

This author's PDF version corresponds to the article as it appeared upon acceptance. Fully formatted PDF versions will be made available soon.

## **Obesity risk and preference for high dietary fat intake are determined by FTO rs9939609 gene polymorphism in selected Indonesian adults**

doi: 10.6133/apjcn.201811/PP.0002

Published online: November 2018

**Running title:** Obesity, dietary fat intake, and FTO rs9939609

Mulianah Daya MD, MS<sup>1</sup>, Dwi Ari Pujianto SSi, M.Biomed, PhD<sup>2</sup>, Fiastuti Witjaksono MD, PhD<sup>1</sup>, Lidwina Priliani M.Si<sup>3</sup>, Jimmy Susanto PhD<sup>4</sup>, Widjaja Lukito MD, PhD<sup>5</sup>, Safarina G Malik, DVM, MS, PhD<sup>3</sup>

<sup>1</sup>Department of Nutrition, Faculty of Medicine, Universitas Indonesia/Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia

<sup>2</sup>Department of Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

<sup>3</sup>Eijkman Institute for Molecular Biology, Ministry of Research, Technology and Higher Education, Jakarta, Indonesia

<sup>4</sup>Kalbe Farma Tbk, Jakarta, Indonesia

<sup>5</sup>Human Nutrition Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

### **Authors' email addresses and contributions:**

- Mulianah Daya: conception of the study; protocol development; recruitment of the participants; data collection; assessments of dietary intake, anthropometric and body composition variables, and genotype; data management; data analyses; drafting and finalising the manuscript.
- Dwi Ari Pujianto: conception of the study; protocol development; monitoring data collection; data management; data analyses; drafting and finalising the manuscript. Email address: dwi.ari@ui.ac.id
- Fiastuti Witjaksono: conception of the study; protocol development; monitoring data collection; data management; data analyses; drafting and finalising the manuscript.
- Lidwina Priliani: supervise and provide inputs for genotype data collection; data analysis and interpretation; revise the final manuscript; Email address: wina@eijkman.go.id
- Jimmy Susanto: coordinator for in-kind contribution by Kalbe Farma, Tbk and facilitate the coordination for DNA extraction and blood-sampling collection; data collection; supervise DNA collection.
- Widjaja Lukito: provide inputs during the conception of the study and protocol development; facilitate the coordination for genotyping laboratories; monitoring data collection; data analysis and interpretation; provide inputs during manuscript development; revise the final manuscript.
- Safarina G. Malik: provide inputs for genetic association protocol; supervise and provide inputs for genotype data collection; data analysis and interpretation; provide inputs during manuscript development; revise the final manuscript; Email address: ina@eijkman.go.id (institutional).

**Corresponding Author:** Dr Safarina G Malik, Eijkman Institute for Molecular Biology, Ministry of Research, Technology and Higher Education, Jalan Diponegoro no. 69, Jakarta 10430, Indonesia. Tel: +62 21-3148695/3917131. Email: ina@eijkman.go.id

## ABSTRACT

**Background and Objectives:** Data suggest that genetic factors are associated with BMI. The fat mass and obesity-associated (FTO) gene modulates adipogenesis through alternative splicing and m6A demethylation. Individuals with FTO rs9939609 gene polymorphism have a preference for energy-dense foods. This study investigates the relationship between FTO rs9939609 and obesity and preference for dietary fat intake among selected Indonesian adults.

**Methods and Study Design:** A total of 40 non-obese and 40 obese participants aged 19–59 living in Jakarta were recruited. Body composition measurements included body weight, height, BMI, waist circumference, and body fat mass. Dietary intake was assessed using a semiquantitative food frequency questionnaire and food recall over 2 × 24-h periods. Genetic variation was determined using amplification-refractory mutation system polymerase chain reaction.

**Results:** The genotype distribution of the FTO gene (rs9939609) was at Hardy–Weinberg equilibrium ( $p=1$ ) with minor allele frequency =0.19. Individuals with AT/AA genotypes had 3.72 times higher risk of obesity ( $p=0.009$ ) and 5.98 times higher dietary fat intake ( $p=0.02$ ) than those with TT genotype. Obese participants with the AT/AA genotypes had 1.40 times higher dietary fat intake than those with the TT genotype ( $p=0.016$ ).

**Conclusions:** These findings suggest that Indonesian adults with AT/AA genotypes of the FTO rs9939609 have higher obesity risks and preferences for high dietary fat intake than those with TT genotype.

**Key Words:** obesity, dietary fat intake, FTO rs9939609, polymorphism, Indonesian

## INTRODUCTION

Obesity is a worldwide epidemic. Its prevalence has doubled since 1980 in more than 70 countries and has continuously increased in most other countries. In 2015, obesity affected 603.7 million adults worldwide.<sup>1</sup> Indonesian Baseline Health Research demonstrated that the prevalence of obesity in Indonesian adults aged  $\geq 18$  years in 2013 was 15.4% higher than that in 2007 (10.3%).<sup>2</sup> The International Obesity Task Force (IOTF) defined obesity for Asians as those who have a body mass index (BMI) of  $\geq 25$  kg/m<sup>2</sup>.<sup>3</sup>

The fundamental cause of obesity is environment or behaviour, including excessive energy-dense food intake and physical inactivity. Indonesian Baseline Health Research data show that the average distribution of dietary fat intake for Indonesian adults is 25.6% of the total daily energy intake, and the prevalence of physical inactivity (exercise for <150 min per week) is approximately 48.2%. Provincial data reveal that Daerah Khusus Ibukota (DKI) Jakarta has

the highest dietary fat intake (30% of total daily energy intake) combined with the highest prevalence of physical inactivity (54.7% in 2007 and 44.2% in 2013).<sup>2,4,5</sup> Nevertheless, genetic factors play significant roles in the etiopathogenesis of obesity. People with the same dietary patterns can trend differently towards a spectrum of body compositions. Genetic factors account for 40% of BMI status.<sup>6</sup>

