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Polymorphism of Uncoupling Protein 2 (UCP2) Gene in Obes People with the Family History of Type 2 Diabetes Mellitus

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Abstract

History with type 2 diabetes mellitus (T2DM) is manifested by the presence of insulin resistance and β-cell dysfunction which prerequisites for T2DM development involving unbalanced energy intake and expenditure. Uncoupling gene protein 2 is the main regulator of energy balance. This study aims to determine the UCP2 -866G/A genetic variation in obese individuals with a family history of T2DM and without a family history of T2DM. The study was a case control design in which the case subjects (n = 60) were obese individuals with a family history of T2DM and control subjects (n = 60) without a family history of T2DM. Polymorphism was analyzed with PCR-RFLP. The value of HOMA-IR and HOMA-β was calculated by the HOMA formula. Data was analyzed by Independent Sample t-test, Mann Whitney U-test, Chi-Square test and Kruskal-Wallis test with a significance level of $p < 0.05$. The frequency of genotype and alleles in obese individuals with a family history of T2DM and without a history of T2DM did not differ significantly, GA+AA (56.7 %) and A (32.5 %) allele was higher in individuals with a family history of T2DM. The GG+AA genotype in the male group with a family history of T2DM could significantly increase the risk of UCP2 gene polymorphism in T2DM by 2.23 times (CI 95 % 0.64 - 8.14) whereas in the female group there was no risk of T2DM. HOMA-IR and HOMA-β value in both family background did not differ significantly. The value of HOMA-β in the female gender group had significant relationship between obese individuals with a family history of T2DM ($p = 0.04$). Conclusion: The result suggests obese individuals with a family history of T2DM have a higher risk of getting GA+AA genotypes and A allele than individuals without a family history of T2DM.

Keywords: Obesity, Type 2 diabetes mellitus, Polymorphism -866G/A UCP2 gene, Genotype, Insulin resistance

Introduction

Obesity has been a serious global health challenge in the last 2 decades [1]. The *Riset Kesehatan Dasar* (Riskesdas) stated that the prevalence of obesity increased from 2007, 2010 and 2013 in which for male adult population reported 13.9, 17.8 and 19.7 %, while those of adult female population were 13.9; 15.5 and 32.9 %, respectively [2]. The problem of obesity in adults has begun to appear in Central Sulawesi with a prevalence of 18.2 %. All districts/cities have a high prevalence of obesity in adults. The prevalence of obesity in Palu is 25.5 % and ranks highest in Central Sulawesi, with prevalence above 15 years in men 12.4 % and women 23.7 % [2]. Energy intake that exceeds energy expenditure will cause body fat mass to increase when a state of positive energy balance occurs. In the long term, a positive energy balance will result in obesity.

Environmental factors and genetic factors that are known to play a role in the pathological mechanism of obesity, through energy balance mechanisms, especially thermogenesis and glucose homeostasis that link the condition of obesity with genetics. The genetic component for obesity is associated with relative risks between family relationships and body composition relationships. Among obesity comorbid, insulin resistance is one of the most important [3] because insulin resistance is an important link between obesity and other diseases such as type 2 diabetes mellitus (T2DM). Insulin resistance and β-cell dysfunction are prerequisites for the development of type 2 diabetes [4-6].

The risk of DMT2 increases 2 to 6 times if parents or siblings experience T2DM. Early onset of diabetes is found in patients who have a family history of diabetes, with a higher risk if both parents have diabetes [7]. The prevalence of insulin resistance is influenced by interactions between environmental factors and genetic factors. Genes associated with insulin resistance and contribute to the pathogenesis of obesity are uncoupling proteins (UCP) [8].

Uncoupling protein 2 (UCP2) which is the main regulating protein of energy balance, is a transporter protein in the inner membrane of the mitochondria. In addition to mitochondria, UCP2 is expressed on various tissues, including adipose tissue, skeletal muscle, liver and pancreatic islet cells [9]. Uncoupling protein 2 releases energy reserves in the form of heat and has the potential to prevent obesity by mediating the leakage of protons across the inner membrane, thereby reducing ATP production through the mitochondrial respiratory chain. As a result, high UCP2 activity can suppress glucose-stimulated insulin secretion, which is regulated by the ATP/ADP ratio [6]. The UCP2 gene with genotype -866A/A on the promoter of the UCP2 gene is associated with insulin resistance in individuals predisposed to T2DM, suggesting that UCP2 gene genetic variance can contribute to T2DM by affecting both cell function and insulin action.

