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Polymorphism UCP2 Gene in Predicting the Occurrence of type 2 Diabetes Mellitus in Respondents Cohort Study of Risk Factors for non-communicable Diseases in Bogor, West Java, Indonesia

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ABSTRACT

Diabetes mellitus type 2 (DM) is a risk factor for other non-communicable diseases and is a problem, along with the increasing prevalence in Indonesia. One of the proteins involved in the occurrence of diabetes is UCP2, which is produced by the UCP2 gene. So that the polymorphism in this gene is likely to affect the incidence of diabetes. This study uses a cross-sectional method in a population study cohort of risk factors for non-communicable diseases (RF-NCD) conducted in the city of Bogor, West Java, Indonesia. This analysis aims to determine the relationship of UCP2 gene polymorphisms rs660339 on the incidence of DM and pre-DM. SNP examination was performed using the Taqman method and analyzed by Taqman Genotyper. Hardy-Weinberg Equilibrium (HWE) analysis and odds ratios (OR) using online software from www.oege.org. Taqman Genotyper analysis results show the minor alleles in the UCP2 rs660339 gene are A and the lowest frequency is the A/A genotype. The calculation of OR shows that the G/G genotype in the UCP2 rs660339 gene has the potential to become pre-DM.

Keywords*: SNP, UCP2, rs660339, diabetes, pre diabetes*

1. INTRODUCTION

Diabetes mellitus (DM) is a risk factor for other non-communicable diseases and it is a health problem, along with the increasing prevalence in Indonesia. The results of blood glucose tests in the Basic Health Research (BHR) conducted by the National Institute for Health Research and Development (NIHRD) showed an increase the prevalence of DM in Indonesia in 2018 compared to 2013. The prevalence of DM in all regions of Indonesia according to the consensus of PERKENI 2011 to 8.5% (BHR, 2018), higher than before at 6.9% (BHR, 2013). This increase also occurred in cases of prediabetes (pre DM) especially impaired glucose tolerance (IGT), which was 10.2% (BHR, 2007) to 29.9% (BHR, 2013)

Pre DM is a condition of the body with a metabolic disorder characterized by an increase in blood sugar levels exceeding the normal value but has not yet reached the DM stage. Prediabetes includes impaired fasting glucose (IFG) and IGT. Whereas exclusive prediabetes includes isolated IFG and isolated IGT, with the following criteria:

1. Isolated IFG, i.e. fasting plasma glucose between 100-125mg/dL and 2 hours after glucose 75gr oral <140mg/dL.

2. Isolated IGT, i.e. plasma glucose 2 hours after glucose 75gr oral between 140-199mg/dL and fasting plasma glucose <100mg/dL.

In cohort study of risk factors for non-communicable diseases by NIHRD (RF-NCD Cohort Study) conducted in Bogor, Indonesia at 2013 found a prevalence of IGT of 19.4%. It is estimated that there are 314 million people with pre-DM worldwide and will increase to 418 million by 2025 (Manaf, 2013). Pre DM is also a risk factor for stroke, heart disease and blood vessels

DM and pre DM are influenced by various factors, especially energy metabolism in the body and it's characterized by an increase in blood sugar levels. The main energy source is derived from mitochondria. In the mitochondria, there is a conversion of energy substrate to ATP. Some of the energy produced will be released as heat. This process is facilitated by the carrier protein in the mitochondria, the uncoupling proteins (UCP). UCP is a member of mitochondrial anion carrier proteins (MACP), the inner mitochondrial membrane protein that plays a role in reducing the mitochondrial membrane potential. UCP facilitates the movement of anions from the inner membrane to the outer membrane mitochondrial and the return of protons from the outer to the inner mitochondrial membrane by releasing oxidation substrates from ATP synthesis, thereby reducing ATP production by the mitochondrial respiration chain. UCP also controls the production of reactive oxygen species (ROS) generated

from electron transport in the process of energy formation. The structure of various types of UCP is similar to the ATP / ADP carrier protein. (Donadelli, Massimo; Dando, Ilaria; Fiorini, Claudia; Palmieri, Marta., 2014).
There are 5 types of UCP found in mammals, namely UCP1-5. UCP1 is mainly expressed in brown fat tissue, UCP2 is found in several tissues such as liver, brain, pancreas, fat, immune cells, spleen, kidney and central nervous system. UCP3 in adipose tissue and skeletal muscle, related to lipid metabolism. While UCP4-5 is expressed in various specific networks. UCP2 in pancreatic tissue related to oxidative stress and insulin secretion. In experiments using mice that did not have the UCP2 gene, it was seen that UCP2 in pancreatic β cells played a role in oxidative stress, controlling ROS levels in cells and related to insulin secretion stimulated by glucose levels, and mediating changes in glucose, pancreatic α cells and glucagon levels. The absence of UCP2 results in alpha cell morphology changes, which is getting bigger and increasing glucagon secretion. In a normal state, glucagon only increases in hypoglycemia. (Robson-Doucette, Christine A; Sultan, Sobia; Allister, Emma M; Wikstrom, Jakob D; Koshkin, Vasilij; et al., 2011) UCP2 activity can suppress insulin secretion stimulated by an increase in glucose levels, which is regulated based on the ATP ratio / ADP.