Gene polymorphisms associated with obesity may be categorised into three major groups: those that affect the central nervous system,<sup>7-9</sup> those that affect adipogenesis,<sup>7,10</sup> and those that affect the regulation of energy balance.<sup>11,12</sup> The fat mass and obesity-associated (FTO) gene, located on chromosome 16q12.2 in intron 1,<sup>13</sup> associates with obesity by modulating adipogenesis with alternative splicing through m<sup>6</sup>A demethylation,<sup>10,13-16</sup> which further triggers mitotic clonal expansion during early adipogenesis.<sup>10,17</sup> Overexpression of the FTO gene is also related to adipogenesis through triggering the expression of nearby genes that play a role in energy balance and the formation of white adipocytes.<sup>13</sup> Individuals with FTO rs9939609 gene polymorphism (T/A substitution) have preference for energy-dense foods, including high-fat foods, leading to excess body weight<sup>15</sup> due to alteration of FTO gene expression in the hypothalamus.<sup>18</sup>

The literature that describes the relations between genetic factors and obesity is extensive. Meta-analyses conducted by Peng et al<sup>19</sup> concluded that 21 of the 29 studies demonstrated correlations between gene FTO rs9939609 polymorphism and obesity in Hispanic, Caucasian, and Asian ethnicities. The current study was designed for two reasons: firstly, given the diversity of Asian populations, we generated a hypothesis whether relations between FTO rs9939609 gene polymorphism and obesity exist in selected Indonesian adults; secondly, although several studies have described a correlation between FTO rs9939609 gene polymorphism and preference for energy-dense foods, further exploration is needed of whether FTO rs9939609 relates to high dietary fat intakes given that this particular macronutrient contributes to variations in the development of obesity in Indonesian adults.

## **MATERIALS AND METHODS**

### ***Participants and sample size***

The sample size was calculated based on the difference in the proportion of variation of the gene FTO rs9939609 allele A between non-obese and obese participants, which Wey estimated to be approximately 6% for populations from Malaysia.<sup>20</sup> Using a power of 80% and a level of significance of 5%, we required 38 non-obese and 38 obese participants.

Therefore, in the current study, we rounded the sample size to 40 non-obese and 40 obese participants.

### ***Participant recruitment***

Recruitment was undertaken through the instant messaging application WhatsApp by the Principal Investigator (MD). Information about the inclusion criteria was provided. The following inclusion criteria were adopted: men or women, aged 19–59 years, living around DKI Jakarta, BMI matching the IOTF definitions for Asian obesity ( $\geq 25$  kg/m<sup>2</sup>) and for non-obesity ( $< 23$  kg/m<sup>2</sup>), and willing to provide informed consent. Those who responded and agreed with the WhatsApp-based entry criteria were invited to visit Seruni Clinic at the Department of Nutrition, Faculty of Medicine, Universitas Indonesia, Jakarta, for verification. Participants with a history of gastrointestinal resection, hormonal therapy, or weight loss programmes using fat absorption inhibitors and appetite inhibitors as well as pregnant and lactating women were excluded from the study.

Of the 114 participants who visited the clinic, 34 did not meet the inclusion criteria (24 participants had BMIs of 23–24.9 kg/m<sup>2</sup>, 2 participants were breastfeeding, 2 participants were undergoing hormonal therapy, and 6 participants were on weight loss programmes using fat absorption inhibitors. The process of recruitment of the participants is illustrated in Figure 1.

The study protocol was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No.1137/UN2/F1/ETIK/2017, protocol number 17-12-1212). Prior to study commencement, the experimental procedure was explained to each participant, and individual informed consent was obtained.

### ***General questionnaires***

Sociodemographic information such as age, gender, and residence was obtained from each participant's Resident Identity Card (Kartu Tanda Penduduk). A structured questionnaire was prepared to obtain information on family history of obesity, weight loss programmes with medicine, hormonal therapy, pregnancy, or breastfeeding.

### ***Dietary intake assessment***

Dietary intake was assessed by the MD using a semiquantitative food frequency questionnaire and food recall over 2 × 24-h periods. Food models and pictures were used for identification of portion size. Each food item had a corresponding serving size, and each serving size had a

corresponding weight in grams. The amount of food consumed was quantified by multiplying the frequency of consumption by the number of serving portions consumed and their corresponding weight in grams. The categorisation of food consumed was based on the Food-Based Dietary Guidelines of Southeast Asian Countries,<sup>21</sup> such as cereals and products and tubers; vegetables; fruits; milk and milk products; fish, poultry, meat, eggs, and legumes; and fat and oil. The latter category was defined as additional fat and oil used for spread, cooking, frying, and flavouring.<sup>22</sup> The vegetables and fruits categories were combined into one category due to the small sample size. Food-to-nutrient conversion was conducted using NutriSurvey 2007. NutriSurvey is the English translation of the professional German nutrition software (EBISPro) and has been supplemented with the Indonesian Food Database 2007.

To demonstrate the level of dietary fat intake, we defined a cut-off value for the percentage contribution of total dietary fat to energy intake according to Labayen et al.<sup>23</sup> Participants with a dietary fat intake  $\geq 30\%$  total energy intake were classified into the high dietary fat intake category. Those with a dietary fat intake  $< 30\%$  total energy intake were classified into the low dietary fat intake category. This cut-off value is in line with the data of the Indonesian Baseline Health Study in 2010, where the average intake of total dietary fat for residents of Jakarta was 30% of total energy intake.

