Association of *FTO* rs9939609 and *CD36* rs1761667 with Visceral Obesity

Stephen SALIM1, Felicia KARTAWIDJAJAPUTRA2,* and Antonius SUWANTO1

1 Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jalan Jendral Sudirman 51 Jakarta Selatan, Jakarta, Indonesia 12930
2 Nutrifood Research Center, PT Nutrifood Indonesia, Jalan Rawabali II No.3 Kawasan Industri Pulogadung Jakarta Timur, Jakarta, Indonesia 13920

(Received June 22, 2019)

**Summary** In 2016, more than 1.9 billion adults were overweight; of which, over 650 million of adults were obese. Genetics and lifestyle play important roles in the development of obesity. Studies have shown that genetic variants contribute in developing obesity; such as *FTO* and *CD36*, which regulate metabolism and food preferences. Many researches have also emphasized the importance of lifestyle in obesity prevention. However, the interactions of both factors were still underexplored. Therefore, this study was aimed to assess the interaction between *FTO*-*CD36* variants and fat consumption on the metabolic status of healthy Indonesians. Twenty-one females and seventeen males were involved in this cross-sectional study. *CD36* rs1761667 and *FTO* rs9939609 genotypes were identified from blood samples using PCR-RFLP. Data were compared with dietary patterns (24-h food recall), physical activities (IPAQ), medical records, and body compositions (InBody720). Results: *CD36* rs1761667 AA and AG group showed higher—but not significant—fat consumption, WHR, and VFA compared to GG. The trend persisted after gender and physical activity adjustment. Meanwhile, *FTO* rs9939609 AT group showed significant higher WC, WHR and VFA in male subjects after gender and energy balance adjustment: WC (TT: 74.40±3.85, AT: 85.50±5.92, p=0.011), WHR (TT: 0.85±0.02, AT: 0.92±0.04, p=0.010), and VFA (TT: 48.65±10.61, AT: 78.48±15.18, p=0.010). *CD36* rs1761667 might be correlated with higher fat consumption and visceral obesity; while *FTO* rs9939609 showed a significant association with male visceral obesity. These results indicates that both genetic variants were potential as visceral obesity markers.

**Key Words** *CD36* rs1761667, *FTO* rs9939609, Visceral obesity, Fat consumption, BMI

Recent data from WHO shows that in the last three decades the number of worldwide obesity has raised more than twofold. In 2014, at least 600 million adults were obese. This number is expected to increase further if there is no action taken to overcome this epidemic. Obesity raises the risk of other diseases, such as cardiovascular diseases, diabetes, and hypertension. Every year, the mortality of more than 2.800.000 people worldwide is due to the health complications associated with obesity; and at least 300.000 of them are from South-East Asia (1,2).

Nowadays, consumption of high-fat diet and low physical activity are the main reasons for developing obesity. However, some researches have suggested that genetics plays an important role in the development of obesity. The fatty acid receptor gene single nucleotide polymorphism (SNP), *CD36* rs1761667 has been identified in human tongue and directly linked to obesity due to the fat overconsumption in risk allele (3). The rs1761667 is a polymorphism at the promoter of *CD36*. A allele variation of this polymorphism has a lower *CD36* expression compared to the GG genotype (4–6). Therefore, A allele has lower fatty acid sensitivity and more prone to fat overconsumption compared to the GG genotype (7). The fat overconsumption in A allele is associated with higher BMI compared to the GG genotype (8).

The high-risk variation within *FTO* rs9939609 SNP has also been reported in a human study to be associated with obesity. *FTO* encodes for FTO enzyme, an α-ketoglutarate dependent dioxygenase. FTO has an oxidative demethylation activity towards N6-methyladenosine (m6A). Ghrelin can only be synthesized after m6A is demethylated by FTO. The synthesized ghrelin will then penetrate the blood-brain barrier to exert its effect on the hypothalamus to increase appetite (9,10). *FTO* rs9939609, the first intron polymorphism is associated with obesity due to the higher BMI and food imaging activity in A allele. Karra et al. suggested that this was due to the *FTO* upregulation in A allele. However, a recent study concluded that *FTO* SNP did not influence *FTO* expression (11). A diminished resting energy expenditure (REE) in A allele of *FTO* might be the plausible reason on how it had higher BMI compared with the TT genotype (12).