UCP2 also regulates the process of phagocytosis of apoptotic cells. Compared to UCP1, UCP2 is spread over more tissues including several phagocytic cell types. UCP2 levels increase after incubation with apoptotic cells. The loss of UCP2 reduces the ability of phagocytic capacity. While increased expression of UCP2 increases phagocytosis of apoptotic cells. Mutational and pharmacological studies indicate a direct role of mitochondrial function mediated by UCP2 in phagocytosis. Macrophages from mice with UCP2 deficiency experienced phagocytic failure in vitro and deficient mice showed in vivo defects in clearing dead cells in the tymus and testes. These data indicate that the mitochondrial membrane potential and UCP2 are molecular keys that influence the clearance of apoptotic cells. UCP2 is also related to metabolic disease and atherosclerosis, due to the ability to clear apoptotic cells. . (Park, Daeho; Han, Claudia Z; Elliott, Michael R; Kinchen, Jason M; Trampont, Paul C; et al., 2011)

Polymorphisms in the UCP2 gene often occur in promoters -866G> A (rs659366); 45-bp polymorphisms of insertions / deletions (rs1800795) of 3 'UTR at exon 8; and missens polymorphisms in codon 55 exon 4, change amino acid alanine to valine (Ala55Val, rs660339, G> A, C> T). Several studies in several countries have shown an association between UCP2 polymorphisms and several diseases, including obesity, T2DM, myocardial infarction, multiple sclerosis, etc. (Massimo Donadelli · Ilaria Dando · Claudia Fiorini · Marta Palmieri, 2014). There is no data yet on the influence of UCP2 gene polymorphisms that represent the Indonesian population.

The objectives of this study were to describe genotype of UCP2 rs660339 in the population of RF-NCD cohort study in Bogor, Indonesia and the association of polymorphisms (Single Nucleotide Polymorphism = SNP) UCP2 genes rs660339 with the incidence of type-2 DM and pre-DM.

2. METHOD

This study uses a cross-sectional method in the RF-NCD cohort study population conducted in the city of Bogor, West Java. Ethical approval obtained from ethics commission of NIHRD with No. LB.02.01/2/KE.235/2017. Number of subjects was 199 (DM), 633 (pre DM) and 643 (normal). Single Nucleus Polimorphisme (SNP) examination was carried out using the Taqman method and analyzed by Taqman Genotyper, Hardy-Weinberg Equilibrium (HWE) analysis and odds ratio (OR) using online software from www.oege.org.

3. RESULTS AND DISCUSSION

Proportion of UCP2 Genotype in DM, Pre DM and Normal

Taqman Genotyper analysis results showed the minor alleles in the UCP2 rs660339 gene were A and the lowest frequency was the A/A genotype. The frequency of G/G genotypes in DM is more than normal and it's greatest in pre-DM (Figure 1).

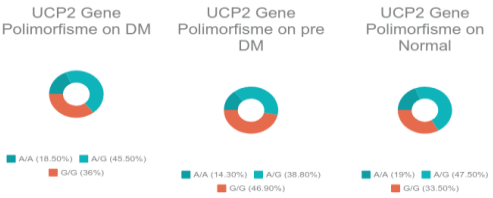

Figure 1. Proportion genotype in DM, pre DM and normal

There is a polymorphism of the UCP2 missens gene in codon 55 exon 4 chromosome 11, G> A, C> T which results in the conversion of amino acids from alanine to valine (Ala55Val, rs660339). Uncoupling of protein 2 (UCP2) is reported to have an important role in the regulation of energy metabolism, through its function as a proton gradient remover that produces a change in ATP to heat. So the variation in the UCP2 gene is expected to affect its potential as a modulator in energy balance.
Figure 1 shows that there was no significant difference between the DM and normal groups, where the highest frequency of genotype was A/G, but there was a tendency for the G allele frequency in the DM group to be higher than normal. A different picture is seen in the pre-DM group, where the largest in this group was the G/G genotype. The results of this study was consistent with data on genebank where the minor alleles in the UCP2 Ala55Val gene, rs660339 are A and the lowest frequency was the A/A genotype.

Table 1. Odds Ratio Genotype on UCP2 rs660339 at DM, Pre DM and Normal
[Hardy-Weinberg Equilibrium (HWE) Analysis and Odds Ratio]

Test	Normal vs DM			Normal vs preDM			Normal vs (DM+preDM)		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
A/G vs G/G	0,88	0,62-1,25	>0,05	0,58	0,46-0,74	<0,001	0,65	0,52-0,82	<0,001
A/A vs A/G	1,03	0,67-1,58	>0,05	0,92	0,66-1,28	>0,05	0,95	0,71-1,28	>0,05
A/A vs G/G	0,91	0,58-1,42	>0,05	0,54	0,39-0,75	<0,001	0,62	0,46-0,84	<0,001