To demonstrate the pattern of food choices contributing to total dietary fat intake, a reverse calculation from dietary fat to food categories was performed using Microsoft Excel 2010 (14.0.4756.1000) MSO (14.0.4756.1000), part of Microsoft Office Standard 2010 (Microsoft Corporation, CA, USA).

### ***Anthropometric and body composition assessment***

Body weight was measured using bioelectrical impedance analysis (BIA) with a Tanita SC-330 (Tanita, Japan). Participants were advised to fast for at least 4 h prior to the measurement using BIA. The soles were cleaned with water-based wipes before standing on the scale. Participants were asked to stand upright with their heads slightly tilted, their eyes looking at a defined spot, and their legs apart. Body weight was measured to the nearest 0.1 kg with very light clothes on. No adjustment was made for the light clothes.

Height was measured in centimetres to the nearest 0.1 cm in standing position with socks on and shoes removed using a microtoise (GEA, Germany).

Waist circumference was measured in centimetres to the nearest 0.1 cm using a flexible nonelastic tape (Roche, Switzerland) at the midway region between the lowest rib margin and

the iliac crest in standing position with abdomen relaxed, feet together, and body weight equally divided over both legs.

Fat mass (kg) and fat mass percentage (%) variables were obtained from the readings provided by BIA with a Tanita SC-330. All anthropometric and body compositional variables were taken twice, and the average values were adopted for data analyses.

### ***DNA extraction and genotyping***

Blood specimens were collected in the morning after 4-h fasting as required for BIA. A total of 5-mL venous blood was withdrawn from each participant into EDTA tubes and immediately placed in a dark cool box during transport to the laboratory. DNA was extracted immediately from buffy coat using Qiagen (QIAamp DNA mini kit) at Kalgen Innolab Clinical Laboratory (approved by the Ministry of Health of the Republic of Indonesia, in line with the Minister of Health Regulation Number 411/MENKES/PER/111/2010). Subsequently, DNA purity was measured using a NanoDrop ND-1000 (Thermo Scientific, USA). FTO rs9939609 gene examination was performed using the amplification-refractory mutation system (ARMS) polymerase chain reaction (PCR) method, as reported by Ye et al,<sup>24</sup> at the Eijkman Institute for Molecular Biology, Ministry of Research, Technology and Higher Education, Jakarta, Indonesia. ARMS PCR was performed using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The primer sets used in this study were as previously described by Fawwad et al.<sup>25</sup> In total, 15  $\mu$ L of PCR reaction contained 10 $\times$  PCR buffer, 0.6  $\mu$ M of each primer, 200  $\mu$ M of each dNTPs, 0.05 U of Taq DNA polymerase (New England BioLabs, Ipswich, MA, USA), and 50 ng of genomic DNA as template. The PCR protocol was as follows: predenaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 61°C for 35 s, and extension at 68°C for 30 s, followed by final extension at 68°C for 5 min. The amplified PCR products were visualised with 2% agarose gel electrophoresis (Lonza, Basel, Switzerland) using Gel Doc XR System (BioRad, USA) (Figure 2).

### ***Statistical analyses***

R Studio version 1.1.491 (genetics, Hardy–Weinberg packages) was used to observe the genotype distribution of the FTO rs9939609 and calculate the minor allele frequency (MAF). Overall data were analysed using SPSS version 20. Data normality was tested using Shapiro–Wilk. Continuous variables with normal distribution were presented as means  $\pm$  SD, and those with abnormal distributions were presented as median (minimum–maximum). Differences in

values between non-obese and obese participants were calculated using unpaired t test for normally distributed data and Mann–Whitney U test for abnormally distributed data.

For categorical variables, chi-squared analyses were conducted to calculate the differences. Logistic regression analyses were chosen to measure how much variations of the independent variables affected the dependent variable.

Two obese participants were excluded during the final analyses due to extreme under-reporting of dietary intake data.

## RESULTS

The basic characteristics of the participants are shown in Table 1. Both non-obese and obese groups were comparable in age, dietary intake, and macronutrients intakes such as carbohydrates, total fats, and protein intakes. Gender differences were observed in both groups, and these differences were more pronounced in the non-obese groups than the obese groups. Number of participants with family history of obesity was higher in the obese group than in the non-obese group. The obese group had higher values of body weight, height, BMI, waist circumference, and fat mass than the non-obese group.

The frequency of the genotype FTO rs9939609 of the non-obese and obese groups is shown in Table 2. The MAF of the FTO gene rs9939609 was 0.19, and the overall genotype distribution was at Hardy–Weinberg equilibrium ( $p=1$ ). The genotype and heterozygosity distribution were moderately equal between the non-obese and obese groups. Both the non-obese and obese groups were in Hardy–Weinberg equilibrium ( $p=0.55$  and  $p=0.65$  for the non-obese and obese groups, respectively).

The association between the FTO rs9939609 gene polymorphism and dietary fat intake in the non-obese and obese groups is presented in Table 3. In the obese group, individuals with the AT/AA genotypes had 1.40 times higher dietary fat intake those with the TT genotype did.

Results of logistic regression analyses demonstrating the determinants of obesity and dietary fat intake are available in Tables 4 and 5, respectively. Participants with the AT/AA genotypes had 3.72 times higher risk of obesity and 5.98 times higher dietary fat intake than those with the TT genotype did. Women and a history of obesity in the family were also associated with increased risk of obesity.

