The Association Between DNA Methylation of CASZ1 Gene and Overweight/obesity in the Chinese Han Population

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Abstract

Objective

The aim of this study was to examine the potential association between castor zinc finger 1 (CASZ1) gene methylation and overweight/obesity, and investigate the interaction effects between CASZ1 methylation levels and some environmental factors on overweight/obesity.

Methods

This study included 1,029 individuals. The methylation levels of CpG sites at CASZ1 promoter were measured with bisulfite sequencing method. Multiple linear regression and logistic regression models were used to analyze the association between CASZ1 methylation and body mass index (BMI) and overweight/obesity, respectively; while additive scale and multiplicative scale were used to evaluate the interaction effects between methylation levels and environmental factors on overweight/obesity.

Results

The levels of CASZ1 methylation at CpG51, 70, 72, 98, 157, 165 were associated with BMI after adjustments. There were differences between cases and controls in the levels of DNA methylation at CpG58, 72, 98, 116, 157, 163, 165. Methylation at CpG72, 98, 116, 157, 163 were associated with overweight/obesity after adjustments. The association between methylation levels of CpG98 (Odds ratio=1.06, 95% Confidence interval: 1.02-1.10) and CpG165 (Odds ratio =1.15, 95% Confidence interval: 1.07-1.42) and overweight/obesity was significant after considering multiple testing (P < 0.003). Besides, we found significant additive interaction effects of drinking status and triglyceride level with CpG163 on BMI.

Conclusion

The methylation levels of CpG sites in CASZ1 gene, as well as the interactions with some environmental factors, were associated with overweight/obesity in the Chinese Han population.

1. Introduction

Increasing prevalence of obesity has become a serious health problem all around the world, approximately 2.28 billion children and adults are estimated to be overweight or obesity [1]. Globally, the proportion of adults with a body mass index (BMI) of 25 kg/m² or greater increased from 28.8% in 1980 to 36.9% in 2013 for men, and from 29.8% to 38.0% for women. The substantial increases of obesity were observed in both developed and developing countries [2]. A study in the US suggested that obesity is associated with decreases in life expectancy significantly, especially among young adults[3]. Furthermore, excessive weight gain can increase the burden of many different diseases, such as cardiovascular diseases, diabetes, and cancers [4]. Obesity is caused not only by unhealthy diet and irregular exercise, but also by genes and gene expression [5].

Obesity is of highly heritability [6]. A genome-wide association meta-analysis of 2499,796 individuals of European descent confirmed 14 known obesity-susceptibility loci and revealed 18 new loci associated with BMI, and it is speculated that there may be more than 250 common variants affecting BMI that have not yet been identified [7]. Epigenetic markers involved in regulating gene expression, including DNA methylation, histone modifications, chromatin remodeling and microRNA [8]. DNA methylation is one of the most stable and prevalent epigenetic markers. A epigenome-wide study has associated DNA methylation at CpG loci with obesity, based on BMI and waist circumference (WC) [9]. However, epigenetic factors associated with obesity are largely unknown.

Human castor zinc finger 1 (CASZ1) gene, which codes for a zinc finger transcription factor, is mapped on chromosome 1p36 [10]. CASZ1a with 11 zinc fingers and CASZ1b with 5 zinc fingers were both encoded by CASZ1 gene. CASZ1b is more evolutionally conserved and could express with CASZ1a during neurogenesis [11], which is associated with obesity [12]. In addition, CASZ1 gene plays an important role in suppressing neuroblastoma (NB) cell growth[13]. A Korean female case showed that rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation (ROHHAD) syndrome associates with neuroblastoma [14]. In neuroblastoma N2A cells, PAS kinase (PASK) was found to be important for feeding behavior, which may associate with obesity [15]. Besides, CASZ1 has been found to associate with blood pressure (BP) and hypertension in Chinese[16] and Japanese population[17]. So far, numerous studies have demonstrated that BP is associated with increasing weight gain[18]. A whole-genome linkage scan showed that 1p36 was associated with BMI [19]. According to the latest study, the CASZ1 methylation in CD4+ T cells was strongly associated with the amount of visceral adipose tissue [20]. In this context, CASZ1 gene methylation may play a role in obesity. Therefore, further researches and analyses are necessary to
reveal the association between CASZ1 gene methylation and obesity. In this study, we examined the association between CASZ1 gene methylation level and overweight/obesity. In addition, we investigated the interaction effects between CASZ1 gene methylation levels and some environmental factors on overweight/obesity.

