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Description of FTO Gene (Fat Mass and Obesity-Associated) Polymorphism rs9939609 in Obesity and Non-Obesity Subjects

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Abstract

The FTO gene (Fat Mass and Obesity-associated) has been identified to influence the incidence of obesity based on Genome-Wide Association Studies. Hence, the present study examined the polymorphism rs9939609 of the FTO gene in a sample of 15 obese subjects and 15 non-obese subjects among employees of Prodia Pusat Jakarta. Descriptive research was conducted to characterize the polymorphism or variation of the FTO gene rs9939609 in obese and non-obese individuals. Among the non-obese subjects, 100% of the AT alleles were observed, indicating a potential genetic risk factor for obesity. Among the obese subjects, 60% had the TT alleles, which genetically should not be a risk allele for obesity. Lifestyle or diet might contribute to this finding. Additionally, the AA allele was found in 6.67% of obese subjects, indicating an increased risk of obesity. In conclusion, the FTO gene polymorphism rs9939609 demonstrated a 100% AT alleles prevalence in obese subjects and 33.33% in non-obese subjects among employees of Prodia Pusat Jakarta. The AA allele was present in 6.67% of obese subjects, while the TT allele was found in 60% of obese subjects.

Keywords: Obesity, FTO rs9939609, Body mass index

Introduction

Obesity is a significant risk factor for numerous chronic diseases and imposes a substantial health and economic burden on society (Flegal KM, 2012; M. Ng, 2013). The etiology of obesity is multifactorial and influenced by a complex interplay of environmental, lifestyle, and genetic factors (Frayling, 2007) (A. van der Klaauw and I. S. Farooqi, 2015). According to the World Health Organization, obesity is defined as the abnormal or excessive accumulation of fat that poses a health risk ((WHO)., 2016).

The FTO gene (Fat Mass and Obesity-Associated) has been identified as a gene that influences the incidence of obesity based on Genome-Wide Association Studies (Frayling, 2007). Population genetic studies have reported an association between FTO gene variations and BMI, body fat composition, metabolic parameters, and metabolic abnormalities related to obesity (Q. Qi, 2014.).

In 2007, a genetic study was conducted on European populations involving 38,759 subjects. It identified a common variant of the FTO gene (rs9939609), located on the first intron, exhibiting a significant relationship with type 2 diabetes mellitus (DM). The association between the FTO gene variant and type 2 DM is influenced by Body Mass Index (BMI). The correlation between environmental and genetic variables can affect an individual's predisposition to obesity. The FTO gene is frequently associated with increased BMI (Maharani C,

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2019). However, once BMI is controlled for, the association between FTO gene variants and type 2 DM disappears. Homozygous individuals carrying the A allele in the FTO gene variant rs9939609 have a higher BMI than those of heterozygous (K. Hotta, 2008.) (Y. Sun, 2010).

The FTO gene (rs9939609) is associated with obesity. Individuals who are homozygous for the risk allele (AA) weigh approximately 3 kg more and have a 1.7-fold higher risk of obesity compared to those who do not carry the risk allele (TT) (Frayling, 2007) (Sudargo T, 2014). Research supports these findings, indicating that individuals with the AA genotype exhibit decreased satiety, make poor food choices, and consume more energy. However, the FTO SNP gene associated with obesity does not appear to affect energy expenditure, as evidence suggests that carrying the risk allele does not lead to a decrease in basal metabolic rate or level of physical activity (T. Berentzen, 2008). Variations in the FTO gene play a significant role in developing early-onset obesity. The A-allele of the rs9939609 variant is associated with a 31% increased risk of obesity, highlighting the fact that this variant is linked to a higher risk of both obesity and type 2 diabetes. In European populations, the A-allele variant of rs9939609 has been reported to have the most substantial effect. However, unlike Asian residents, the A-allele is less predisposed to obesity, resulting in a weaker effect of the rs9939609 variant (J. M. McCarery, 2012) (Nurhasanah, 2022). One study reported that 16% of adults carrying the homozygous risk allele (AA) had a 3 kg higher body weight and a 1.7 times higher risk of obesity than those without the risk allele (TT). Additionally, research conducted by Priyambodo et al. (2012) suggested that the FTO polymorphism rs9939609 was the most significant risk factor for body fat accumulation and obesity compared to other polymorphisms. Furthermore, the rs9939609 polymorphism located in the first intron of the FTO gene has been found to impact the components of metabolic syndrome, with the AA genotype and A allele conferring higher risk factors than the TT genotype and T allele (Seto P, 2013).

