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 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 in 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. fasting plasma 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
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.oegge.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](image-url)
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 homozgyot 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. (Yincheng 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. Fenbia1, Ketut Suastika2, Made R. Saraswati2, Pande Dwipayana2, Wirbo 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 polymorphism with DM.

Metaanalysis result of Sauza BM et al showed pholimorphism the UCP2 Ala55Val associated with DM only in Asians. (de Souza BM, Brondani LA, Bouças AP, Sortica DA, Kramer CK, Canani LH, Letiáoo 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.

### Table 1. Odds Ratio Genotype on UCP2 rs660339 at DM, Pre DM and Normal

| 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 |
4. CONCLUSION

In conclusion, the smallest genotype frequency in the 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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