2. Methods And Material

2.1 Subjects

The study included 1,029 individuals that were randomly selected from a study on the prevention and treatment of metabolic syndrome in community population of Jiangsu Province. The study was conducted in 2007–2008 in Changshu City, Jiangsu Province, China. Participants who had chronic kidney disease, definite diagnosis of heart disease, acute infectious disease or chronic wasting disease or serious liver disease were excluded. A standard questionnaire was designed including demographic characteristics, lifestyle risk factors, personal medical history, and family history of hypertension for all participants, and conducted by trained staff. After resting for 5 minutes, each participant had been measured sitting BP for three times by the trained observers using a standard mercury sphygmomanometer, three measurements were taken at 30 seconds intervals. The first and fifth Korotkoff sounds were recorded as the systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively. The mean of the three BP measurements was used in the analysis. The trained observers measured body weight, height and waist circumference (WC) using the standard protocols, and BMI was calculated as the weight in kilograms divided by the square of the height in meters. Overweight and obesity were defined by BMI cutoff points according to the Chinese criterion: 24.0 kg/m² ≤ BMI ≤ 27.9 kg/m² for overweight and BMI ≥ 28.0 kg/m² for obesity [16]. We classified overweight/obesity participants as cases (n = 288) and underweight or normal weight participants as controls (n = 741) according to the Chinese BMI classification. Smoking was defined as ever having smoked at least 100 cigarettes. Drinking was defined as consuming any type of alcohol beverage at least 12 times during the past 1 year. Fasting blood samples were collected by professional laboratory staffs and placed in EDTA anticoagulant tubes. After centrifugation at 3,000 r/min for 10 min, white blood cells were separated and placed in a 1.5 ml EP tube and stored at −80 °C until further processing. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and fasting blood glucose (FBG) were measured by laboratory tests. The study was approved by the Ethics committee of Soochow University, and informed consent was signed with all the individuals.

2.2 DNA methylation sequencing

DNA was extracted from white blood cell. DNA methylation profiling was performed with bisulfite sequencing according to the targeted bisulfite sequencing (MethylTarget™) developed by Genesky Biotechnologies Inc. (Shanghai, China). CpG islands close to the promoter region (from 2 kilo bases upstream of the transcription start site to 1 kilo bases downstream of the first exon) of CASZ1 gene were analyzed, the targets which based on these CpG islands (Percent C + Percent G > 50.00%, Observed/Expected ratio > 0.60, Length > 200bp) were identified and sequenced. Genomic DNA through quality control (concentration ≥ 20ng/μL, total DNA ≥ 1μg, OD260/280 = 1.7 ~ 2.0, OD260/230 ≥ 1.8) were subjected to bisulphite modification using the EZ DNA Methylation-Gold Kit (ZYMO, CA, USA). Polymerase chain reaction was used for amplifying the targeted DNA sequences. Using an Illumina MiSeq benchtop sequencer (Illumina, CA, United States) to sequence the CpG islands. Methylation levels of each CpG site were calculated as the percentage of the methylated cytosines in the total measured cytosines. The median of methylation levels of all measured CpG sites within the gene fragment was used to represent the methylation level of CASZ1. 20 samples were randomly selected for duplicate detection in order to test the reliability of methylation level for each CpG site.

2.3 Statistical Analysis

To compare the anthropometric and biochemical differences between cases and controls, the continuous variables were analyzed by independent samples t test or nonparametric test, and presented as the mean ± standard deviation (SD) or median (P_{50}). The categorical variables were tested by chi-square analyses, and presented as number (percentages) of participants. Multiple linear regression analyses were conducted to investigate the association between CASZ1 methylation levels and BMI after adjusting for confounding factors. The differences of CASZ1 methylation levels between the cases and controls were analyzed by nonparametric test. Unconditional logistic regression analyses were conducted to investigate the association between CASZ1 methylation levels and overweight/obesity after adjusting for co-variates. Odds ratios (ORs) and the corresponding confidence intervals (CIs) for 1-percent increase in methylation levels were calculated. Besides, we estimated the interaction effects between the levels of CASZ1 gene methylation and other risk factors on a multiplicative scale[21] and an additive scale[22] in regression models. Bonferroni method was used for multiple testing adjustment. All analyses were performed using SAS statistical software (version 9.4, Cary, North Carolina, USA).