Prodia Pusat Jakarta employs approximately 300 individuals, including both obese and non-obese people. Given the background information provided, it is essential to conduct research on the FTO gene polymorphism (rs9939609) to determine the differences between obese and non-obese employees.

Research Method

This study employed a descriptive research design to elucidate the polymorphisms or variations in the FTO gene (Fat Mass and Obesity-Associated) rs9939609 among obese and non-obese subjects who were employees of Prodia Pusat Jakarta.

Research Instruments

The researchers utilized various tools and materials, including 3 cc syringes, K3EDTA vacuum tubes, plasters, alcohol swabs, tourniquets, variable micropipettes, sterile blue and yellow tips with filters, sterile Fisher cups, centrifuges, and the CFX96 Touch Real-Time PCR Detection System (Bio-Rad).

Procedure

DNA isolation

DNA isolation was performed throughout the following steps: 300 µL of whole blood was transferred to a 1.5 mL

microtube. 900 µL of RBC Lysis Buffer was added, and the mixture was gently mixed by inversion (avoiding a vortex usage). The microtube was incubated at room temperature for 10 minutes. After incubation, centrifugation was carried out for 5 minutes at 3,000 x g, and the supernatant was carefully discarded. The pellets were resuspended by adding 100 µL of RBC Lysis Buffer, and the cell lysis stage immediately proceeded. 200 µL of GB Buffer was added to the sample, and the 1.5 mL microtube was vortexed at full speed. The microtube was then incubated at 60°C for 10 minutes to clarify the lysate sample solution. During incubation, the microtube was inverted once every 3 minutes. Meanwhile, the Elution Buffer (120 µL per sample) was preheated to 60°C for Step 4: DNA Elution. After the incubation at 60°C, the microtube was left to stand at room temperature for 5 minutes. Subsequently, 200 µL of absolute ethanol was added to the lysate sample solution, and the microtube was vortexed at full speed for 10 seconds. Suppose any precipitate was present; it was crushed using a pipette. Afterward, a GD Column was prepared and placed in a 2 mL Collection Tube. The mixture in the microtube and any existing precipitate was transferred to the GD Column, filling it up to 1.5 mL. The column was then centrifuged for 5 minutes at a speed of 16,000 x g. The 2 mL Collection Tube was removed, and the GD Column was placed into a new 2 mL Collection Tube. The dry GD Column was transferred to a fresh 1.5 mL microtube. Next, 100 µL of preheated Elution Buffer was added to the center of the column matrix. The microtube was left for at least 3 minutes to ensure the Elution Buffer seeped into the column matrix. The microtube was then centrifuged for 30 seconds at 16,000 x g to elute the purified DNA. The resulting DNA extract was stored at -70°C until further use (Geneaid., 2017) (Kartika, 2018).

qPCR

The PCR reaction was performed using TaqMan GTXpress Master Mix (2X) reagent with a total volume of 25 µL. The reaction mixture consisted of 12.5 µL TaqMan GTXpress Master Mix (2X), 1.25 µL of a 20X working stock SNP Genotyping Assay, 6.25 µL ddH2O, and 5 µL of DNA template (DNA extract) at a concentration of 1-10 ng/well. Amplification was carried out using the CFX 96 Touch™ Real-Time PCR (Bio-Rad) instrument with the following standard protocol: initial enzyme activation at 95°C for 20 seconds, followed by 40 cycles of amplification consisting of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute.