This study was designed to evaluate polymorphism of uncoupling protein 2 (UCP2) gene in obes people with the family history of type 2 diabetes mellitus in Palu Central Sulawesi-Indonesia.

Materials and methods

Design and participants

This research used a case control study. Subjects with obesity and family history of T2DM were a case group and obese subjects without a family history of T2DM were a control group. This study obtained the permission of the Medical and Health Research Ethics Committee of the Faculty of Medicine, Tadulako University based on a statement of ethical eligibility with number 1274/UN28.1.30/KL/2019 on March 6, 2019.

Anthropometry and subject screening

Determination of subjects in the case group and the control group or not included in the study group was adjusted to the inclusion and exclusion criteria that were known through direct examination and subject personal data. Determined BMI by measuring weight and height, assessed body composition using a body composition monitor (Karada Scan HBF-375, Omron) subjects used thin clothes without using shoes.

Laboratory examination

The subjects were fasted 8 h before venous blood collection, which was taken from the subject's anterior median venous vein. Subject blood testing consisted of fasting glucose levels measured using the chemical analysis method of hexokinase, and fasting insulin hormones were measured using enzyme-linked immunosorbent assays (ELISA) (Calbiotech).

Calculation of insulin resistance and β-cell function

The homeostatic assessment model of insulin resistance (HOMA-IR) was calculated for evaluation of insulin resistance, and the homeostatic model assessment of insulin secretion (HOMA-β) was calculated as basal insulin release.

DNA isolation and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

DNA isolation using Genomic DNA Mini Kit (Blood/Cultured Cell) (GeneAid) based on the protocol from the manufacturer. The DNA concentration is quantified using a spectrophotometer and was made into concentration of 100 ng/μL. Analysis of -866G/A genetic variation used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The DNA mixture used Taq Master Mix (GoTaq®Green Master Mix, Cat. No. M7122) was carried out with the addition of primary forward 5'- CACGCTGCTTCTGCCAGGAC-3' and reverse primer 5'-AGGCGTCAGGAGATGGACCG-3' then PCR was conducted with following conditions 95 °C for 5 min (initial denaturation), followed by 35 cycles of PCR (denaturation) at 95 °C for 30 s, annealing at 67 °C for 40 s, extensions at 72 °C for 50 s, final extension at 72 °C for 5 min and stored at 4 °C.

Cutting PCR products with RFLP used the Mlu1 restriction enzyme (ThermoFisher Scientific), incubated for 1 h at 37 °C. PCR products were analyzed in 3 % agarose gel with DNA ladder (Bioron,

Germany, Cat. 306009) by electrophoresis (Cosma Bio and Mupid-EXu Submarine Electrophoresis System). Electrophoresis results were seen in the UV transilluminator (Pro Logic 21 pro (UV light)) using GeneSys software.

Statistical analysis

Data was analyzed using IBM® SPSS® Statistics. The normality test of the data used the Kolmogorov Smirnov Test. Normal distributed data used unpaired t-test. If the data was not normally distributed, the Mann-Whitney U test was used, after log-transformation was performed. Descriptive analysis was used to determine the characteristics of the research subject. Chi-Square test was used to compare the genotypic frequency and allele -866G/A UCP2 gene between the case and control groups. Mann-Whitney U test to compare mean HOMA-IR and HOMA-β values between case and control groups. The Kruskall-Wallis test compared the mean values of HOMA-IR and HOMA-β among genotypic groups in the study subjects.

Results and discussion

The case group consisted of 19 men and 41 women, while the control group consisted of 20 men and 40 women. The abnormal data distribution was found at age, body weight (BW), body height (BH), body mass index (BMI), and visceral fat (VF) and after log-transformation normal distribution data was obtained for only visceral fat, while other distributed data remains abnormal. The results of the Mann-Whitney U test showed that there were no significant differences between age, body weight (BW), body height (BH) and BMI in the case and control groups. While data distribution was normal in fasting blood glucose (GDP), fasting insulin, subcutaneous fat (SF), total body fat (TBF), and skeletal muscle (BC). Results for difference in Independent samples t-test showed no significant differences in GDP, SF, TBF and SM in cases and controls. The characteristics of case and control groups are seen in **Table 1**.