The food choices contributing to total dietary fat intake are described in Table 6. In both the non-obese and obese groups, the TT and AT/AA genotypes had comparable intakes of cereals and products and tubers; vegetables and fruits; milk and milk products; and fish, poultry, meat, eggs, and legumes. In the non-obese group, participants with the AT/AA

genotypes had higher percentages of total energy intake from fat and oil than those with the TT genotype did. A trend towards higher fat and oil intake was observed in both the non-obese and obese groups carrying the AT/AA genotypes, although this was not statistically significant.

## DISCUSSION

The link between gene and obesity development and its underlying factors such as high consumption of energy-dense foods has been widely studied. Despite differences in obesogenic foods in terms of the sociocultural factors for the development of obesity among Indonesians, nutrigenetics-based studies on obese Indonesians are limited. However, diversity of dietary patterns may offer improved health outcomes despite emerging evidence of nutrigenomic approaches.<sup>26</sup>

Among other gene polymorphisms, FTO rs9939609 is strongly associated with obesity by genome-wide association studies.<sup>27</sup> FTO rs9939609 is related to obesity by synergistically acting at both the central and peripheral levels. At the central level (the hypothalamus), expression of FTO rs9939609 leads to high consumption of energy-dense foods; at the peripheral level, FTO rs9939609 modulates adipogenesis. The current clinical study elaborates these links in selected Indonesian adults living in DKI Jakarta, which was selected given that the prevalence of central obesity is 39.7% with an average dietary fat intake of 30%, which is the highest in Indonesia.<sup>2,5</sup>

This study did not select the participants by gender. Therefore, women outnumbered men in prevalence of obesity. This finding is in line with that in the Indonesian Baseline Health Research in 2010 and 2013; the prevalence of obesity in women was higher (15.5% in 2010 and 32.7% in 2013) than that in men (7.8% in 2010 and 19.7% in 2013).<sup>2,5</sup> This study also found that the risk of developing obesity was higher among those with a family history of obesity, which was also reported by a study in Brazil.<sup>28</sup>

FTO rs9939609 increases predisposition to obesity by almost 22% in various reported populations, especially in Europe and North America. In this study, the MAF of 19% was similar to the study conducted by Susmiati et al<sup>29</sup> in Minangkabau, Indonesia (22%), and Wey et al<sup>20</sup> in Malaysia (19.9%) across various ethnicities (Malay, Chinese, and Indian).

Our findings have strengthened those of Labayen et al<sup>23</sup> regarding the effect of FTO rs9939609 on adiposity indices increase in accordance with an increased proportion of total dietary fat intake accounting for  $\geq 30\%$  of total energy. In another Indonesian study on community-based adolescent girls of Minangkabau ethnicity, Susmiati et al<sup>29</sup> demonstrated

that such girls with AT/AA genotypes consumed more fried foods and had lower intake of fruits than those with the TT genotype did.

Our results are explainable through eating behaviour pathways. Cecil et al<sup>16</sup> reported that FTO rs9939609 gene polymorphism played crucial roles in appetite regulation, characterised by hyperphagic phenotype and a tendency to choose energy-dense food. Energy-dense foods are defined by their levels of dietary fat. A further study performed by Tanofsky et al<sup>15</sup> demonstrated that the FTO rs9939609 minor allele is characterised by high-energy food choices, leading to an increase in body weight. Individuals with one or two A alleles experience loss of control over eating behaviour, and this occurred in both children and adults.

In this study, we also observed food sources contributing to total dietary fat intake. We focused on food sources of dietary fat given the strong link between FTO rs9939609 polymorphism and high dietary fat intake as observed in the current study. The relatively high contribution of additional fat and oil to percentage total energy intake apparent in this study is due to the regular use of fat and oil for cooking and frying in Indonesian food.<sup>22</sup> Using different approaches for the categorisation of foods, this study partly supports the findings of Susmiati et al regarding AT/AA genotypes.

Brunkwall et al<sup>14</sup> reported that FTO rs9939609 is associated with higher intake of certain foods, in particular with energy-dense foods such as biscuits and pastries. In their study, biscuits were categorised into sweets and cakes, which may indicate sugar and fat-rich foods.<sup>30</sup> However, our sample size did not allow us to further analyse food sources contributing to total carbohydrate intake, which, to some extent, may be related to energy-dense foods in Indonesian food culture.<sup>5</sup>

However, not all studies support the link between FTO rs9939609 polymorphism and a high dietary fat diet. In one meta-analysis including 177,330 adult participants, Qi et al<sup>31</sup> reported that participants with the A allele had a low-carbohydrate and high-protein diet but not a high-total fat diet. Therefore, the relationships between FTO rs9939609 gene polymorphism and high dietary fat intake remain controversial, and further comprehensive and analytical studies are necessary.

Together with the literature, this study has built on a conceptual framework as described in Figure 3. In line with previous other studies, it confirmed the influence of FTO rs9939609 gene polymorphism in modulating preferences for higher fat intake and overall obesity risk.

## ***Conclusion***

Results of this study indicate that FTO rs9939609 gene polymorphism is linked to obesity risk and preferences for energy-dense foods, such as those high in dietary fat, in selected Indonesian adults. Furthermore, FTO rs9939609 gene polymorphism partly supports high consumption of additional fat and oil, which often constitute large parts of the energy-dense foods in Indonesian food culture.