Data analysis

The data were analyzed both qualitatively and quantitatively. The qualitative analysis involved examining the results of the PCR-RFLP on FTO gene rs9939609 on gel electrophoresis, while the quantitative analysis included calculating allele and genotype frequencies.

Research Results and Discussion

Based on the process conducted over a predetermined period, 30 samples were obtained from all respondents. Among them, 15 samples belonged to obese

employees, and the remaining were categorized as non-obese. Table 1 displays that out of the 30 respondents from Prodia Pusat Jakarta employees, 15 individuals (50%) were male, and 15 others (50%) were female. It indicates a balanced representation of male and female patients, with no dominance of either gender.

Table 1. Respondents' Characteristics Based on Gender

Gender	Quantity	Percentage
Male	15	50%
Female	15	50%
Total	30	100%

(Source: Primary Data, 2023)

Table 2. Respondents' Characteristics Based on Body Mass Index (BMI)

Category	Quantity	Min	Max	Average	SD
Non-obese	15	18.9	21.8	20.72	1.004
Obese	15	27.2	30.1	28.18	0.9

(Source: Primary Data, 2023)

Table 3. Characteristics of Non-Obese Respondents Based on FTO Gene Variant rs9939609

No.	Sample Code	Gender	Body Mass Index (BMI)	FTO gene variant rs9939609
1	NOB1	M	21.9	AT
2	NOB2	F	20.2	AT
3	NOB3	F	19.0	AT
4	NOB4	F	19.7	AT
5	NOB5	M	22.0	AT
6	NOB6	M	21.3	AT
7	NOB7	M	21.6	AT
8	NOB8	F	20.4	AT
9	NOB9	M	20.9	AT
10	NOB10	M	21.8	AT
11	NOB11	F	22.0	AT
12	NOB12	M	21.5	AT
13	NOB13	F	19.7	AT
14	NOB14	F	18.9	AT
15	NOB15	M	21.0	AT

(Source: Primary Data, 2023)

Table 4. Characteristics of Obese Respondents Based on FTO Gene Variant rs9939609

No.	Sample Code	Gender	Body Mass Index (BMI)	FTO gene variant rs9939609
1	OBS1	F	27.2	TT
2	OBS2	M	29.0	AT
3	OBS3	M	30.1	TT
4	OBS4	M	28.5	TT
5	OBS5	F	28.0	TT
6	OBS6	F	27.5	AT
7	OBS7	M	28.3	AA
8	OBS8	F	27.6	TT
9	OBS9	M	28.2	AT
10	OBS10	F	27.4	TT
11	OBS11	F	28.6	TT
12	OBS12	M	29.5	TT
13	OBS13	M	28.7	AT
14	OBS14	F	27.4	AT
15	OBS15	F	27.3	TT

(Source: Primary Data, 2023)

Table 2 shows that for non-obese employees, the lowest BMI is 18.9, while the highest is 22.0, with an average value of 20.72 and a standard deviation of 1.004. In the obese category, the lowest BMI is 27.2, whereas the highest is 30.1, with an average value of 28.18 and a standard deviation of 0.9.

Tables 3 and 4 reveal that the percentage of AT alleles among non-obese employees is 100%. Among obese employees, the percentage of TT alleles is 60%, the percentage of AT alleles is 33.33%, and the percentage of AA alleles is 6.67%. In this context, obesity is a condition characterized by an excessive accumulation of body fat, resulting in a weight significantly above the normal range. Furthermore, genetic factors are perceived as one of the causes of obesity (J. Wardle, 2008).Genetic factors play a significant role in the incidence of obesity, with approximately 60% of susceptibility to obesity attributed to genotype differences (Maharani C, 2019). Among the genes associated with obesity, the Fat Mass and Obesity-associated (FTO) gene is widely recognized (Merra G, 2020). FTO gene polymorphisms have also been linked to metabolic syndrome, hypertension, type 2 diabetes mellitus (DM), atherosclerosis, and levels of C-reactive protein, among other conditions. Nonetheless, the precise mechanism by which variations in the FTO gene contribute to the development of obesity remains uncertain (Maharani C, 2019).