Table 1 Characteristics of obese individuals with family history of type 2 diabetes mellitus (case) and without family history of type 2 diabetes mellitus (control).

Variabel	Case (n = 60)	Control (n = 60)	*p* (CI 95 %)
Age (years old)	20 (18 - 40)	20 (18 - 36)	0.575*
Sex (M/F)	19/41	21/39	0.699**
Body weight (kg)	66.5 (51.8 - 90.6)	64.0 (55.6 - 99.4)	0.741*
Body height (cm)	156 (142 - 175)	155 (142 - 179)	0.823*
IMT BMI (kg/m^2)	26.33 (25.01 - 36.17)	26.52 (25.02 - 34.39)	0.321*
GDP (mg/dL)	80.40 ± 12.63	83.12 ± 12.57	0.290#
Fasting Insulin (mU/mL)	6.99 ± 3.51	6.45 ± 2.97	0.420#
VF (%)	5.42 ± 4.27	5.09 ± 4.40	0.664*
SF (%)	22.14 ± 6.77	22.39 ± 7.16	0.844#
TBF (%)	26.65 ± 6.26	27.31 ± 7.18	0.595#
SM (%)	20.70 ± 6.12	20.66 ± 7.30	0.975#

Data was reported in the form of mean ± SD (standard deviation) or median (minimum - maximum) for data not normally distributed. 95 % CI: Confidence Interval. The data distribution was tested by Kolmogorov-Smirnov: $p > 0.05$: Data was normally distributed. #Independent samples t-test: $p < 0.05$: Significantly different.

The results of the genotype -866G/A examination of the UCP2 gene carried out with the polymerase chain reaction restriction length polymorphism (PCR-RFLP) can be seen in **Figure 1**. Obese individuals with a family history of type 2 diabetes mellitus and without a family history of type 2 diabetes mellitus were with found GG genotype (wild-type), GA genotype (heterozygous mutant) and AA genotype (homozygous mutant).

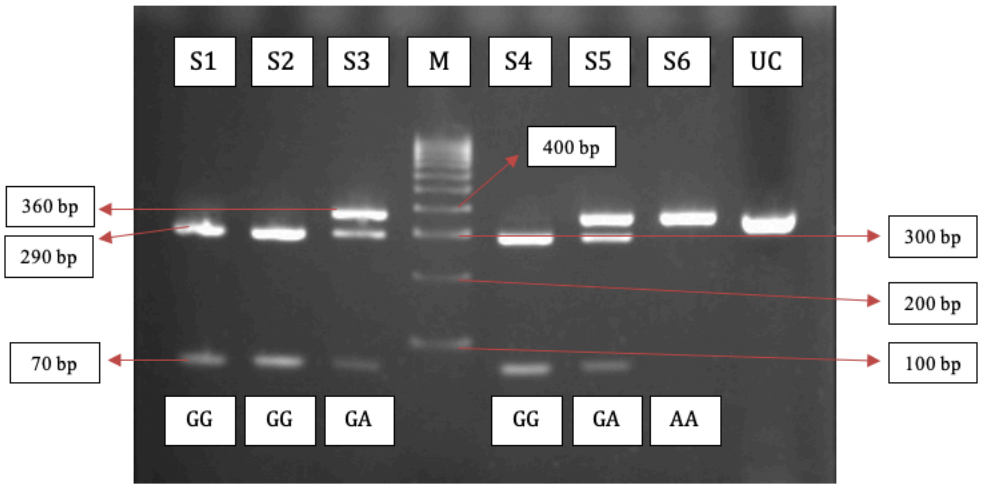

Figure 1 Genotyping results of genetic variations in -866G/A UCP2 gene. M = Marker; UC = uncut DNA; GG genotype = wild type genotype has a fragment length of 290 and 70 bp, GA genotype = heterozygous mutant with 360, 290 and 70 bp fragment length, and AA genotype = homozygous mutant with a 360 bp fragment length. (the arrow in the gel has been corrected and synchronized).