3. Results
3.1 Characteristics of the cases and controls

The Chinese BMI obese criterion was applied to divide the participants into cases and controls. General characteristics of cases and controls were shown in Table 1. There was no difference between cases and controls in gender, height, smoking proportion or drinking proportion. Nevertheless, the cases had higher levels of weight, WC, BMI, FBG, TC, TG, LDL-C, SBP, DBP, HTN proportion and lower levels of age, HDL-C as compared with controls (P<0.05) (Table 1).

3.2 Association between CASZ1 methylation levels and BMI

We obtained methylation levels for 17 CpG sites in CASZ1. Multiple linear regression models were used to evaluate the association of CASZ1 methylation levels with BMI. In Table 2, after adjustment for age, sex, smoking and drinking status, CpG51, 67, 70, 72, 94, 98, 116, 157, 165 were associated with BMI, respectively (P<0.05). The associations between methylation levels of CpG72 and 165 and BMI were significant after considering multiple testing (Bonferroni correction, P<0.003 = 0.05/17). In model 2, adjusting for age, sex, smoking and drinking status, SBP, FBG and TC, there were associations between CpG51, 70, 72, 98, 157, 165 and BMI (P<0.05). The remaining CpG sites were not associated with BMI (P>0.05). The association between methylation level of CpG 165 and BMI was significant after considering multiple testing in model 2 (P = 0.001). The median of all of the tested CpG sites in CASZ1 was associated with BMI both in model 1 (P=0.002) and model 2 (P=0.032) (Table 2).

3.3 Association between CASZ1 methylation and overweight/obesity

As shown in figure 1, the CASZ1 methylation levels of 17 CpG sites were expressed as the median. There was a significant difference of CASZ1 DNA methylation values for CpG58, 72, 98, 116, 157, 163, 165 and median of all CpG sites (P<0.05). The median methylation level of these CpG sites of the cases were higher than those of the controls (Table 3). The level of CpG165 had a significant difference between cases and controls both in male and female as well (P=0.004 and 0.003, respectively) (figure 2). In table 3, after adjustment for age, sex, smoking and drinking status, CpG70, 72, 98, 116, 163, 165 were associated with overweight/obesity cases which were defined by BMI obesity criterion markedly (P<0.05). Besides, CpG72, 98, 163, 165 were related to overweight/obesity (P<0.05) after adjustment for age, sex, smoking and drinking status, SBP, FBG and TC. In the two models, the median of methylation level at CpG sites was associated with overweight/obesity divided by BMI obese criterion (P<0.05). The associations between methylation level of CpG98 (OR=1.06, 95%CI: 1.02-1.10) and CpG 165 (OR=1.15, 95%CI: 1.07-1.42) and overweight/obesity were significant after considering multiple testing (P<0.003).

3.4 The additive interaction effects on BMI with adjustment for environmental factors and CpG sites

We tested the interaction effects of methylation levels of CASZ1 CpG sites and risk factors on BMI using an additive model. Interaction effects between the CpG74, 98 and age on BMI were observed after adjustment for age, sex, smoking and drinking status, SBP, FBG and TC (β=-0.004, -0.004, Pinteraction = 0.031, 0.040, respectively). There was a similar interaction effect between CpG163 and sex on BMI(β=-0.177, Pinteraction= 0.043). CpG163 and drinking status had an significant additive interaction effect on BMI as well(β=-0.285, Pinteraction= 0.001). Besides, CpG70 and SBP had an interaction effect on BMI(β=0.002, Pinteraction= 0.045). There were also interaction effects between CpG35, 94 and FBG on BMI (β=-0.046 and -0.072, Pinteraction = 0.019 and 0.033, respectively). Interactions of CpG163 and TG, CpG72 and HDL-C were found for decreasing BMI (β=-0.080 and -0.200, Pinteraction = 0.002 and 0.011, respectively) (Table 4). The multiplicative interaction effects between the CASZ1 CpG sites and phenotypes on overweight/obesity were conducted after adjusting for age, sex, smoking and drinking status, SBP, FBG and TC as well. However, these interactions were not significant after considering multiple testing.