*To whom correspondence should be addressed.*
E-mail: felicia@nutrifood.co.id
Both genes are the main focus of this study due to their well-known effect on several obesity parameters throughout various populations (6, 7, 13). However, the studies that associate CD36 and FTO with body composition and the dietary pattern is still absent in Indonesia. Since human genetic architecture varies among different population, the effect of these SNPs in Indonesia might produce different results and generate a new understanding.

Planning effective strategies to overcome this epidemic will require insights from both genetics and lifestyle. However, the research is still inadequate in Indonesia. Therefore, this study aimed to investigate the interaction between genetics variants (CD36 and FTO) and lifestyle (dietary intake and physical activity) on other phenotype characteristics in Indonesian population.

MATERIALS AND METHODS

Subjects recruitment. This study involved 38 subjects (21 females and 17 males) who were selected based on the following criteria: aged between 20–30 y old, did not suffer any chronic diseases or fever, did not smoke, did not drink alcohol frequently (five times or more in the past month), did not take any medication or treatment, not being pregnant and not breastfeeding.

Body composition analysis and medical data collection. The subjects had to undergo body composition measurement and the medical checkup procedure before participating in this study. The subject’s body composition was analyzed using InBody720 (Biospace, Gangnam-gu, Seoul, Korea) which was based on bioelectrical impedance method. The instrument predicts body fat mass and non-fat mass by measuring total water value and low electric current resistance within the human body (14). Other body compositions, such as body mass index (BMI), waist-hip ratio (WHR), percent body fat (PBF), visceral fat area (VFA), and basal metabolic rate (BMR) were also analyzed using this instrument. The medical data collected from subjects were total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, blood glucose, systolic and diastolic blood pressure.

Twenty-four-hour food recall and physical activity survey. To investigate subjects’ nutrient intake in the last 24 h, all subjects were interviewed using 24-hour food recall. Subjects were required to memorize the type and amount of food and drinks they had consumed for the last 24 h; and informed it to the interviewer. Food models were used as visual aid to facilitate this procedure. The 24-hour food recall was performed three times; twice on the weekdays and once on the weekend. The 24-hour food recall data were further processed using Food Processor SQL 10.1.1; ESHA, Salem, Oregon to generate detailed nutrient intake information.

Energy intake information was adjusted for energy balance. The adjustment for energy balance was based on modified Schofield equation. Total energy expenditure (TEE) was calculated by adding 30% of the Basal metabolic rate (BMR) calories to BMR for low physical activity, 50% of BMR calories for moderate physical activity and 100% of BMR calories for high physical activity. In contrast to the conventional Schofield equation, BMR data in this study was obtained from InBody720 (Biospace, Gangnam-gu, Seoul, Korea) (15).

International Physical Activity Questionnaire (IPAQ) was used to analyze the activity level. This questionnaire was used to calculate the physical activity Metabolic equivalents (METs) value taken for the past week. The physical activity METs were calculated based on the intensity and duration of an activity. METs are described as the amount of oxygen consumed when sitting at rest (1 METs= 3.5 mL O₂·kg⁻¹·min⁻¹). The expended energy can be determined by dividing relative oxygen cost of the activity (mL O/ kg/min) by 3.5. We obtained the METs values of various activities from the previous study by Ainsworth et al. (16). The subjects were classified based on their METs into low, moderate, and high activity categories (17).

CD36 and FTO genotyping. Blood samples were collected from all subjects after 8 h of fasting. The DNAs were isolated from the blood samples using phenol-chloroform method and the concentration was determined using NanoDrop 2000 (Thermo Scientific, Massachusetts, United States). The DNAs were then amplified in PCR reaction using PCR GS 482 (G-Storm, Somerset, United Kingdom). The CD36 rs1761667 and FTO rs9939609 primer set were obtained from Banerjee et al. 2010 and Shahid et al. 2013 respectively (18, 19). CD36 PCR cycling conditions consists of initial denaturation at 95˚C for 5 min, followed by 35 cycles of denaturation at 95˚C for 30 s, annealing at 54˚C for 30 s, and extension at 72˚C for 30 s. Whereas, the FTO PCR cycling conditions were initial denaturation at 95˚C for 5 min, followed by 35 cycles of denaturation at 95˚C for 30 s, annealing at 58˚C for 30 s, and extension at 72˚C for 30 s. The amplified genes were subjected to restriction fragment length polymorphism (RFLP) method using HhaI enzyme for CD36 rs1761667 and Scal enzyme for FTO rs9939609. The genotypes were determined using agarose gel electrophoresis.

