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Polymorphism of Fat Mass and Obesity Associated (FTO) gene as a risk Factor for Type 2 Diabetes Mellitus with Metabolic Syndrome at DR Sardjito Hospital Yogyakarta

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ABSTRACT

Introduction The metabolic syndrome is a group of metabolic risk factors found in an individual and has been known a multidimensional risk factor for cardiovascular disease. Metabolic syndrome associated with an increased risk of diabetes mellitus (DM) type 2, which is a major risk factor for cardiovascular disease . Metabolic syndrome is characterized by obesity (especially central obesity), dyslipidemia, hyperglycemia, and metabolic syndrome simultaneously. FTO gen is one of the new recently studied and still requires a lot of studies on the role of this gen on the incidence of metabolic syndrome. FTO rs 9939609 polymorphism in some populations have mentioned as the risk factors for obesity, type 2 diabetes mellitus and metabolic syndrome. Protein FTO role in demethylations of the genes in the hypothalamus that helps maintain energy intake and expenditure. In addition, FTO polymorphism rs 9939609 also affect the components of the syndrome.The objective of this study is to examine rs 9939609 FTO polymorphism as a risk factor for metabolic syndrome and it's components in type 2 diabetes in Yogyakarta.

Methods This study used a case- control design with type 2 DM subjects with metabolic syndrome (n = 40) as cases and type 2 diabetes mellitus without metabolic syndrome (n = 40) as a control from DR.Sardjito Hospital Yogyakarta 2009-2010. FTO gen rs9939609 polymorphism was analyzed by PCR-RFLP. Chi square test and odds ratio test are used to examine FTO rs9939609 polymorphism and the relationship with the incidence of type 2 diabetes with metabolic syndrome and it's components.

Results The frequency of TT genotype in subjects with type 2 DM patients with metabolic syndrome 2(5%), AT genotype 35 (87.5%) and AA genotype 3 (7.5%) (n = 40). In subjects with type 2 DM patients without metabolic syndrome TT genotype frequency 6 (15%), AT genotype 32 (80%) and AA genotype 2 (5%) (n = 40). Odds ratio (OR) AT /AA genotip for metabolic syndrome isn't significant, OR are significant for sistolic hypertension (OR=0,039) and for HDL plasma level (OR=0,046) as the components of metabolic syndrome.

Conclusion The FTO gen rs9939609 polymorphism is the risk factor for hypertension and high HDL plasma level as the components of metabolic syndrome at DR Sardjito Hospital Yogyakarta.

Keywords : Type 2 Diabetes Mellitus with metabolic syndrome, rs9939609 polymorphism, FTO gene

INTRODUCTION

Metabolic syndrome is a group of metabolic risk factors found in an individual and is known to be a multidimensional risk factor for cardiovascular disease. Metabolic syndrome is associated with an increased risk of developing type 2 diabetes mellitus (DM), which is a major risk factor for cardiovascular disease. The metabolic syndrome is characterized by the presence of obesity (especially central obesity), dyslipidemia, hyperglycemia, and hypertension simultaneously.[1]

The incidence of type 2 DM with metabolic syndrome is influenced by the balance of energy intake and energy expenditure. Many genes that influence the incidence of metabolic syndrome include Insulin Receptor Substrate (IRS-1), Estrogen receptor, Leptin receptor and Melanocortin 4 Receptor (MC4R). One of the genes that play a role in the development of DM related to energy intake and expenditure is fatso/fat mass and obesity associated (FTO). The FTO gene is one of the genes that has recently been studied and still requires a lot of studies regarding the role of this gene in the incidence of metabolic syndrome. Research has proven that there is mRNA expression of the FTO gene in the hypothalamus which is related to the energy regulation center and the function of these genes by demethylating DNA.[2] With demethylation, these genes will be easier to express because of changes in the charge on DNA due to demethylation. FTO protein plays a role in demethylation of genes that affect energy intake such as

Thyroid Releasing Hormone (TRH), Gonadotropin Releasing Hormone (GnRH),Melanin Concentrating Hormone (MCH).[3] Many single nucleotide polymorphisms are found in the FTO gene. One of the intronic polymorphisms of the FTO gene is the rs 9939609 polymorphism which may affect the mRNA expression of the FTO gene in the energy regulatory center in the hypothalamus. 2 The presence of polymorphisms in the FTO gene will reduce the expression of these genes so that it affects adiposity, insulin resistance, and body weight regulation, including energy intake and lipolysis.[4,5,6]