4. Discussion

In this study we examined the association between the methylation levels of CpG sites in CASZ1 gene and obesity in the Chinese Han population. There was a difference between obese and normal weight individuals in the level of CASZ1 DNA methylation. The methylation level of CASZ1 was associated with BMI and overweight/obesity. Besides, we have found several CpG sites of CASZ1 gene have significant additive interaction effects with some environmental factors, such as age, drinking status, SBP, FBG, TC, TG, HDL-C on BMI. This is the first study to report the association between CASZ1 methylation levels and obesity.

In an epigenome-wide association study, it was confirmed that 164 CpGs were associated with BMI [23]. However, there may still be some undiscovered CpG sites associated with BMI. Genome-wide association studies could only explain part of genetic variability, and epigenetics could be conducive to explain the missing variability. CASZ1 expression is essential for cardiomyocyte cell-cycle progression in the cardiovascular system [24]. A research has shown that CASZ1 gene was associated with BP [25], and methylation in CASZ1 may be associated with cardiovascular patients in regulating mortality [26]. It was widely known that BP is significantly associated with weight
gain [18]. In the current study, we found that CASZ1 methylation is associated with overweight/obesity, but it was still unclear whether the higher level of CASZ1 methylation caused obesity. Recent investigation indicated that changes in DNA methylation were consequences of adiposity at the majority of the identified CpG sites [27].

The mechanism by which obesity may be associated with methylation of CASZ1 was unclear. The methylation at a specific CpG (cg17177074) of CASZ1 gene was identified to be associated with visceral adipose tissue in CD4+ T cells [20]. CASZ1 gene was highly expressed in CD4+ T cells, and can promote the expression of T-helper type 17 (Th17) genes by influencing chromatin modifications in Th17 lineage genes [28]. Th17 cell, a proinflammatory T cell subset, is vital for secreting Interleukin 17 (IL-17) [29]. Recent study indicated that IL-17 has a crucial role in the creation of inflammation in adipose tissues of obese individuals [30]. IL-17- producing CD4(+) T cells was enriched in adipose tissue of type 2 diabetic obese patients, and the adipose tissue macrophages express IL-17 receptors, indicating IL-17 was associated with obesity [31]. Besides, CASZ1 could regulate gene transcription by means of recruiting NuRD complex [32]. Mi-2/NuRD was associated with Macrophage Immune Function, and macrophages play important roles in causing obesity [33]. In addition, CASZ1b could express during neurogenesis [11], and adult neurogenesis is associated with fat mass and obesity-associated protein [34]. CASZ1b lengthens a NB cell cycle progression by activating restoring pRb, which caused the suppression of NB [35]. A critical region at the CASZ1 N terminus (AAs 23-40) was identified to suppress NB tumorigenesis by mediating the interaction between CASZ1b nuclear localization and NuRD, [36]. A case had ROHHAD syndrome showed that obesity may be associated with neuroblastoma, but the mechanism has still not been clarified [20]. To confirm the mechanism between CASZ1 methylation and obesity, further research remains to be conducted.

There were some potential limitations to this study. Firstly, although the association between CASZ1 methylation and obesity was detected in this study, the validation in independent samples was not performed. Secondly, this study was conducted methylation detection only in the peripheral blood, the methylation detection of fat tissue may get more meaningful CpG sites. Thirdly, this study could not provide direct evidence of the expression of CASZ1 due to the lack of gene expression data. Further study should be conducted to measure gene expression data for clarifying the causal relationship between methylation and obesity.

5. Conclusion

In summary, this study first showed that blood cell DNA methylation level of CpGs in CASZ1 gene was associated with overweight/obesity in the Chinese Han population. The level of CASZ1 gene methylation and some environmental factors had significant interaction effects on overweight/obesity. The results of this study not only provide a new clue for the study of CASZ1 methylation, but also provide a basis for the future obesity researches, and have certain reference value for the interaction effects between CASZ1 methylation and environmental factors on obesity. Further studies are needed to confirm the association and unfold the mechanism.