Genotypic frequency distribution (GG, GA, AA) and allele (G, A) of UCP2 genes in cases and controls can be seen in **Table 2**. The frequency of case genotypes is GG (43.3 %), GA (48.3 %) and AA (8.3 %) while the control was GG (46.7 %), GA (45 %) and AA (8.3 %). The allele frequency distribution in the case was G (67.5 %) and A (32.5 %) while the control was G (69.2 %) and A (30.8 %).

Table 2 Genotype frequency distribution (GG, GA, and AA) and alleles (G and A) UCP2 genes in obese individuals with family history of type 2 diabetes mellitus (cases) and without family history of type 2 diabetes mellitus (control).

Variable		Case (n = 60)	Control (n = 60)	OR (CI 95 %)	*p*
Genotype	GG	26 (43.4 %)	28 (46.7 %)		
	GA	29 (48.3 %)	27 (45 %)	OR 0.86 (0.40 - 1.82)	0.703*
	AA	5 (8.3 %)	5 (8.3 %)	OR 1.07 (0.27 - 4.15)	0.914*
Genotype	GG	26 (43.3 %)	28 (46.7 %)		
	GA+AA	34 (56.7 %)	32 (53.3 %)	OR 1.14 (0.55 - 2.35)	0.714*
Allel	G	81 (67.5 %)	83 (69.2 %)		
	A	39 (32.5 %)	37 (30.8 %)	OR 1.08 (0.2 - 1.86)	0.781*

*Chi-square test: $p < 0.05$: Significantly different.

Based on the results of Chi-Square analysis, the genotypes of GG, GA and AA in the 2 groups were not significantly different ($p \geq 0.05$; $p = 0.930$). Allele frequencies also showed results that were not significantly different in the 2 groups with a *p*-value = 0.781. The results of the odds ratio (OR) for genotypes and alleles showed less risky effects of the UCP2 gene. The genotypic odds ratio is 0.714 and for alleles is 0.781.

Table 3 Genotype frequency distribution (GG, GA and AA) and alleles (G and A) UCP2 genes in obese individuals with family history of type 2 diabetes mellitus (cases) and without family history of type 2 diabetes mellitus (control) in female subjects.

Variable		Case (n = 41)	Control (n = 39)	OR (CI 95 %)	*p*
Genotype	GG	19 (46.3 %)	16 (41.0 %)		
	GA	17 (41.5 %)	20 (51.3 %)	OR 1.39 (0.55 - 3.53)	0.479*
	AA	5 (12.2 %)	3 (7.7 %)	OR 1.40 (0.29 - 6.80)	0.673*
Genotype	GG	19 (46.3 %)	16 (41.0 %)		
	GA + AA	22 (53.7 %)	23 (59.0 %)	OR 0.80 (0.33 - 1.95)	0.632*
Allel	G	55 (67.0 %)	52 (66.6 %)		
	A	27 (33.0 %)	26 (33.3 %)	OR 0.98 (0.50 - 1.89)	0.956*

*Chi-square test: $p < 0.05$: Significantly different.

Table 4 Genotype frequency distribution (GG, GA, and AA) and alleles (G and A) UCP2 genes in obese individuals with family history of type 2 diabetes mellitus (cases) and without family history of type 2 diabetes mellitus (control) in male subjects.

Variable		Case (n = 19)	Control (n = 21)	OR (CI 95 %)	*p*
Genotype	GG	7 (36.8 %)	12 (57.1 %)		
	GA	12 (63.2 %)	7 (33.1 %)	OR 0.34 (0.09 - 1.27)	0.105*
	AA	0 (0 %)	2 (9.5 %)	OR 1.16 (0.94 - 1.44)	0.293*
Genotype	GG	7 (43.3 %)	12 (46.7 %)		
	GA + AA	12 (56.7 %)	9 (53.3 %)	OR 2.28 (0.64 - 8.14)	0.199*
Allel	G	26 (67.5 %)	31 (69.2 %)		
	A	12 (32.5 %)	11 (30.8 %)	OR 1.30 (0.49 - 3.43)	0.595*

*Chi-square test: $p < 0.05$: Significantly different.

Tables 3 and **4** represent genotypes (GG, GA and AA) and alleles (G and A) in groups with a family history of T2DM and without a history of T2DM after gender stratification.