The things mentioned above caused by the FTO gene polymorphism can be a risk factor for the occurrence of metabolic syndrome. 7 Research on the FTO gene rs 9939609 polymorphism in several populations states that this polymorphism is the biggest risk factor compared to other polymorphisms for body fat accumulation.4 In addition, the first intron polymorphism of rs 9939609 of the FTO gene also affects the components of the metabolic syndrome with the AA genotype and the A allele having higher risk factors than the TT genotype and the T allele. FTO gene and type 2 diabetes mellitus with metabolic syndrome in Yogyakarta, especially in women who have adiposity, are more at risk for suffering from metabolic syndrome.8

METHOD

Case-control study, type 2 DM patients with metabolic syndrome as the case group and type 2 DM patients without metabolic syndrome as controls from DR.Sardjito Hospital Yogyakarta 2009-2010

1.Variable Independent : Genotypes AA, AT and TT gene FTO
2.Variable Dependent : Metabolic syndrome, waist circumference, HDL levels, triglyceride levels and blood pressure

Definition Of Research Variables

1. Patients with type 2 DM are patients diagnosed as type 2 DM at Dr. Sardjito General Hospital with the diagnostic criteria for fasting plasma glucose levels 126 mg/dl (7.0 mmol/l) or plasma glucose levels 2 hours after loading glucose 75 g 200 mg/dl (11.1 mmol/l) .9

2. Metabolic syndrome is a group of metabolic risk factors found in an individual and is known to be a multidimensional risk factor for cardiovascular disease.Kriteria diagnosis sindrom metabolik sebagai berikut :

Tabel 1. Definition of Metabolic syndrome according to NCEP ATP III (modified IDF 2005)

If there are main criteria and 2 additional criteria	
Main criteria:	
Waist Circumference (waist circumference) (cm)	Male >102cm Female>88cm
Additional criteria:	
Triglyceride Level (mg/dl)	150mg/dL
HDL cholesterol (mg/dl)	Male < 40mg/dL Female< 50mg/dL
The waist circumference of the Asian population is lower, the cutoff is 90cm for male and 80 for female (IDF modification): the main criteria is waist circumference and 2 additional criteria	

The FTO gene polymorphism rs9939609 polymorphism (T>A) determined by the PCR-RFLP method.

Research subject

Case subjects (group 1) were taken from type 2 DM patients with metabolic syndrome, while control subjects (group II) were taken from type 2 DM patients without metabolic syndrome. Both groups were patients who underwent outpatient treatment at the Endocrinology polyclinic, RSUP. DR. Sardjito Yogyakarta.

Exclusion criteria for both groups :
Pregnant, suffering from hyperthyroidism, kidney and liver disease (based on medical records).

Genotyping Polimorfisme rs9939609 FTO gene

1. DNA Amplification

Amplification DNA with :
- forward primer

5′-GGT TCC TTG CGA CTG CTG TGA AAT T-3′
- reverse primer

5′-GCT TTT ATG CTC TCC CAC TC-3′

PCR

a. PCR mixture: 2 µl of genomic DNA, plus 15 µl of PCR master mix, 11 µl of H2O and 2 µl of primer mix, so that the total volume becomes 30µl.

b. Polymerase Chain Reaction (PCR) cycle temperature conditions:

Initial denaturation at 95oC (5 minutes) denaturation

(34 cycles) at 94oC (30 seconds) annealing at 54oC (30 seconds) extension at 71oC (30 seconds) final extension at 72oC (5 minutes).

2. RFLP (Restriction Fragment Length Polymorphism)

Incubation at 37 degrees Celsius for 16 hours with Apo1 cleaving enzyme: T allele was split by 2 fragments of 85 bp and 20 bp. Allele A was not split by 105 bp (success rate 98.4%) .[10]

3. Electroforesis

The results of DNA digestion by the restriction enzyme Apo1 were electrophoresed on 3% agarose gel visualized with ethidium bromide. Electrophoresis for 35 minutes, 100 volts and the results viewed under UVs light. The T allele (wild type) was cut by 2 fragments of 85 bp and 20 bp. Allele A (mutant) was not cut (105 bp) .