Declarations

Acknowledges

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Ethics approval and consent to participate

The study was approved by the Ethics committee of Soochow University, and informed consent was signed with all the individuals.

Competing interests

The authors declare that they have no competing interests.

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### Tables

Table 1 Characteristics of the cases and controls

| Characteristics | Cases(n=288) | Controls(n=741) | t/χ² | P Value |
|-----------------|--------------|-----------------|------|---------|
| Age(years)      | 59.03±11.07  | 61.01±12.18     | -2.401 | 0.016   |
| Man, number (%) | 154(53.47)   | 415(56.01)      | 0.538 | 0.463   |
| Height(cm)      | 159.63±8.50  | 159.33±8.78     | -0.506 | 0.613   |
| Weight(kg)      | 66.66±8.59   | 52.40±7.90      | -25.355 | P<0.001 |
| WC(cm)          | 86.41±6.74   | 73.12±7.46      | -20.314 | P<0.001 |
| BMI(kg/m²)      | 26.09±1.95   | 20.57±2.08      | -24.933 | P<0.001 |
| FBG(mmol/L)     | 5.25±1.26    | 4.93±0.72       | -4.018 | P<0.001 |
| TC(mmol/L)      | 4.79±0.96    | 4.52±0.92       | -4.185 | P<0.001 |
| TG(mmol/L)      | 1.91±1.43    | 1.32±0.81       | -8.960 | P<0.001 |
| HDL-C(mmol/L)   | 1.24±0.30    | 1.41±0.32       | 7.505  | P<0.001 |
| LDL-C(mmol/L)   | 2.75±0.76    | 2.53±0.75       | -4.266 | P<0.001 |
| SBP(mm Hg)      | 140.30±24.04 | 130.81±22.32    | -5.809 | P<0.001 |
| DBP(mm Hg)      | 86.45±13.36  | 77.79±11.77     | -9.309 | P<0.001 |
| Smoking, number (%) | 109(38.11)   | 300(40.87)      | 0.653  | 0.419   |
| Drinking, number (%) | 81(28.13)   | 194(26.18)      | 0.400  | 0.527   |
| HTN             | 156(54.17)   | 225(30.36)      | 50.392 | P<0.001 |
Table 2 Association between CASZ1 methylation levels and BMI