The proportion of GA genotypes in female subjects in the control group was greater than in groups with a history of T2DM. In female subjects with a family history of T2DM GG genotype was more in the control group. Genotypic frequency distribution in the female subjects of the 2 groups were not significantly different ($p \geq 0.05$; $p = 0.622$). The frequency of GG genotype in the UCP2 gene in the group of female subjects with a family history of T2DM was 46.3 % and in the control group it was 41.1 %. In female subjects GA genotype frequency for the control group was 51.3 % and groups with family history of T2DM was 41.5 %. The distribution of UCP2 gene alleles in female subjects in groups with family history of T2DM and the control group did not differ significantly with $p = 0.956$. The frequency of G allele was more common in groups with a family history of T2DM of 67 % compared to the control group at 66.6 %. The frequency of A allele was found in the control group at 33.3 and 33 % in the group with a family history of T2DM. The odds ratio of 0.80 for genotypes showed a less risky effect of the UCP2 gene towards DMT2 and the odds ratio of 0.98 (95 % KI 0.50 - 1.89) on the allele showed less risk of the influence of the UCP2 gene towards DMT2.

In male subjects, the GG genotype frequency in the control group was more than the group with a history of T2DM. In male subjects with a family history of GA genotype T2DM were more than the control group. The genotypic frequency distribution of male subjects in the 2 groups was not significantly different ($p \geq 0.05$; $p = 0.103$). The genotype frequency of the GA UCP2 gene in the male subject group with a family history of T2DM was 63.2 % and in the control group was 33.1 %. In male subjects the GG genotype frequency control group was 57.1 % and the group with DMT2 family history was 36.8 %. The distribution of UCP2 gene alleles in male subjects in groups with a family history of T2DM and the control group did not differ significantly with a value of $p = 0.595$. The frequency of G allele was more common in the control group at 69.2 %, in groups with a family history of T2DM of 67.5 %. The

frequency of allele A was found in groups with a family history of T2DM of 32.5 and 30.8 % in the control group. The odds ratio of 2.28 for genotypes showed significant effect of the UCP2 gene towards DMT2 and the odds ratio of 1.30 (95 % KI 0.49 - 3.43) on the allele showed a less significant influence of the UCP2 gene towards DMT2. Functional polymorphism can affect gene expression and regulate the amount of final protein in certain tissues [8].

The mean HOMA-IR in cases and controls can be seen in **Table 5**. The mean HOMA-IR in obese individuals with a family history of type 2 diabetes mellitus is higher than obese individuals without a family history of type 2 diabetes mellitus but not significantly different between groups ($p = 0.583$).

Table 5 HOMA-IR difference between case subjects and control subjects.

Variable	Case (n = 47)	Control (n = 49)	*p*
HOMA-IR	1.36 (0.06 - 2.00)	1.34 (0.42 - 2.00)	0.583

Data was reported in the form of a median (minimum - maximum) for data not normally distributed. Data distribution was tested with Saphiro-Wilk: $p > 0.05$: Data was normally distributed. *The Mann-Whitney U test after data transformation was unsuccessful: $p < 0.05$: Significantly different.

Table 6 Difference in HOMA-IR between case subjects and control subjects in women and men.

Variable	Case (n = 33)	Control (n = 30)	*p*
Female			
HOMA-IR	1.42 (0.06 - 2.00)	1.34 (0.42 - 1.98)	0.283*
Variable	**Case (n = 15)**	**Control (n = 19)**	***p***
Male			
HOMA-IR	1.19 ± 0.69	1.33 ± 0.42	0.463#

Data was reported in the form of a mean ± SD (standard deviation) or median (minimum - maximum) for data not normally distributed. Data distribution was tested with Saphiro-Wilk: $p > 0.05$: Data was normally distributed. *The Mann-Whitney U test after data transformation was unsuccessful: $p < 0.05$: Significantly different. #Independent Samples t-test: $p < 0.05$: Significantly different.

The mean HOMA-IR in obese female subjects with a family history of T2DM was higher (1.42) compared to obese female individuals without a family history of T2DM (1.34) but statistically the 2 groups were not significantly different ($p = 0.283$). HOMA-IR values in obese male subjects without a history of T2DM were higher (1.33 ± 0.42) compared to obese male individuals with a family history of T2DM (1.19 ± 0.69) but statistically the 2 groups were not significantly different ($p = 0.463$).