Alleles are genes located at corresponding loci on homologous chromosomes. Regarding their influence on phenotype, they occupy the same or nearly identical locus and have similar

functions.

The research results are presented in Tables 4.5 and 4.6, providing information on the FTO gene variant rs9939609 in obese and non-obese subjects. Prodia Pusat employees in Jakarta obtained the AT allele in 100% of non-obese employees, the TT allele in 60% of obese employees, the AT allele in 33.33% of obese employees, and the AA allele in 6.67% of obese employees.

This study' proportions were similar to those found in research by Nurhasanah et al. (2022) on 80 students in Riau. The percentage of the TT allele in obese subjects was 38.75%, the percentage of the AT allele was 6.25%, and the percentage of the AA allele was 2.5%. In non-obese subjects, the percentage of the AA allele was 2.5% (Nurhasanah, 2022). Correspondingly, the A allele was considered risky for obesity. In this study, non-obese subjects demonstrated a 100% occurrence of the AT allele, indicating a potential genetic risk factor for obesity. Among obese subjects, 60% had the TT allele, contradicting the genetic expectation of it not being a risk allele for obesity. This circumstance could be attributed to lifestyle or diet factors. Additionally, the study found that the AA allele occurred in 6.67% of the participants, suggesting a higher risk of obesity (Septiyanti, 2020).

The FTO gene variation significantly contributes to obesity, with the A allele of the variant rs9939609 associated with an increased risk. In European populations, the A allele of variant rs9939609 has been reported to have the most substantial effect. However, in Asian populations, the A allele is less predisposed to obesity, resulting in a weaker effect of the variant rs9939609 (Ursu RI, 2015;).

Other studies indicated that the distribution of AA alleles was higher in people with obesity than individuals with average weight. Furthermore, some others revealed that carriers of the AA allele had twice the risk of obesity compared to those with the TT and AT alleles.

Overweight or obesity can be influenced by various factors such as age, ethnicity, marital status, education level, and health conditions, including diabetes, hypertension, and hypercholesterolemia. In men, the prevalence of obesity is higher in the upper middle age group, specifically among individuals aged 40 to 49 years, and tends to decrease with advancing age, particularly among the elderly population aged 60 years and above, when compared to teenagers and young adults. In women, advancing age is significantly associated with obesity, particularly among those of childbearing age, as they are at a higher risk of weight gain. Furthermore, middle-aged and older women may experience increased body weight and central fat distribution due to hormonal changes during the menopausal transition. In addition, educational attainment can also influence the likelihood of being obese. Individuals with higher levels of education often have sedentary jobs or occupations that involve minimal physical activity, leading to a less active lifestyle (Chan, 2017) (Chey, 2013.)

In this regard, the pipetting technique is a significant factor in medical laboratory analysis. It significantly impacts research success, especially in the molecular field, where small-volume pipetting is commonly employed

(Widayat, 2019). Incorrect application can lead to unreadable extraction results or necessitate sample repetition, requiring additional costs. To minimize the need for recurrence, researchers should possess proficiency in pipetting techniques for molecular research (Rahardianti, 2017).

Conclusion

Based on the present research findings, the FTO gene variant rs9939609 among obese and non-obese individuals in Prodia Pusat employees in Jakarta revealed certain allele frequencies. Specifically, the AT allele was observed in 100% of non-obese employees, the TT allele in 60% of obese employees, the AT allele in 33.33%, and the AA allele in 6.67%. For future researchers, it is recommended to expand the sample size to obtain a more representative population and include additional screening tests such as Diabetes Mellitus or cholesterol tests. It will contribute to a comprehensive understanding of the subject matter. Moreover, clinical laboratory institutions should emphasize the significance of genetic testing for early obesity prevention by providing relevant information and guidance.

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