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PRELIMINARY STUDY: IDENTIFICATION OF DNA VARIATION WITH SNP NUMBERS RS1137101 AND RS8050136 IN PATIENT'S TYPE 2 DIABETES MELLITUS AT SALSABILA CLINIC BOGOR – INDONESIA

CHRIS ADHIYANTO, NARILA MUTIA NASIR, FLORI R. SARI, RISFY GUSTI PAMUNGKAS, INTAN AZIS, ZETI HARRIYATI, WITRI ARDINI, HARI HENDARTO, NOUVAL SHAHAB, ENDAH WULANDARI, KARYADI AND FASE BADRIAH

Faculty of Medicine and Faculty of Health Sciences, Universitas Islam Negeri, Syarif Hidayatullah, Jakarta, Indonesia

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Abstract – Type 2 Diabetes Mellitus (T2DM) has become a worldwide pandemic. Data from Indonesia Basic Health Research show the prevalence of diabetes in Indonesia in 2017 is 6.3% which ranked 6th in the world. The Genome-Wide Association Studies (GWAS) has obtained more than 250 genetic loci that are assumed to play a role in the pathogenesis of obesity and T2DM. We have identified the DNA variation in T2DM patients, specifically the LEPR and FTO genes that thought to be linked with T2DM. We chose Rs 1137101 (LEPR Q223R, exon 6 A>G) and rs8050136 (FT0 intron variant C>A) because these SNP variations are common in the Asian region. The result show T2DM subjects had a higher genotype GG for LEPR Rs1137101, and AC for FTO Rs8050136, and it had associated between allele risk Rs1137101 (G) and Rs 8050136 (A) ($p<0.0001$). In conclusion the research found associated between SNP Rs1137101 and Rs 8050136 in patients T2DM.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) has become a worldwide pandemic (Meshkani *et al.*, 2016). Data from Indonesia Basic Health Research shows the prevalence of diabetes in Indonesia in 2017 is 6.3% and put Indonesia 6th rank in the world. It is estimated that 1,670 million people whose age 20 to 39 years old suffer from T2DM. Meanwhile, the sufferer for the ages of 40 to 59 years are 4,651,000 people. Type 2 Diabetes mellitus (T2DM) is caused by multifactor. The interaction between genetic factors and environmental factors such as diet, smoking habit, and physical activity might cause the occurrence T2DM. The Genome-Wide Association Studies (GWAS) has obtained more than 250 genetic loci that are assumed to play a role in the pathogenesis of obesity and T2DM. Various studies have been conducted to prove the effects of these genetic risk factors on the increased risk of T2DM. Variants mutation of PPARG, TCF7L2, and FTO genes are show the most consistent relationships gene with an increased risk of T2DM. Variants of other genes, such as ADIPOQ, LEPR, GCKR and KCNQ1, which are widely studied in the Asian population, also show an association with an increased risk of T2DM.

The number of the genetic studies of T2DM is rarely conducted in Indonesia. Puspitaningrum *et al* have identified 16sRNA and ND1 gene mutations in T2DM patients in Java (Puspitaningrum, *et al.*, 2014). These mutations are a related factor to T2DM. In our study, we have identified the DNA variation in T2DM patients, specifically the LEPR (leptin receptor) and FTO (fat mas and obesity) genes that considered to be linked with obesity and T2DM. We chose Rs 1137101 (LEPR Q223R, exon 6 A>G) and Rs8050136 (FT0 intron variant C>A) because these SNP variations are common in the Asian region. The presence of individuals who have variant alleles increases the risk of developing T2DM (Hastuti *et al.*, 2016).

MATERIALS AND METHODS

Collection and Isolation of DNA samples

Eighty respondents were participated as the research subject in this study. Thirty-five of them were T2DM patient aged 30 to 60 years old who routinely visit the mini hospital (Clinic) Salsabila Medical Center in Bogor – West Java. As for the control group, we recruited 45 healthy subjects that decided after physically - laboratory observation by medical doctor. Venous blood (3 mL) samples were collected (Blood –EDTA) from each subject groups. All participants must signed informed consent form before they involve in this study. Three hundred milliliters blood-EDTA treated with lysis buffer (Genomic DNA Mini Kit from Geneaid (Cat#GB300)). After finishing with isolated the genome, the purity and concentration of each sample were measured using a spectrophotometer method with Nano-drop Denovix.

Genetic – SNP analyses

LEPR SNP: Rs1137101 and FTO SNP: Rs8050136 were selected for analysis based on others researcher in Asia. Two-microliter sample mixed with master-mix buffer contained probe (Taqman-SNP) and transferred to plate chamber, then measured the fluorescence intensity and did the analysis used LC480-Roche. The statistical analysis was performed using MEDCAL Software and Easy Calculation.com for calculated of allele frequencies.