Statistical analysis. Statistical analyses were performed with SPSS Statistics 21 (IBM, Armonk, New York, United States). The mean difference between groups was analyzed using Student’s t-test or Mann-Whitney test. Goodness of fit test was performed using Hardy-Weinberg equilibrium calculator to determine whether the observed population is significantly different from the expected population of Hardy-Weinberg Equilibrium (HWE) (20). All p values reported in this study were two-tailed and p<0.05 were considered as statistically significant.

Statement of ethics. The informed consent was obtained from all subjects according to the Helsinki Declaration. This study was reviewed and approved by Ethical Commissions of Atma Jaya Catholic University of Indonesia. approval number: 1468/III/LPPM-PM. 10.05/09/2016. Furthermore, this study was registered in Australian New Zealand Clinical Trials Registry (ANZCTR), Trial Id: ACTRN12618000579291.
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RESULTS

AG and AA genotypes of CD36 rs1761667 contributed in more fat consumption, and had higher WC and VFA: The genotype distribution of CD36 rs1761667 was as follow: GG (58%), AG and AA (42%) (Fig. 1A). The allelic frequency of CD36 rs1761667 conforms to HWE and there was no observed of statistically significant deviation from HWE ($\chi^2 = 2.90 < 3.84$, $p = 0.005$, df=1). The fat consumption was not significantly different between genotypes; however, there were consistent trends that subjects with AA and AG genotypes consumed a higher amount of fat compared to the GG genotype. The trends persisted even after adjusting for gender (Fig. 1B & 1C) and physical activity (Fig. 1D). Interestingly, the trend was more obvious in males (Fig. 1B) and in the high physical activity group (Fig. 1D). Despite higher fat consumption, the BMI value (Fig. 2A) of AG and AA groups were slightly lower than GG group; although WHR (Fig. 2B) and VFA (Fig. 2C) values were higher. However, the difference was not statistically significant.

AT genotype of FTO rs9939609 had lower energy intake and increased visceral fat deposition: The genotype distribution of FTO rs9939609 polymorphism was as follows: TT (66%) and AT (34%) (Fig. 3A). No statistically significant deviation from HWE was observed in allelic frequency of FTO rs9939609 ($\chi^2 = 1.62 < 3.84$, $p = 0.005$, df=1). AT group had lower calorie consumption and the trend persisted even after adjustment by gender (Fig. 3B). Further physical activity adjustment also showed higher calorie consumption in AT group; except for the high activity group, in which AT had slightly higher calorie consumption than TT subjects (Fig. 3C). However, the BMI was higher in AT compared to TT group, even after being adjusted for gender (Fig. 4A), gender and energy balance, according to modified Schofield equation (Fig. 4B), and physical activity level.
Moreover, the AT group also showed higher WC, WHR and VFA value; although the values were only significant in male subjects: WC (TT: 74.40 \pm 3.85, AT: 85.50 \pm 5.92, \( p = 0.011 \)) (Fig. 5A), WHR (TT: 0.85 \pm 0.02, AT: 0.92 \pm 0.04, \( p = 0.010 \)) (Fig. 5B), and VFA (TT: 48.65 \pm 10.61, AT: 78.48 \pm 15.18, \( p = 0.010 \)) (Fig. 5C).

**DISCUSSION**

The result showed that the CD36 rs1761667 genotype in this population study was comparable to the distribution in Japanese population; which was 59.6% for
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GG, 36.5% for AG, and 3.8% for AA (21). However, the result was different compared to Mexican, African-American, and Egyptian populations. Lopez et al. reported the allele distribution in Mexican population to be GG (19%), AG (47.4%), and AA (33.6%) (6). Keller et al. reported the distribution of African-American population genotypes were GG (31.5%), AG (49.4%), and AA (19.2%) (7). Bayoumy et al. found that the Egyptian population genotype distribution to be GG (3%), AG (59%), and AA (38%) (22).

