilibrium [20]. However, an in-depth study of this genomic context is required to verify the varying effects of the CIDEA V115F polymorphism among population groups.

The functional difference caused by the V115F substitution has not yet been studied biochemically. Thus, we examined the possible impact of this amino acid substitution on the structure and function of the CIDEA protein using POLYPHEN software [21]. The result showed this substitution would induce a benign effect on the CIDEA protein, suggesting there might be other causal variants. Functional studies of CIDEA proteins with V and F at codon 115 are warranted.

There are some limitations of our study. The small sample size limited the power of analysis. Confounding factors, such as dietary and environmental factors, have not been considered. Since MetS is not monogenic, other genetic factors should have been considered. Despite these limitations, the present study detected a positive association between the CIDEA gene V115F (G/T) genotype and metabolic disorders in a Chinese population. Larger studies are needed to determine the association between CIDEA gene polymorphisms and metabolic disorders in different races. Also, it is important to clarify the biochemical function of this non-synonymous SNP.

Our study used the IDF criteria to define MetS because it requires central obesity as a requisite for its diagnosis. The recommended cutoff points for waist circumference in the NCEP definition is inappropriate for Asian populations because Asian people tend to have a higher percentage of body fat, particularly abdominal visceral fat, than white people with the same body mass index. It is documented that central obesity is a more precise predictor for MetS compared with overall obesity, at least in Asia [22,23]. Thus the use of the NCEP criteria in a Chinese population could underestimate the prevalence of MetS [24].

7. Conclusion

In summary, our study showed that the T allele of the CIDEA gene V115F (G/T) polymorphism is a risk factor for MetS and its related metabolic phenotypes in a Chinese population. CIDEA polymorphisms and their relation to metabolic disorders warrant further study.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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