The mean HOMA-β in cases and controls can be seen in **Table 7**. The mean HOMA-β in obese individuals without a family history of T2DM was lower than for obese individuals with a family history of T2DM but statistically the 2 groups were not significantly different ($p = 0.100$).

Table 7 HOMA-β difference between case subjects and control subjects.

Variable	Case (n = 47)	Control (n = 49)	*p*
HOMA-β	102.60 (12.1 - 282.8)	85.7 (19.9 - 219.8)	0.100*

Data was reported in the form of a median (minimum - maximum) for data not normally distributed. Data distribution was tested with Saphiro-Wilk: $p > 0.05$: Data was normally distributed. *The Mann-Whitney U test after data transformation was unsuccessful: $p < 0.05$: Significantly different.

Table 8 Difference in HOMA-β between case subjects and control subjects in women and men.

Variabel	Case (n = 33)	Control (n = 30)	*p*
Female			
HOMA-β	121.94 ± 63.87	91.89 ± 51.51	0.04[#]
Variabel	**Case (n = 15)**	**Control (n = 19)**	***p***
Male			
HOMA-β	114.98 ± 87.76	96.30 ± 55.49	0.454[#]

Data was reported in the form of a median (minimum - maximum) for data not normally distributed. Data distribution was tested with Saphiro-Wilk: $p > 0.05$: Data was normally distributed. *The Mann-Whitney U test after data transformation was unsuccessful: $p < 0.05$: Significantly different.

The mean HOMA-β in obese female subjects without a family history of DMT2 was lower (91.89 ± 51.51) compared to obese female subjects with a family history of T2DM (121.94 ± 63.87) and statistically the 2 groups were significantly different ($p = 0.04$). This result meant that family history of T2DM is significantly related to HOMA-β in women. The HOMA-β values in obese male subjects without a history of T2DM was also lower (96.30 ± 55.49) compared to obese male individuals with a family history of T2DM (114.98 ± 87.76) but statistically the 2 groups did not differ significantly ($p = 0.454$).

The assessment of HOMA-IR distribution was carried out by the Test of Normality Shapiro-Wilk found that the data was not normally distributed. The mean HOMA-IR in GG, GA and AA genotypes can be seen in **Table 9**. The mean HOMA-IR in subjects carrying AA genotypes was higher than GG and GA genotype readers but not statistically significant ($p = 0.986$).

Table 9 Mean difference of HOMA-IR Value between GG, GA and AA genotypes in research subjects.

Variable	GG (n = 41)	GA (n = 46)	AA (n = 9)	*p*	GA+AA (n = 55)
HOMA-IR	1.39 (0.06 - 1.98)	1.27 (0.09 - 2.00)	1.50 (0.58 - 1.98)	0.986[*]	1.27 (0.09 - 2.00) ($p = 0.937$) [#]

Data was reported in the form of a median (minimum - maximum) for data not normally distributed. Data distribution was tested by Shapiro-Wilk: $p > 0.05$: Data was normally distributed. *Kruskal-Wallis test: $p < 0.05$: Significantly different. #Independence samples t-test: $p < 0.05$: Significantly different.

The assessment of HOMA-β distribution was carried out by the Test of Normality Shapiro-Wilk of data was not normally distributed. The mean HOMA-β in the GG, GA and AA genotypes can be seen in **Table 9**. The mean HOMA-β in subjects carrying the GA genotype was lower than the GG and AA genotype but not statistically significant ($p = 0.357$).

Table 10 Mean difference of HOMA-β Value between GG, GA and AA genotypes in research subjects.

Variable	GG (n = 41)	GA (n = 46)	AA (n = 9)	*p*	GA+AA (n = 55)
HOMA-β	107.25 (12.6 - 282.8)	81.75 (12.1 - 273.9)	117.32 ± 64.50	0.357[*]	102.20 (12.1 - 273.9) ($p = 0.416$)[#]

Data was reported in the form of a median (minimum - maximum) for data not normally distributed. Data distribution was tested by Shapiro-Wilk: $p > 0.05$: Data was normally distributed. *Kruskal-Wallis test: $p < 0.05$: Significantly different. #Independence asmples t-test: $p < 0.05$: Significantly different.