## **ACKNOWLEDGEMENTS**

We are indebted to Ms. Sukma Oktavianthi, SSi, Mbiomed, Ms. Rut Christine Inggriani, SSi and Ms. Hazrina Tiyas Nussa, SSi of the Eijkman Institute for Molecular Biology for their assistance in ARMS PCR procedure, and R analyses application. We are very grateful to Mr. Farid Sastra Negara, SSi and the technical staff of the Kalgen Innolab for their support during blood collection and DNA extraction. We wish to thank Prof. Saptawati Bardosono for her continuous advice of the study. We highly appreciate the personal consultation provided by Sutantik Endang Wasih Kasunjatan, MD and Melvin Lukito, MD in data management and statistical analyses. We are grateful to Ms. Lindawati Wibowo, SSi, MSc. for her assistance in food categorisation following the Food-based Dietary Guidelines of Southeast Asian Countries. This study is part of a Master Degree thesis of MD. Therefore, we recognize the value of the in-kind contributions made by the Eijkman Institute for Molecular Biology and Kalbe Farma Tbk. Finally, we truthfully thank and appreciate the participants for taking part in this study. Without their participation, no data could be presented, and, further, no scientific evidence could be generated.

## **AUTHOR DISCLOSURE**

JS is currently employed by Kalbe Farma Tbk. Other authors have no conflict of interest in regards to this paper.

## **REFERENCES**

1. GBD 2015 Obesity Collaborators. Health effects of overweight and obesity in 195 countries over 25 years. Gates Foundation Author Manuscript. *New Engl J Med.* 2017;377:13-27. doi 10.1056/NEJMoa1614362.
2. National Health Research and Development Agency. Baseline Health Research 2013 (Indonesian language). National Health Research and Development Agency, Ministry of Health, Republic of Indonesia; 2013. p223-7.
3. WHO/IASO/IOTF. The Asia-Pacific perspective: redefining obesity and its treatment. Melbourne: Health Communication Australia; 2000. p18-20.

4. National Health Research and Development Agency. Baseline Health Research 2007 (Indonesian language). National Health Research and Development Agency, Ministry of Health, Republic of Indonesia; 2007. p106-11.
5. National Health Research and Development Agency. Baseline Health Research 2010 (Indonesian language). National Health Research and Development Agency, Ministry of Health, Republic of Indonesia; 2010. p192-3.
6. Wardle J, Carnell S, Haworth CMA, Farooqi IS, O'Rahilly S, Plomin R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. *Am J Clin Nutr.* 2008;87:398-404. doi: 10.1210/jc.2008-0472.
7. Herrera BM, Lindgren CM. The genetic of obesity. *Curr Diab Rep.* 2010;10:498-505. doi 10.1007/s11892-010-0153-z.
8. Ronkainen J, Huusko TJ, Soininen R, Mondini E, Cinti F, Makela KA et al. Fat mass- and obesity-associated gene FTO affects the dietary response in mouse white adipose tissue. *Sci Rep.* 2015;5:9233. doi: 10.1038/srep09233.
9. Karra E, Zelaya FO, Batterham R. A link between FTO, ghrelin, and impaired brain food-cue responsiveness. *J Clin Invest.* 2013;123:3539-51. doi: 10.1172/JCI44403.
10. Merkestein M, Sellayah D. Role of FTO in adipocyte development and function: Recent insights. *Int J Endocrinol.* 2015;6:1-4. doi.10.1038/ncomms7792.
11. Gropper SS, Smith JL. Advanced nutrition and human metabolism. 6th edition. Wadsworth: Cengage Learning; 2013. p295-96.
12. Dalgaard LT. Genetic variance in Uncoupling Protein 2 in relation to obesity, type 2 diabetes, and related metabolic traits: Focus on the functional -866G>A promotor variant (rs659366). *J Obes.* 2011;2011:1-12. doi:10.1155/2011/340241.
13. Yang Q, Xiao T, Guo J, Su Z. Complex relationship between obesity and the fat mass and obesity locus. *Int J Biol Sci.* 2017;13:615-29. doi: 10.7150/ijbs.17051.
14. Brunkwall L, Erickson U, Hellstrand S, Gullberg B, Orho-Melander M, Sonestedt E. Genetic variant in the fat mass and obesity-associated gene (FTO) in association with food preferences in healthy adults. *Food Nutr Res.* 2013;57:1-22. doi: 10.3402/fnr.v57i0.20028.
15. Tanofsky-kraff M, Han JC, Anandalingam K, Shomaker LB, Columbo KM, Wolkoff LE et al. The FTO gene rs9939609 obesity-risk allele and loss of control over eating. *Am J Clin Nutr.* 2009;90:1483-88. doi: 10.3945/ajcn/2009.28439.
16. Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CNA. An obesity associated FTO gene variant and increased energy intake in children. *N Engl J Med.* 2008;24:2558-66. doi: 10.1056/NEJMoa0803839.
17. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. *Physiol Rev.* 1998;78:783-809. doi: 10.1152/physrev.1998.78.3.783.