RESULTS AND DISCUSSION

Thirty-five T2DM and 45 normal subjects (T2DM) were signed informed consent to donate the blood sample. The distribution SNPs parameters of the subjects are showed in Fig. 1 and 2. The T2DM

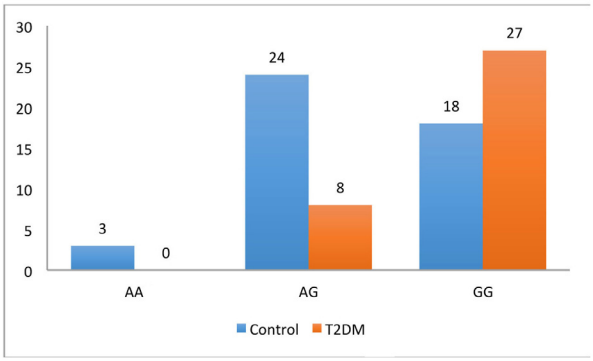

Fig. 1. Distribution of LEPR gene rs1137101 in our samples; (AA= normal; AG=1.25xT2DM; GG=1.5 × T2DM (SNPedia.com))

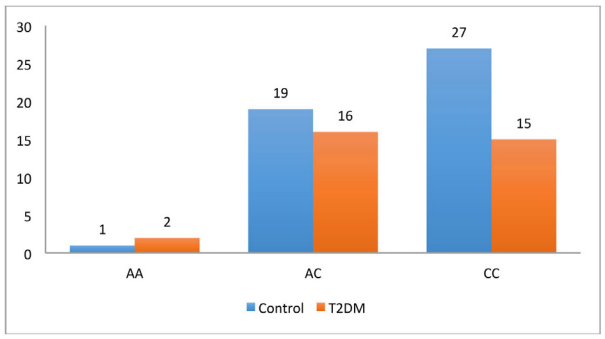

Fig. 2. Distribution of FTO gene rs 8050136 in our samples; (CC= normal; AC=1.2xT2DM; AA= 1.4 × T2DM (SNPedia.com))

subjects had a higher genotype GG for LEPR Rs1137101, and AC for FTO Rs8050136. Meanwhile, non-T2DM or control had a higher genotype AG for LEPR, and CC for FTO.

Table 1 and 2 shows the allele frequencies of LEPR and FTO and the association of T2DM with the risk allele. The risk allele frequencies of Rs 1137101 for LEPR and Rs8050136 for FTO in normal subjects were 0.663 and 0.223 versus 0.885 and 0.303 in T2DM respectively.

Table 1. Allele Frequency

LEPR	Control	T2DM
A (non risk allele)	0.333	0.114
G (risk allele)	0.667	0.885
FTO	Control	T2DM
A (risk allele)	0.223	0.303
C (non risk allele)	0.777	0.697

The allele are summarized in Table 1 and 2. The risk allele of LEPR rs1137101 (G) was strongly associated with T2DM ($p<0.0001$). Meanwhile, the risk allele Rs8050136 (A) was borderline significant associated with T2DM ($p=0.0001$).

The incidence of T2DM in Indonesia is quite high, which is ranked 6th in the world. The increasing cases of T2DM in Indonesia may be due to lifestyle patterns, especially in the younger generation. Numerous studies have reported the influence of genetic and T2DM in the world (Saif-Ali *et al.*, 2011; Tan *et al.*, 2009; Wu *et al.*, 2014; Babu *et al.*, 2018; Stoger, 2012). However, there is the lack genetic information of T2DM in Indonesia which made the reason why we conducted this study. LEPR Rs1137101 and FTO Rs8050136 genes in relation to T2DM are widely studied in Asia. In our study, we

Table 2. Association risk allele of LEPR and FTO with T2DM

SNP	Group	Risk allele (frequency)	Odds Ratio		Risk Ratio	
			OR (95% CI)	P value	RR (95% CL)	P value
LEPR Rs 1137101	normal	G	3.8768		1.3282 (1.26)	
		0.667	(3.0644 to	p<0.0001		p<0.0001
	T2DM	0.885	4.9013)		to 1.39	
	normal	A	15140		1.3587(1.171)	
		0.777	(1.2390 to	p=0.0001		P=0.0001
	T2DM	0.697	1.8520		to 1.577)	

LEPR SNP: rs1137101; FTO SNP: rs8050136

found the variant of LEPR and FTO that showed the association of Rs1137101 and Rs8050136 with risk of T2DM. The risk allele of the rs1137101 (G) is a higher distribution in our samples. This result is similar and consistent to the result conducted by other researchers in Asia (Hastuti *et al.*, 2016; Hussein and Hassan, 2017).