Discussion

Uncoupling protein 2 (UCP2) which is the main regulating protein for energy balance. UCP2 activity high in skeletal muscle when increased can suppress insulin secretion and suppress thermogenesis and increase lipid use so that increased lipid oxidation causes peripheral insulin resistance. Another UCP2 function is regulating insulin secretion by β-pancreatic cells. Insulin secretion by β-pancreatic cells depends on ATP. ATP resulting from coupling of oxidative phosphorylation and ATP synthesis will increase the ATP/ADP ratio which causes closure of K-ATP channels in the plasma membrane of β-pancreatic cells. As a result, β-pancreatic cells are depolarized. This depolarization is followed by the opening of the Ca^{2+} channel which causes the entry of Ca^{2+} ions into β-pancreatic cells. Ca^{2+} ion then stimulates insulin secretion by β-pancreatic cells. By reducing ATP production through oxidative phosphorylation from ATP synthesis, UCP2 has an important role in inhibiting insulin secretion by β-pancreatic cells [6,9,10].

Uncoupling protein 2 separates oxidative phosphorylation from ATP synthesis, with energy dissipated as heat. UCP2 facilitates the transfer of anions from the mitochondrial inter membrane space to the mitochondrial matrix. With uncoupling of oxidative phosphorylation from ATP synthesis, UCP2 decreases ATP production [1,10,11]. UCP2 is important in regulating insulin secretion which stimulates glucose and protects mitochondria from oxidative damage so that small changes in UCP2 expression will significantly affect UCP2 function. The results acquired in this research showed that the AA genotype in both groups had the same frequency, which meant obese individuals with and without a family history of T2DM had the same amount of AA genotype. Difference in geography, ethnic, environmental factors, and specific genetic backgrounds could contribute towards the variation of genotypes and allele frequency in a certain population. Hence polymorphism of the UCP2 gene becomes a T2DM risk factor for certain populations but not for other populations.

Zhang *et al.* [12] reported that UCP2 gene KO rats experienced decreased levels of UCP2 mRNA and resulted in increased ATP concentrations, increased insulin secretion which stimulated glucose, decreased blood glucose levels, and higher serum insulin compared to wild type rats. Meanwhile, obese mice, experienced pancreatic dysfunction, increased levels of UCP2 mRNA and protein, blood glucose levels, and hyperinsulinemia. This shows that changes in UCP2 gene expression have contributed to regulating insulin secretion which stimulates glucose [6,9,10].

The incidence of obesity and insulin resistance is associated with the presence of TNF-α which will increase the expression of UCP2, the regulator of mitochondria which increases the leakage of protons across the inner membrane to separate respiration from ATP synthesis and reduce generation of ROS. TNF-α has an important role in increasing ROS production observed in steatotic liver. The key components of TNF-α signaling include cramps, which affect the mitochondrial electron transport chain and generate excess hydrogen oxidation. Another potential trigger for cell death caused by TNF-α is the release of mitochondrial respiration. Regulated UCP2 can endanger cellular ATP levels and worsen liver damage by increasing cell death, or may be protective by reducing the level of ROS [6,9,10]. The results in this research showed a higher HOMA-IR value in subjects with a history of T2DM compared to subjects without a history of T2DM, but based on statistical analysis were not significantly different (p = 0.583). Higher HOMA-IR value in subjects with a history of T2DM could indicate the beginning of endothelial dysfunction.

Polymorphism in the UCP2 gene promoter region, -866G/A polymorphisms in the UCP2 gene affect UCP2 transcriptional activity. The INS-1 β cell line infected with vectors containing UCP2 promoter DNA fragments with allele -866A was reported to have higher promoter activity compared to INS-1 β cell lines infected with vectors containing UCP2 DNA promoter fragments with alleles - 866G [12]. The consequence of changes in UCP2 gene transcription activity is modulation of insulin secretion by β-pancreatic cells. Higher promoter activity in DM patients with the -866A allele in Japan produces lower insulin secretion compared to patients with allele -866G [13]. The same in individuals with -866A/A had lower insulin secretion than individuals with -866G/A or -866G/G. This is evidenced by measuring insulin secretion of isolated pancreatic islands, where individuals with genotype -866A/A have lower insulin secretion [14].