18. McTaggart JS, Lee S, Iberl M, Church C, Cox RD, Ashcroft FM. FTO is expressed in neurones throughout the brain and its expression is unaltered by fasting. *PLoS One*. 2011;6:e27968. doi: 10.1371/journal.pone.0027968.
19. Peng S, Zhu Y, Xu F, Ren X, Li X, Lai M. FTO gene polymorphisms and obesity risk: a meta-analysis. *BMC Med*. 2011;9:71. doi: 10.1186/1741-7015-9-71.
20. Chey W, Fan S, Say Y. Association of fat mass and obesity-associated (FTO) gene rs9939609 variant with obesity among multi-ethnic Malaysians in Kampar, Perak. *Sains Malaysiana*. 2013;42:365-71.
21. Florentino RF, Tee ES, Hardinsyah R, Ismail MN, Suthutvoravut U, and Hop LT. Food-Based Dietary Guidelines of Southeast Asian Countries: Part 2 – Analysis of Pictorial Food Guides. *Mal J Nutr*. 2016;22:S49-S65.
22. Hanafiah A, Karyadi D, Lukito W, Muhilal, Supari F. Desirable intakes of polyunsaturated fatty acids in Indonesian adults. *Asia Pac J Clin Nutr*. 2007;16:632-40.
23. Labayen I, Ruiz JR, Huybrechts I, Ortega FB, Arenaza L, Gonzales-Gross M et al. Dietary fat intake modifies the influence of the FTO rs9939609 polymorphism on adiposity in adolescents: The HELENA cross-sectional study. *Nutr Metab Cardiovasc Dis*. 2016;26:937-43. doi: 10.1016/j.numecd.2016.07.010.
24. Ye S, Dhillon S, Ke X, Collins AR, Day INM. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acid Res*. 2001;29:1-8.
25. Fawwad A, Siddiqui IA, Zeeshan NF, Shahid SM, Basit A. Association of SNP rs9939609 in FTO gene with metabolic syndrome in type 2 diabetic subjects recruited from a tertiary care unit of Karachi, Pakistan. *Pak J Med Sci*. 2015;31:140-5. doi:10.12669/pjms.311.6524.
26. Wahlqvist ML. The rise of clinical nutrition science in North-East Asia. *Asia Pac J Clin Nutr*. 2016;25:437-43. doi: 10.6133/apjcn.072016.02.
27. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM et al. Index and Predisposes to Childhood and Adult Obesity. *Science*. 2007;316:889-94. doi:10.1126/science.1141634.
28. Reuter CP, Burgos MS, Bernhard JC, Tornquist D, Klinger EI, Borges TS, Renner JDP, Valim ARM, Mello ED. Association between overweight and obesity in schoolchildren with rs9939609 polymorphism (FTO) and family history for obesity. *J Pediatr (Rio J)*. 2016;92:493-8. doi: 10.1016/j.jped.2015.11.005.
29. Susmiati, Lipoeto NI, Surono IS, Jamsari J. Association of fat mass and obesity-associated rs9939609 polymorphisms and eating behaviour and food preferences in adolescent Minangkabau girls. *Pak J Nutr*. 2018;17:471-9. doi:10.3923/pjn.2018.471.479.
30. Hlebowicz J, Persson M, Gullberg B, Sonestedt E, Wallstrom P, Drake I, Nilsson J, Hedblad B, Wirfalt E. Food pattern, inflammation markers and incidence of cardiovascular diseases: the Malmo Diet and Cancer study. *J Intern Med*. 2011;270:365-76. doi: 10.1111/j.1365-2796.2011.02382.x
31. Qi Q, Kilpelainen TO, Downer MK, Tanaka T, Smith CE, Sluijs I et al. FTO genetic variants, dietary intake and body mass index: insights from 177,330 individuals. *Hum Mol Genet*. 2014;23:6961-72. doi: 10.1093/hmg/ddu411.

**Table 1.** Basic characteristics of non-obese and obese groups<sup>†</sup>

Variable	Non-obese group (n=40)	Obese group (n=38)	<i>p</i>
Age (years)	31 (21–53)	33 (19–52)	0.630
Gender (n (%))			0.008
Men	6 (15.0)	16 (42.1)	
Woman	34 (85.0)	22 (57.9)	
With family history of obesity	21 (52.5)	31 (76.3)	0.028
Anthropometric and body composition			
Body weight (kg)	51.22±6.78	87.63±16.5	<0.001
Height (cm)	159.05±7.69	163.26±8.68	0.039
BMI (kg/m <sup>2</sup> )	20.42 (15.65–22.8)	31.95 (25.86–50.6)	<0.001
Waist circumference (cm)			
Men	74.80 (62.1–80.95)	104.10 (94.2–125.75)	<0.001
Woman	73.50 (60.6–86.0)	95.93 (80.05–133.45)	<0.001
Fat mass			
kg	12.55 (5.10–18.0)	33.25 (18.1–77.2)	<0.001
%	25.10 (10.3–33.3)	38.70 (24.1–53.3)	<0.001
Dietary intake			
Total energy/day (Kcal/day)	1599.41 (754.15–2695.68)	1603.79 (1007.13–5158.25)	0.760
Carbohydrate intake			
g	167.89 (59.04–357.91)	184.66 (52.45–558.55)	0.300
% energy	40.68±5.43	42.43±6.66	0.120
Fat intake			
g	61.00 (23.68–124.55)	66.18 (34.40–247.85)	0.490
% energy	33.53±6.59	34.98±6.19	0.330
Protein intake			
g	51.22 (21.43–171.11)	47.41 (16.56–157.74)	0.500
% energy	14.63 (10.5–25.5)	14.38 (9.25–26.0)	0.290

<sup>†</sup>Data are presented as mean ± SD for normally distributed data and median (minimum–maximum) for abnormally distributed data.

**Table 2.** Genotype and allele frequency of FTO rs9939609 in non-obese and obese groups<sup>†</sup>

Genotype	Total, n (%) n=80	<i>p</i> -HWE	Non-obese group, n (%) n=40	<i>p</i> -HWE	Obese group, n (%) n=38	<i>p</i> -HWE
TT	51 (65)	1.00	30 (75)	0.55	21 (55)	0.650
AT	27 (32)		9 (22)		16 (42)	
AA	2 (3)		1 (2)		1 (3)	
Allele T	81%		86%		76%	
Allele A	19%		14%		24%	

*p*-HWE: *p* value of Hardy–Weinberg equilibrium; MAF: minor allele frequency.

<sup>†</sup>Statistical test: RStudio 1.1.491 genetics packages, Hardy–Weinberg.