Our study showed the frequency of variant allele LEPR Rs137101 were 0.885 in the T2DM patients, meanwhile wild allele were 0.114. Leptin and leptin receptors are involved in regulating satiety and energy metabolism through central and peripheral mechanisms (Wu and Sun, 2017; Fan and Say, 2014). The association of leptin and leptin receptors with T2DM begins with obesity (Dasgupta *et al.*, 2015; Rejeb *et al.*, 2012). Leptin will prevent obesity through the leptin receptor by stimulating glucose uptake and fat oxidation in the muscles and liver, and inhibits insulin secretion by pancreatic beta cells. Various studies have shown genetic variation in the LEPR gene between others are related to obesity, hyperinsulinemia, and T2DM. The meta-analysis by Yang *et al* on 5143 patients with T2DM and 5021 controls from 14 studies showed that minor alleles (G) in Rs 1137101 were associated with an increased risk of T2DM (OR 1.21, 95% CI 1.13 to 1.42). Similar results were obtained by Li *et al* through a meta-analysis of 7 studies in the Chinese population involving 3,376 subjects, that revealed the minor allele (G) Rs 1137101 was associated with an increased risk of T2DM (OR 1.432, 95% CI 1.211-1.694). Our preliminary study results are also not different from the studies conducted by other researchers, where the OD score and RR allele (G) which are minor alleles in LEPR Rs1137101 indicate an association with T2DM risk.

Variant Fat and Obesity-associated gene (FTO) is one of the polymorphisms that are closely related to obesity and T2DM. The FTO gene is a gene that encodes the alpha-ketoglutarate dependent dioxygenase enzyme. FTO is expressed in all body tissues, but especially in the brain and hypothalamus. The physiological function of this gene is not yet fully known, but various studies in animals and humans show the role of these genes in the nervous and cardiovascular systems, and are associated with body mass index (BMI), risk of obesity, and risk of T2DM (Peng *et al.*, 2011; Tan *et al.*, 2008; Chey, Wei-Wei; Fan, Sook-Ha, Say, 2013). From GWAS it was found that SNPs of FTO were strongly associated with increased BMI and adipocytes (Wu *et al.*, 2014; Cheung and Yeo, 2011). Some researchers have reported that subjects with AA genotypes at FTO Rs8050136 (located in intron 1) have a risk of obesity than the CC genotype (Park *et al.*, 2013; Qian *et al.*, 2013). Unlike the previous studies, in our study we found that the forty-five percent of the T2DM respondents have CC (non risk allele) genotype. This shows the SNP Rs 8050136 at the FTO gene does not have a major cause with our T2DM subject. Only six percent of the total T2DM subjects carry AA genotypes. It is mean that there is no independent association of Rs 8050136 with T2DM. However, it appears that allele A is the dominant risk allele where forty-eight percent of T2DM subjects have AC genotype. Forty-eight percent of T2DM-FTO subjects with AC genotypes have a combination of GG and AG for LEPR. It seems that the combination of genotypes is a possible cause of T2DM in our study subjects. This is supported by the results of a meta-analysis that genetic linkages and T2DM are not only caused by one gene variant SNP, but more than one variant (Hale *et al.*, 2012; Elbein, 2006; Radha and Mohan, 2007; Qian *et al.*, 2013).

In conclusion, the two of polymorphism Rs1137101 A/G of the LEPR and Rs8050136 C/A of the FTO were associated with T2DM in our patients.

REFERENCES

Babu, G. R. 2018. Association of obesity with hypertension

and type 2 diabetes mellitus in India/: A meta-analysis of observational studies. *World J Diabetes*. 9(1) : 40–52. doi: 10.4239/wjd.v9.i1.40.

Cheung, M. M. and Yeo, G. S. H. 2011. FTO biology and obesity/: why do a billion of us weigh 3 kg more/?. *Frontier in Endocrinology*. 2: 1–9. doi: 10.3389/fendo.2011.00004.

Chey, Wei-Wei, Fan, Sook-Ha and Say, Y. H. 2013. Association of Fat Mass and Obesity-Associated (FTO) Gene Rs 9939609 Variant with Obesity Among Multi-Ethnic Malaysians in Kampar, Perak. *Sains Malaysiana*. 42 (3) : 365-371.

Dasgupta, S. 2015. Genetic variants in leptin/: Determinants of obesity and leptin levels in South Indian population. *Adipocyte*. 4 (2) : 135–140. doi: http://dx.doi.org/10.4161/21623945.2014.975538.