| Genome Position | Distance to TSS, bp | Type       | Model 1          | Model 2          |
|-----------------|--------------------|------------|------------------|------------------|
|                 |                    |            | β    | SE  | t value | P value | β    | SE  | t value | P value |
| CpG31           | 10856830           | -97        | CG   | -0.002 | 0.023 | -0.10 | 0.917 | -0.009 | 0.021 | -0.41 | 0.685 |
| CpG35           | 10856832           | -99        | CG/rs11810285  | -0.001 | 0.015 | -0.07 | 0.943 | -0.007 | 0.014 | -0.51 | 0.608 |
| CpG51           | 10856838           | -105       | CG   | 0.026 | 0.012 | 2.17  | 0.030 | 0.025 | 0.011 | 2.24  | 0.026 |
| CpG58           | 10856845           | -112       | CG   | 0.036 | 0.023 | 1.58  | 0.115 | 0.016 | 0.021 | 0.76  | 0.445 |
| CpG62           | 10856879           | -146       | CG   | 0.028 | 0.021 | 1.38  | 0.169 | 0.009 | 0.019 | 0.45  | 0.650 |
| CpG67           | 10856897           | -164       | CG   | 0.061 | 0.025 | 2.48  | 0.014 | 0.040 | 0.024 | 1.70  | 0.090 |
| CpG70           | 10856901           | -168       | CG   | 0.049 | 0.020 | 2.45  | 0.015 | 0.044 | 0.019 | 2.30  | 0.022 |
| CpG72           | 10856915           | -182       | CG   | 0.086 | 0.028 | 3.08  | 0.002 | 0.056 | 0.027 | 2.06  | 0.040 |
| CpG74           | 10856921           | -188       | CG   | 0.027 | 0.022 | 1.20  | 0.229 | 0.021 | 0.021 | 1.00  | 0.316 |
| CpG80           | 10856923           | -190       | CG   | 0.020 | 0.024 | 0.85  | 0.397 | 0.007 | 0.022 | 0.31  | 0.760 |
| CpG94           | 10856925           | -192       | CG   | 0.062 | 0.029 | 2.15  | 0.032 | 0.047 | 0.028 | 1.70  | 0.089 |
| CpG98           | 10856928           | -195       | CG   | 0.074 | 0.025 | 2.98  | 0.003 | 0.066 | 0.023 | 2.84  | 0.005 |
| CpG116          | 10856933           | -200       | CG   | 0.065 | 0.033 | 1.98  | 0.048 | 0.023 | 0.031 | 0.73  | 0.465 |
| CpG150          | 10856937           | -204       | CG   | 0.040 | 0.021 | 1.90  | 0.058 | 0.021 | 0.020 | 1.06  | 0.290 |
| CpG157          | 10856944           | -211       | CG   | 0.108 | 0.040 | 2.69  | 0.007 | 0.083 | 0.039 | 2.14  | 0.033 |
| CpG163          | 10856960           | -227       | CG   | 0.071 | 0.044 | 1.62  | 0.107 | 0.048 | 0.042 | 1.12  | 0.263 |
| CpG165          | 10856964           | -231       | CG   | 0.207 | 0.043 | 4.81  | 0.001 | 0.148 | 0.042 | 3.52  | 0.001 |
| Median          | -                  | -          | -    | 0.085 | 0.027 | 3.11  | 0.002 | 0.056 | 0.026 | 2.15  | 0.032 |

Model 1, adjusted for age, sex, smoking and drinking status.

Model 2, adjusted for age, sex, smoking and drinking status, systolic blood pressure, fasting blood glucose, and total cholesterol.

Table 3 Association between CASZ1 methylation and overweight/obesity
replace TC in the model to analysis the multiplicative interaction effect of TG and methylation level.

Adjusted for age, sex, smoking and drinking status, systolic blood pressure, fasting blood glucose, total cholesterol. Using TG or HDL-C replace TC in the model to analysis the multiplicative interaction effect of TG and methylation level.

### Table 4 The additive interaction effects on BMI with adjustment for environmental factors and CpG sites

| Factor | CpG     | β   | SE  | P value | CpG     | β   | SE  | P value | CpG     | β   | SE  | P value |
|--------|---------|-----|-----|---------|---------|-----|-----|---------|---------|-----|-----|---------|
| Age    | CpG74   | -0.048 | 0.018 | 0.009 |         |         |     |         |         |         |     |       |         |
| Age    | CpG98   | -0.050 | 0.017 | 0.003 |         |         |     |         |         |         |     |       |         |
| Sex    | CpG163  | 0.678 | 0.466 | 0.146 |         |         |     |         |         |         |     |       |         |
| Drink  | CpG163  | 1.103 | 0.422 | 0.009 |         |         |     |         |         |         |     |       |         |
| SBP    | CpG70   | 0.018 | 0.009 | 0.053 | -0.181  | 0.114 | 0.111 | 0.002  |         |     |     |         |
| FBG    | CpG35   | 0.985 | 0.283 | 0.001 | 0.222   | 0.099 | 0.025 | -0.046 | 0.020  | 0.019 |     |       |         |
| FBG    | CpG94   | 0.775 | 0.235 | 0.001 | 0.396   | 0.166 | 0.017 | -0.072 | 0.034  | 0.033 |     |       |         |
| TG     | CpG163  | 0.934 | 0.151 | P<0.0001 | 0.165 | 0.059 | 0.005 | -0.080 | 0.025  | 0.002 |     |       |         |
| HDL-C  | CpG72   | -1.657 | 0.546 | 0.003 | 0.332   | 0.109 | 0.003 | -0.200 | 0.078  | 0.011 |     |       |         |

Model 1, adjusted for age, sex, smoking and drinking status.

Model2, adjusted for age, sex, smoking and drinking status, systolic blood pressure, fasting blood glucose, total cholesterol.