**Table 3.** Association between FTO rs9939609 and dietary fat intake in non-obese and obese groups<sup>†</sup>

Genotype	Non-obese group (n=40)				Obese group (n=38)			
	Low fat intake n (%)	High fat intake n (%)	OR	95% CI	Low fat intake n (%)	High fat intake n (%)	OR	95% CI
TT	9 (81.80)	21 (72.40)	0.58	0.103–3.31	6 (100)	15 (46.9)	1.40	1.07–1.84
AT+AA	2 (18.20)	8 (27.60)			0 (0)	17 (53.1)		

<sup>†</sup> Statistical test: Chi-squared test

**Table 4.** Determinants of obesity risks<sup>†</sup>

Risk factor	<i>p</i>	OR	95% CI
AT/AA	0.024	3.72	1.19–11.64
Gender (women)	0.006	6.03	1.68–21.61
Family history of obesity	0.038	3.54	0.09–0.94
Total energy intake (kcal/day)	0.370	1.00	1.00–1.001
Carbohydrate intake (% energy)	0.450	1.05	0.93–1.17
Protein intake (% energy)	0.920	1.01	0.82–1.25

<sup>†</sup>Logistic regression analyses of FTO gene polymorphism with obesity and calculated OR and 95% CI, adjusted for gender, family history of obesity, total energy intake, carbohydrate intake, and protein intake.

**Table 5.** Determinants of daily total dietary fat intake<sup>†</sup>

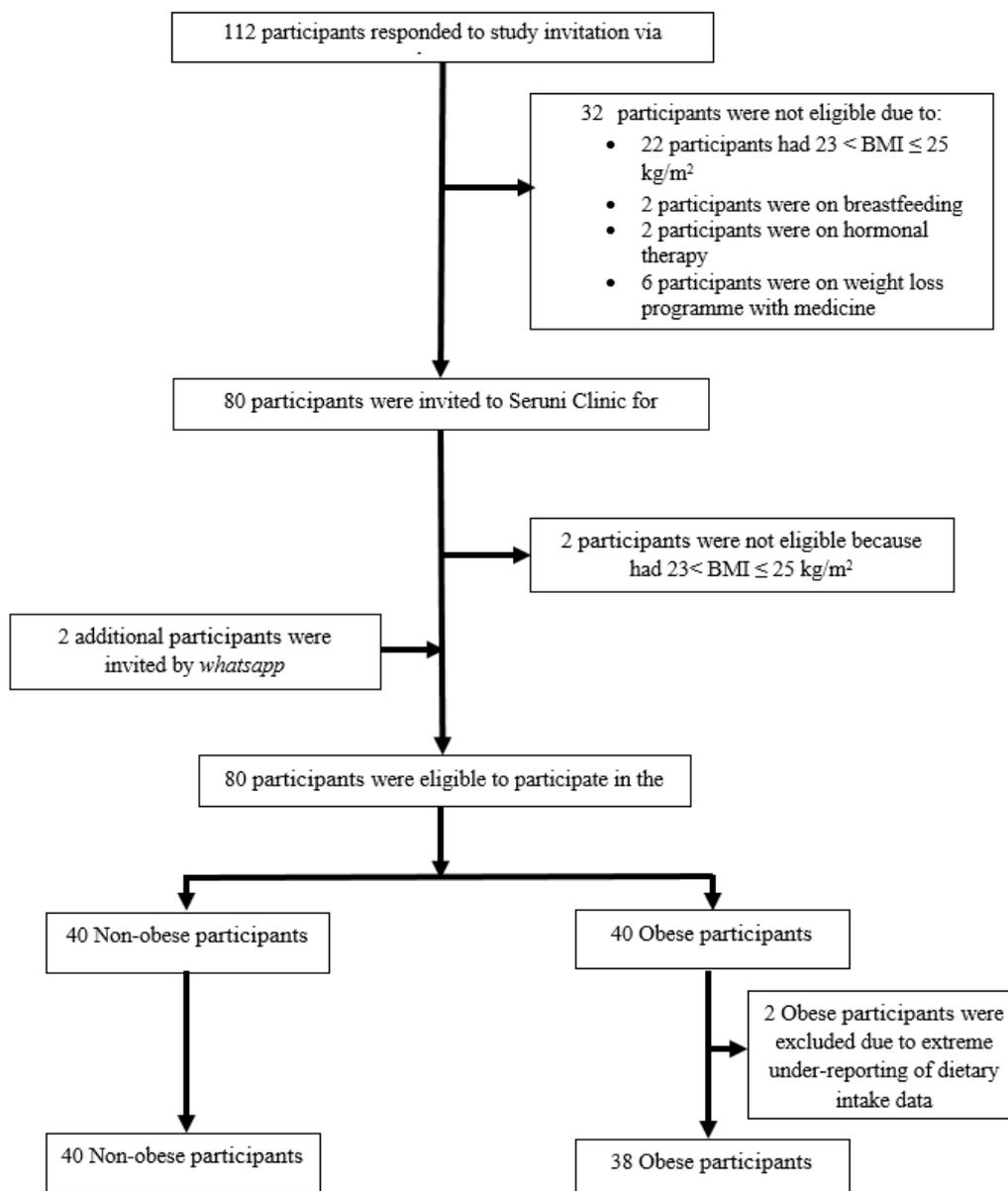
Risk factor	<i>p</i>	OR	95% CI
AT/AA	0.027	5.98	1.22–29.22
Gender (women)	0.150	2.80	0.69–11.41
Family history of obesity	0.710	0.80	0.25–2.57

<sup>†</sup>Logistic regression analyses of FTO gene polymorphism with obesity and calculated OR and 95% CI, adjusted for gender, family history of obesity, total energy intake, carbohydrate intake, and protein intake.