Elbein, S. K. D. and S. C. 2006. The Genetic Basis of Type 2 Diabetes. *Cell science*. 2 (4) : 100-131. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1526773/pdf/nihms11265.pdf.

Fan, S. and Say, Y. 2014. Leptin and leptin receptor gene polymorphisms and their association with plasma leptin levels and obesity in a multi-ethnic Malaysian suburban population. *Journal of Physiological Anthropology*. 33 (1) : 1–10. doi: 10.1186/1880-6805-33-15.

Hale, P. J., López-yunez, A. M. and Chen, J. Y. 2012. Genome-wide meta-analysis of genetic susceptible genes for Type 2 Diabetes. *BMC Systems Biology*. 6(Suppl 3) : S16. doi: 10.1186/1752-0509-6-S3-S16.

Hastuti, P. 2016. Polymorphism in leptin receptor gene was associated with obesity in Yogyakarta, Indonesia', *Egyptian Journal of Medical Human Genetics*.. 17(3): 271–276. doi: 10.1016/j.ejmhg.2015.12.011.

Hussein, I. and Hassan, Z. 2017. SNP rs1137101 Leptin Receptor Gene LEPR as a Risk Factor for Type 2 Diabetes. *American Scientific Research Journal for Engineering, Technology, and Sciences*. 38(2): 341–347. Available at: http://asrjetsjournal.org/.

Meshkani, R. 2016. Association between Leptin G2548A and leptin receptor Q223R polymorphisms with type 2 diabetes in an iranian population. *Clinical Laboratory*. 62 (1–2) : 89–96. doi: 10.7754/Clin.Lab.2015.150535.

Park, S. L. 2013. Association of the FTO Obesity Risk Variant rs8050136 With Percentage of Energy Intake From Fat in Multiple Racial / Ethnic Populations The PAGE Study. *American Journal of Epidemiology*. 178(5): 780–790. doi: 10.1093/aje/kwt028.

Peng, S. 2011. FTO gene polymorphisms and obesity risk/: a meta-analysis. *BMC Medicine*. 9 (1) : 1-15. doi: 10.1186/1741-7015-9-71.

Puspitaningrum, Rini., Adhiyanto, Chris and Hattori, Yukio. 2014. Mutation of mtDNA ND1 Gene in 20 Type 2 Diabetes Mellitus Patients of Gorontalonese and Javanese Ethnicity. *HAYATI Journal of Biosciences*. 21(4): 159–165. doi: 10.4308/hjb.21.4.159.

Qian, Y. 2013. Genetic variant in fat mass and obesity-associated gene associated with type 2 diabetes risk in Han Chinese. *BMC Genetics*. 14 : 2–7. Available at: http://www.biomedcentral.com/1471-2156/14/86.

Radha, V. and Mohan, V. 2007. Genetic predisposition to type 2 diabetes among Asian Indians', *Indian J Med Res*. 125(March): 259–274.

Reinhard Stöger. 2012. *Epigenetic Epidemiology: Epigenetic Epidemiology of Obesity, Type 2 Diabetes, and Metabolic Disorders*. Edited by P. Karin B. Michels, ScD. Dordrecht Heidelberg London: Springer Science+Business Media B.V. New York doi: 10.1007/978-94-007-2495-2.

Rejeb, N. Ben 2012. Relationship Between Leptin G2548A and Leptin Receptor Q223R Gene Polymorphisms and Obesity. *Genetic Testing And Molecular Biomarkers*. 16(7): 726–733. doi: 10.1089/gtmb.2011.0324.

Saif-Ali, R. 2011. KCNQ1 variants associate with type 2 diabetes in Malaysian malay subjects. *Annals of the Academy of Medicine Singapore*. 40(11): 488–492. doi: 10.3390/ijms12095705.

Tan, J. T. 2008. FTO Variants Are Associated With Obesity in the Chinese and Malay Populations in Singapore. *Diabetes*. 57: 2851. doi: 10.2337/db08-0214.J.T.T.

Tan, J. T. 2009. Genetic variation in KCNQ1 associates with fasting glucose and â-cell function: A study of 3,734 subjects comprising three ethnicities living in Singapore. *Diabetes*. 58(6): 1445–1449. doi: 10.2337/db08-1138.

Wu, J. 2014. Association of FTO polymorphisms with obesity and metabolic parameters in Han Chinese adolescents. *PLoS ONE*. 9(6): 1–6. doi: 10.1371/journal.pone.0098984.

Wu, L. and Sun, D. 2017. Leptin receptor gene polymorphism and the risk of cardiovascular disease: A systemic review and meta-analysis', *International Journal of Environmental Research and Public Health*. 14(4) : 375-387. doi: 10.3390/ijerph14040375.