Some of the hereditary factors of parents with type 2 diabetes mellitus are only either father or mother, parents do not carry the UCP2 gene as a risk factor for type 2 diabetes mellitus, and the possibility of other type 2 diabetes mellitus candidate genes is a factor risk from parents. Differences in geographical location, ethnicity, environmental factors, specific genetics and genetic background are the causes of variations in genotypic frequency and alleles in each population. Therefore, UCP2 gene polymorphism in some populations is a risk factor for DMT2 but in other populations it is not a risk factor for T2DM [6,9,15].

Previous studies with genotype analysis and further clinical features showed that subjects with TT genotype rs649446 or subjects with AA rs7109266 genotype at UCP2 had higher HOMA-IR and HOMA-β, subjects with AA rs7109266 genotype also had more waist-hip ratios high, which suggests that subjects with TT genotype rs649446 or subjects with genotype AA rs7109266 are more susceptible to developing insulin resistance [16]. Previous studies have shown that human islets with genotype GA UCP2-866 polymorphisms have reduced glucose-stimulated insulin secretion compared with GG. However, the pathway between UCP2 polymorphism and the HOMA index has not been clearly described [17].

In this study the AA genotype had a higher insulin secretion which meant an association with the response to an increase in insulin resistance, β-cells secrete insulin in a normal physiological state. But individuals which have decreased β-cell function and in cases of severe but stable insulin resistance causing hyperglycemia can result in increased insulin demand. There is a change in β-cell function that causes β-cell hyperplasia [15]. The pathogenesis of β-cell damage is based on an increase in the entry of glucose and fatty acids. Increased glucose and fatty acid metabolism can cause insulin resistance and interfere with β-cell function. The cells show resistance to the effects of insulin because insulin receptors become less responsive to insulin which results in increased insulin release from β-cells and causes hyperinsulinemia [15].

Some hereditary factors of parents who have type 2 diabetes mellitus only one of them from the father or mother, parents do not carry the UCP2 gene as a risk factor for type 2 diabetes mellitus, and the possibility of other genes of type 2 diabetes mellitus candidates being a factor risk from parents. Differences in geographical location, ethnicity, environmental factors, specific genetic and genetic background are the causes of variations in genotype and allele frequencies in each population. Therefore, UCP2 gene polymorphism in some populations is a risk factor for DMT2 but in other populations it is not a risk factor for DMT2 [6,9,16].

The BMI group that developed diabetes showed different levels of obesity, visceral fat, HOMA-β, and HOMA-IR. Asians develop T2DM at lower obesity rates compared to Caucasians. The heterogeneity in the development of obesity before the onset of diabetes in Asians is poorly understood. Factors such as diabetes status, gender, obesity status, and race were found to have a significant correlation between adipose tissue depot/obesity index and insulin resistance, as measured by HOMA. Visceral fat is very important, because it has a strong correlation with the incidence of metabolic syndrome and oxidative stress. Visceral fat is the main place for the imbalance of adipokines and FFA action. Reducing visceral fat has substantial benefits on metabolism and reduces the incidence of insulin resistance and the effects of insulin resistance [17]. In this study it was found that the mean visceral fat was higher in cases than controls so that it could be concluded that obese individuals with a family history had greater insulin resistance than those without a family history, but statistically the results were not significantly different.

Previous research with genotype analysis and further clinical features showed that subjects with the genotype TT rs649446 or subjects with AA rs7109266 genotype on UCP2 had higher HOMA-IR and HOMA-β, subjects with AA genotype rs7109266 also had a higher waist-hip ratio high, which suggests that subjects with TT genotype rs649446 or subjects with AA genotype rs7109266 are more prone to develop insulin resistance [18]. Previous studies have shown that human islet with GA genotype UCP2-866 polymorphism has decreased glucose-stimulated insulin secretion compared with GG. However, the path between the UCP2 polymorphism and the HOMA index has not been clearly described [19].

Conclusions

Based on the results of research that has been conducted, it can be concluded that obese individuals with a family history of T2DM have a higher risk of GA + AA genotype and A allele than individuals without a family history of T2DM. The HOMA-IR and HOMA-β values in AA genotype subjects were significantly higher but HOMA-IR and HOMA-β values were not statistically significant between GG, GA, and AA genotypes.

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