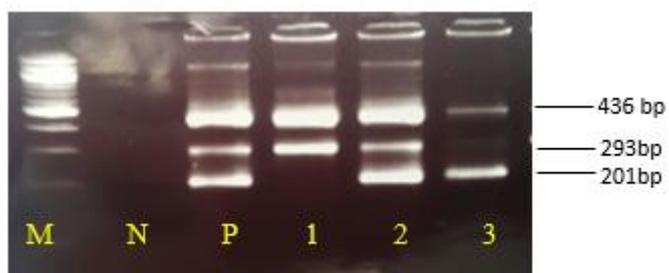
**Table 6.** Food sources contributing to total dietary fat intake of non-obese and obese groups by FTO rs9939609 categories †

Food category	Non-obese group (n=40)			Obese group (n=38)		
	TT	AT/AA	<i>p</i>	TT	AT/AA	<i>p</i>
Cereals and products, tubers						
g	2.25 (0.10–20.4)	1.95 (0.60–4.10)	0.396	2.30 (0.30–12.0)	1.20 (0.20–36.4)	0.352
% total energy intake	1.05 (0.11–9.65)	1.29 (0.30–2.64)	0.612	0.87 (0.14–6.44)	0.64 (0.16–5.95)	0.542
Vegetables and fruits						
g	4.24 (0.40–68.4)	1.65 (0.30–13.3)	0.286	2.20 (0.40–41.2)	1.90 (0.30–17.8)	0.728
% total energy intake	1.84 (0.26–24.0)	1.12 (0.25–5.84)	0.331	1.57 (0.21–20.66)	0.99 (0.18–9.79)	0.750
Milk and milk products						
g	4.30 (0.10–82.1)	3.05 (0.60–6.50)	0.272	1.80 (0.60–19.1)	1.80 (0.40–6.20)	0.601
% total energy intake	2.24 (0.04–22.9)	1.36 (0.54–3.72)	0.286	1.16 (0.27–14.67)	1.02 (0.18–3.72)	0.601
Fish, poultry, meat, eggs, legumes						
g	21.2 (6.30–137.7)	17.3 (9.70–30.2)	0.301	25.2 (3.50–53.7)	20.1 (10.0–67.6)	0.542
% total energy intake	10.66 (5.42–42.37)	10.03 (6.67–15.88)	0.548	8.87 (1.61–30.93)	11.05 (5.36–26.6)	0.416
Fat and oil						
g	29.95 (6.70–73.9)	38.9 (26.9–95.6)	0.067	27.3 (19.5–83.6)	39.0 (20.0–131.2)	0.067
% total energy intake	15.98 (6.17 - 29.29)	22.45 (15.76–42.23)	0.002	20.53 (8.69–29.26)	24.55 (10.37–45.53)	0.399
Total dietary fat						
g	63.86 (23.68 -124.55)	64.65 (34.13–114.58)	0.488	60.4 (34.4–138.9)	66.0 (35.23–247.85)	0.601
% total energy intake	31.77 (21.75–50.75)	36.25 (22.5–45.25)	0.379	33.0 (23.75–49.5)	38.25 (29.0–47.5)	0.036

† Data are presented as median (minimum–maximum). Food classification is based on the Food-Based Dietary Guidelines of Southeast Asian Countries<sup>21</sup>: cereals and products and tubers; vegetables and fruits; milk and milk products; fish, poultry, meat, eggs, and legumes; and fat and oil.



**Figure 1.** Flow diagram of participant recruitment.



**Figure 2.** Result of ARMS PCR [M: marker; N: negative control; P: positive control; 1: homozygote TT (436bp, 293bp); 2: heterozygote AT (436bp, 293bp, 201bp); 3: homozygote AA (436bp, 201bp)].

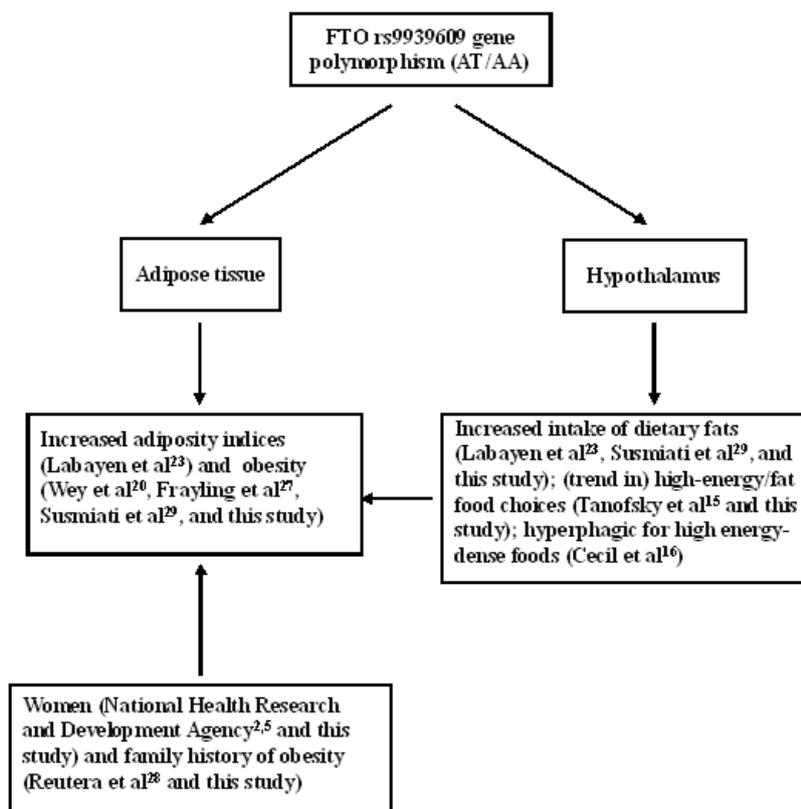


Figure 3. Derived conceptual framework.