This result suggested that there was a difference in the major genotype of CD36 rs1761667 in Asians compared to other races. The GG genotype was dominant in Asian, while AG genotype was dominant in African, Latin American, and Egyptian. It was well known that CD36 is also Plasmodium falciparum receptor in erythrocytes and directly linked to malaria susceptibility. Therefore, the mutation which reduced the CD36 peptide level might increase malaria resistance. In this case, AA and AG have lower CD36 peptide levels, thus resulted in lower chance of being infected by P. falciparum compared to GG. This selective pressure had led to natural selection for AG and AA genotype, where malaria was a major health concern. Hence, AG and AA were more common than GG in countries which had high malaria cases in the past, such as African countries, Mexico, and Egypt (22, 23).

A study suggested that A allele of CD36 rs1761667 was associated with lower BMI (24). This was in line with our results; which showed that, although the A allele had a higher fat consumption, the BMI was lower. Interestingly, it showed higher WC, which suggested a higher risk of visceral obesity. This could explain the plausible effect of excessive fat consumption in A allele subjects because WC and VFA were superior to BMI as obesity measurement, especially when muscle mass was low and visceral adiposity was high (25).

The FTO rs9939609 genotype distribution in our study population was similar to the Han Chinese in HapMap project, which was 76.7% for TT, 23.3% for AT, and 0% for AA. On the other hand, the FTO rs9939609 allele distribution in this study was different from Northern or Western European, which was 22% for TT, 11.9% for AT, and 66.1% for AA (26).

FTO rs9939609 association with BMI has been inconsistent in several populations (27). Since genetic architecture varies across populations, the generated results might also be different. Previous studies suggest that AA and AT groups were associated with higher BMI and obesity risk (10, 11). In this study, a similar trend of higher BMI in AT group was being observed, but the value was not statistically significant. The trend remained consistent even after adjusting for gender and physical activity level.

Furthermore, we classified the subjects into negative and positive energy balance groups. The energy requirement for each individual might vary, and excessive energy intake might also result in higher obesity parameters regardless of the FTO rs9939609 variants (28).

Our results shows that AT genotype of FTO rs9939609 had significantly higher WC (Fig. 5A), WHR (Fig. 5B), and VFA (Fig. 5C) in males; even though there was no difference in energy intake between AT and TT, even after adjustment of energy balance and physical activity.
gender. The female group did not show any statistically significant difference in WC, WHR, and VFA. This could be due to epigenetic regulation of sex-specific loci and hormonal factors which lead females to store more fat in subcutaneous adipose tissue than abdominal adipose tissue (29).

This result implied that individuals with AT genotype might have a defective metabolism, such as the impaired ability to use fat as an energy source and reduced adipocyte thermogenesis. This could be due to high linkage disequilibrium between FTO rs9939609 and IRX3 (30). IRX3 directly inhibited white adipose tissue browning and disrupted energy homeostasis (31). Therefore, FTO rs9939609 A allele might increase IRX3 expression to decrease energy expenditure and increase lipid storage. Interestingly, this study showed that high physical activity could minimize the disadvantageous effect of FTO A allele; thereby reduced the gap between AT and TT BMI, although the AT group had consumed more calorie compared to TT in the high physical activity group. This finding is in line with Kim et al. results (32).

In conclusion, genetic variation might predispose humans into obesity. For instance, risk variants within FTO and CD36 which regulate metabolism and determine fatty food preference respectively. The CD36 rs1761667 AG and AA might be associated with a higher fat intake which led to higher abdominal adiposity. Moreover, AT genotype of FTO rs9939609 showed significantly higher parameters for abdominal obesity, especially in male subjects. Interestingly, the high physical activity could reduce the disadvantageous effects of FTO A allele in the AT genotype individuals.

Therefore, we conclude that genetics might play a role in the development of obesity by regulating dietary pattern and metabolism. Small sample size and lack of case-control group were the limitations in this study. To determine the relationship between the high-fat diet and obesity, it will be necessary to perform a further case-control study by comparing subject’s genotype and high-fat diet exposure frequency between healthy and unhealthy individuals in larger population.

Disclosure of state of COI

FK is an employee of PT. Nutrifood Indonesia. All other authors declared no competing interest.

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