Statistical analysis

Analysis of FTO gene polymorphisms against type 2 DM with metabolic syndrome used descriptive statistics, the frequency of genotypes and allele polymorphisms using the Fisher's Exact test, while to determine the risk factors for FTO gene polymorphisms against type 2 DM with metabolic syndrome using Odds Ratio.

RESULT

Distribution of FTO genotypes and alleles

Table 3. Distribution of genotypes (TT, AT and AA) and alleles (T and A) of the FTO gene in subjects with type 2 diabetes mellitus with metabolic syndrome and type 2 diabetes without metabolic syndrome.

Variable		DM tipe 2 with Metabolic Syndrome	DM tipe 2 without Metabolic syndrome	p (CI= 95 %)
		(N=40)	(N=40)	
Genotype	TT	2(5%)	6 (15%)	
	AT	35(87,5%)	32(80%)	0,311
	AA	3(7,5%)	2(5%)	
Alele	A	41(51,25%)	36(45%)	0,263
	T	39(48,75%)	44(55%)	

Analysis using the Fisher's Exact test, showed that the frequency of the FTO gene genotype between the observed value and the expected value was statistically not significantly different (p = 0.311). Allele A is not yet a risk factor for type 2 DM with metabolic syndrome with an Odds ratio (OR) value of 1.28 (p=0.168). The AA genotype and AT genotype is also not a risk factor for type 2 diabetes mellitus with metabolic syndrome (OR = 3.353) with p = 0.263 , this indicates that the rs9939609 polymorphism of the FTO gene is not yet a risk factor for the occurrence of type 2 diabetes with metabolic syndrome in the population in Yogyakarta. The effect of genotype and allele on the risk of developing type 2 DM with metabolic syndrome is shown in table 4.

Table 4. Risk factors for the genotype and allele of the FTO gene for the occurrence of type 2 DM with metabolic syndrome.

Variable		DM tipe 2 with metabolic syndrome (N=40)	DM tipe 2 without metabolic (N=40)	p	OR
Genotype	TT dan AT	34	38	0,263	3,353
	AA	6	2		
Alele	T	39	44	0,168	1,28
	A	41	36		

The frequency distribution of the genotypic polymorphism of the fto gene based on the criteria for metabolic syndrome

Table 5. Hasil distribusi frekuensi genotip TT dan genotip yang mengandung mutan AT/AA apabila faktor risiko di kategorikan sesuai kriteria IDF .

		TT	AT/AA	OR(95 CI)	p
Lingkar Penggang (cm)	NORMAL	5	23	3,55	0,120
	RISK	3	49		
Systole (mmHg)	NORMAL	7	35	7,4	0,039
	RISK	1	37		
Diastole (mmHg)	NORMAL	8	52	-	0,109
	RISK	0	20		
HDL (mg/dl)	NORMAL	7	36	7	0,046
	RISK	1	36		

Discussion

The frequency of TT genotype in subjects with type 2 DM with metabolic syndrome was 5%, AT genotype was 87.5% and AA genotype was 7.5% (n=40). In subjects with type 2 DM without metabolic syndrome, the frequency of the TT genotype was 15%, the AT genotype was 80% and the AA genotype was 5% (n=40).

In the research on the rs9939609 polymorphism of the FTO gene that has been carried out by Al-Attar, more samples are used when compared to this study. The distribution of genotype frequencies (TT, AT and AA) of the FTO gene, statistically showed no significant difference. In this study all the criteria were not met from the Hardy-Weinberg equilibrium law, namely the small population, because it only used the hospital population and the absence of random marriages only in the Javanese and there was migration that could affect the possibility of the emergence of genotypic variations of the rs9939609 polymorphism. FTO gene.

AA and AT genotype FTO gene polymorphism on the incidence of type 2 DM with metabolic syndrome

In this study, the genotype and allele frequencies of the rs9939609 FTO gene polymorphism, did not find a significant difference between DM type 2 with the metabolic syndrome and type 2 diabetes without the metabolic syndrome. The effect of the AA and AT FTO genotypes on the incidence of type 2 DM with metabolic syndrome was analyzed using the Odds Ratio (OR) yielding a value of 3.353. The frequency of AA and AT genotypes in Indonesia, both in type 2 DM with metabolic syndrome and in type 2 DM without metabolic syndrome, is the highest compared to China and various ethnicities in Canada. 7.11