OR results show that A/A and A/G have a half of the risk to get pre-DM than G/G genotype. The A/A genotype has the same risk as A/G to get DM and pre-DM (Table 1). Ancestral allele on UCP2 rs660339 is G. This gene was not in HWE equilibrium, where the p value in Ala55Val, rs660339 was 0.001, which means that the gene had various possibilities, including natural selection, mutation, migration or random marriage. This is understandable because the respondents in this research came from various ethnic groups, including Sundanese, Betawi, Javanese, Batak, Minang, even Chinese, etc. as description of the population in almost all regions of Indonesia. Octavianthi et al in 2012 reported that in the UCP2 gene SNP study in Bali, the population was in equilibrium of HWE, both urban and rural. This can happen because the Balinese marriage tradition tends to be closed, only married with Balinese and all respondents are also ethnic Balinese.

The OR results (Table 1) show that G/G genotype has the potential to become pre-DM, while A/G and A/A genotypes significant have a protective effect against pre-DM, compared to homozygot G/G (A/A and A/G genotypes have a half of risk to be pre DM than G/G genotype). From this analysis it's appears that the G/G genotype has a risk of being pre-DM twice than the A/G and A/A genotypes.

The results of this study are in line with the review of Donadelli M et al's article stating that Ala55Val polymorphism, rs660339 is associated with reducing the risk of DM in Asia-Indians. (Donadelli, Massimo; Dando, Ilaria; Fiorini, Claudia; Palmieri, Marta., 2014). The results of the Shen Y study in the Chinese population also showed the C or G allele at rs660339 was at risk of developing DM and diabetic retinopathy with significant results. (Yinchen Shen, Zujia Wen, Ning Wang, Zhi Zheng, Kun Liu, Xin Xia, Qing Gu, Yongyong Shi, and Xun Xu, 2014). The results of the metaanalysis of Qian L et al show that the UCP2 Ala55Val polymorphism did not show a significant association with obesity in Asian populations. (Qian L, Xu K, Xu X, Gu R, Liu X, Shan S, Yang T, 2013). As we know the obesity is a risk factor for DM.

The results of Octavianthi et al study in Bali on SNP Ala55Val, rs660339 showed no relationship between all genotypes and all examination results (fasting plasma glucose levels, triglycerides, total cholesterol, LDL and HDL). In urban residents, respondents with A/G and A/A genotypes tend to have a greater BMI than respondents in rural areas. While the results of the study indicated that urban respondents had a greater BMI than rural areas. It is estimated that polymorphism in UCP2 affects if urban environmental factors affect the research subjects. Obesity and DM manifests from a combination of genetic and environmental predisposition, including imbalances in metabolic processes and physical activity. (Sukma Oktavianthi1, Hidayat Trimarsanto1, Clarissa A. Febinia1, Ketut Suastika2, Made R. Saraswati2, Pande Dwipayana2, Wibowo Arindrarto1, Herawati Sudoyo1 and Safarina G. Malik, 2012).

Research with subjects in rural areas in China shows that A/A genotype at rs660339 G>A increases the risk of DM in overweight subjects (OR = 1.48, 95% CI: 0.87-2.52) but decreases risk at normal weight (OR = 0.54, 95 % CI: 0.28-1.05). (Su M, Chen X, Chen Y, Wang C, Li S, Ying X, Xiao T, Wang N, Jiang Q, Fu C, 2018). The limitation in our study is we have not yet to analysis the influence of obesity in the associated polimorphisme with DM.

Metaanalysis result of Sauza BM et al showed pholimorphisme the UCP2 Ala55Val associated with DM only in Asians. (de Souza BM, Brondani LA, Bouças AP, Sortica DA, Kramer CK, Canani LH, Leitão CB, Crispim D., 2013) The reason for the difference of research result in Asia is not yet clear, it is probably because ethnic differences have an environmental or habits or lifestyle differences that affect the sensitivity of genomic variation, thus give the different phenotypes, not only influenced by racial similarities. In the research of Zhou Y et al, the T allele in Ala55Val, rs660339 was related to telomere length. Research subjects with C/C genotype had the shortest leukocyte telomere length (LTL) (1,254 ± 0.187) and T/T genotype had longest LTL (1,297 ± 0,242), while C/T genotype was in the middle (1,292 ± 0.229) in subjects without diabetes. The length of this LTL is also influenced by age. The subjects with the higher age had the shorter LTL. (Yuling Zhou, David Simmons, Brett D. Hambly & Craig S. McLachlan, 2016) LTL length is related to biological age and the risk of getting degenerative diseases. The results of this study are in line with the results of our study, where the genotype of G/G or equal with C/C was more risky to have pre-DM and DM

4. CONCLUSION

In conclusions, smallest genotype frequency in population RF-NCD cohort study in Bogor, Indonesia was A/A, while genotype G/G was greatest in pre DM and there was a tendency for more G/G genotypes in DM than normal subjects. SNP UCP2 rs660339 was significantly reduce risk pre diabetes in this study. This study needs to be continued by involving BMI data and other risk factors to be analyzed.

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