The risk of allele A for the occurrence of type 2 diabetes with metabolic syndrome was 1.28 times higher than the risk of type 2 diabetes without metabolic syndrome, but p value = 0.168. Based on the results of this study, it was shown that the rs9939609 polymorphism of the FTO gene was not a risk factor for the occurrence of type 2 DM with metabolic syndrome in Indonesia, especially in the Javanese. Research on the rs9939609 polymorphism of the FTO gene in various countries has varying OR values. Based on the results of research from the Chinese population (Chang et al, 2008) the OR for the A allele was 1.43 and the OR for South Asian ethnicity was 1.66 and Greenland was 1.33 . [11]

The rs9939609 polymorphism of the FTO gene is a substitution of one nucleotide transversion which acts as a marker because the FTO gene rs9939609 polymorphism is found in introns . This polymorphism can be used as a marker / not causative for mutations in other places in the vicinity which may explain more deeply about changes in the expression and function of the FTO gene itself. Polymorphism of rs 9939609 which may affect the mRNA expression of the FTO gene in the energy regulation center in the hypothalamus.[2] The presence of polymorphisms in the FTO gene will reduce the expression of these genes so that it affects adiposity (Andreasen, 2008)4 insulin resistance, and body weight regulation, including energy intake and lipolysis with AA genotype and A allele have higher risk factors than TT genotype and T allele. [5,6]

In table 5 the results show the criteria for waist circumference, diastolic blood pressure and triglyceride levels there is no difference in the frequency of the TT genotype and the genotype containing the AT/AA mutant, while for the criteria for systolic blood pressure and HDL levels there is a difference in the frequency of each genotype, respectively, with p = 0.039 and p=0.046 and OR=7.4 and OR=7 . This shows that the AT/AA genotype has a risk for systolic blood pressure of 7.4 times compared to the genotype that does not carry the mutant/TT allele.

In this study, the genotype and allele frequencies of the rs9939609 FTO gene polymorphism, found a significant difference between type 2 diabetes with hypertension and type 2 diabetes without

hypertension. The effect of the AA and AT genea FTO genotypes on the incidence of type 2 diabetes with hypertension was analyzed using the Odds Ratio (OR) yielding a value of 7.4. Based on the OR value, it means that the probability of individuals carrying the AA and AT genotypes will suffer from type 2 diabetes with hypertension by 7.4 times greater than with type 2 diabetes without hypertension. The frequency of AA and AT genotypes in Indonesia, both in type 2 DM with hypertension and in type 2 DM without hypertension, was the highest compared to Al-Attar's study of 1.42 for South Asian ethnicity and 1.27 for Chinese ethnicity and 1.33 for Greenlandic ethnicity. .[7,11]

Based on these results, it means that individuals with type 2 diabetes who carry the A allele also have risk factors for suffering from type 2 diabetes with hypertension. Based on the pathogenesis of the metabolic syndrome itself, genetic factors in the form of the AA/AT genotype also have a relationship with the incidence of hypertension because FTO protein indirectly plays a role in regulating energy balance, so FTO also has a role in the accumulation of body fat which also underlies the occurrence of atherosclerosis.

Table 5 also shows that the AA/AT genotype is a risk factor for the category of HDL cholesterol levels with OR = 7 . The AT/AA genotype has a risk for HDL of 7, meaning that individuals with the AA/AT genotype have HDL risk factors at risk, where HDL is a criterion for the incidence of metabolic syndrome. In previous studies, the rs9939609 polymorphism was a risk factor for obesity (BMI), with OR 1.59 , OR 1.43 .[7,11] While the research Sofia from Denmark showed that the FTO gene AA/AT genotype on waist circumference with OR 1.19 . The study also stated that FTO also plays a role in the distribution of body fat and reduces insulin sensitivity . FTO protein indirectly plays a roleIn regulating energy balance, FTO also has a role in the accumulation of body fat.

Conclusions

1. The FTO gene rs9930609 polymorphism was not significantly different in genotype frequency (TT, AT and AA) and allele frequency (T and A) between type 2 diabetes mellitus patients with metabolic syndrome and type 2 diabetes mellitus without metabolic syndrome.
2. The rs9930609 T>A FTO gene polymorphism is a risk factor for the components of metabolic syndrome hypertension and high HDL cholesterol at DR Sardjito Hospital Yogyakarta

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