**CAPN10 SNP-19 is Associated with Susceptibility of Type 2 Diabetes Mellitus: A Javanese Case-control Study**

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**Abstract**

**BACKGROUND:** The health data of Central Java, Indonesia showed that diabetes mellitus (DM) was the second most increasing non-communicable disease in the province. More than 20 genes have been reported to be associated with DM. *Calpain-10 (CAPN10)* polymorphism has been reported to be associated with Type 2 Diabetes Mellitus (T2DM). However, the association between *CAPN10 single nucleotide polymorphism (SNP)-19* and T2DM among Javanese ethnics has never been reported. Therefore, this study was conducted to investigate the association.

**METHODS:** After fasting for 8 hours, blood samples were drawn from veins of 107 T2DM and 107 non-diabetic subjects. A half of the drawn blood was collected for identification of *CAPN10 SNP-19*, and another half for measuring triglycerides and fasting blood glucose (FBG). Identification of *CAPN10 SNP-19* was performed with polymerase chain reaction (PCR) method, while measurement of triglycerides and FBG with colorimetric enzymatic method.

**RESULTS:** The number of T2DM Javanese subjects with 2R/3R and 3R/3R *CAPN10 SNP-19* genotypes was significantly higher than the number of T2DM Javanese subjects with 2R/2R genotype (*p*=0.002). When each number of 2R/3R and 3R/3R T2DM subjects was compared with the number of 2R/2R T2DM subjects, the number of 2R/3R T2DM subjects was significantly higher than the number of 2R/2R T2DM subjects (*p*=0.000).

**CONCLUSION:** Javanese subjects with 2R/3R and 3R/3R *CAPN10 SNP19* genotypes might have susceptibility of T2DM.

**KEYWORDS:** Calpain-10, *CAPN10*, polymorphism, type 2 diabetes mellitus, triglycerides, fasting blood glucose

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**Introduction**

Indonesian lifestyle is continuously changing, which causes the shift from infectious to degenerative diseases, such as diabetes mellitus (DM). DM is metabolic disorder characterized by progressive loss or dysfunction of pancreatic β cell.(1) The health data of Central Java, Indonesia in 2016 showed that DM was the second most increasing non-communicable disease, after hypertension, with a rate of 16.42%. In 2006, 972 new DM cases were found. Its causes were attributed to age, obesity, fat distribution, lack of physical activity, and genetic factors. (2) More than 20 genes have been reported to be associated with DM. One of the genes is *Calpain-10 (CAPN10)*, which is located on chromosome 2q37.3 and encodes CAPN10 protein. A previous study showed that *CAPN10* polymorphism was associated with Type 2 DM (T2DM). (3,4) Calpain-10 was reported to play a role in the insulin vesicle exocytosis process of pancreatic β cell and insulin-stimulated Glucose Transporter 4 (GLUT4) translocation of muscle cell and adipocyte.(5)
Insulin resistance, a condition where targeted cells fail to respond to the insulin stimulus under normal concentration, which can be occurred among patients with T2DM. There is also metabolism dysfunctional among these patients. Decrease in insulin occurred in the fat tissues, leading to a decrease in lipogenesis and an increase in lipolysis. Then this will lead to the dyslipidemia, condition marked by the increase of triglycerides and Low-Density Lipoprotein (LDL), with a decrease in High-Density Lipoprotein (HDL).(6)

Some studies proved that *CAPN10* polymorphism increased the risk of insulin resistance and the level of free fatty acid (FFA). Obese subjects with *CAPN10 single nucleotide polymorphism (SNP)*-43 and G/G genotype were reported to have significant triglyceride level.(7) High level of plasma saturated fatty acids and low glucose effectiveness were also found in metabolic syndrome subjects with G/G genotype.(8) A research on normoglycemic individuals proved that subjects with *CAPN10 SNP-19* or Del/Ins 19 (rs3842570) consisting of 2R/3R and 3R/3R genotypes had significant lower HDL-C levels compared to the 2R/2R genotype.(9) T2DM subjects with homozygous 3R/3R genotypes had a significant higher cholesterol level.(10) *CAPN10 SNP-19* occurred when 2 or 3 repetitions of 32 bp in intron-6.

The involvement of *CAPN10 SNP-19* towards the triglycerides level of Javanese with T2DM has never been reported. Data from Survey of *Aspek Kehidupan Rumah Tangga Indonesia* (Sakerti) or Indonesia Family Life Survey (IFLS) in 2007 showed that Javanese and Madurese ethnic groups had the highest rates of diabetes in Indonesia, by 78.4%.(11) Therefore, this study was conducted to investigate the association between *CAPN10 SNP-19* and T2DM among Javanese ethnics.

### Methods

#### Subject Selection and Sample Collection

A case control study was conducted in 107 T2DM and 107 non-diabetic selected subjects. All subjects were recruited from the Primary Health Care Centre in Semarang. The inclusion criteria were Javanese ethnic, 30-65 years old and having no history of cardiovascular disease. Prior to the selection, all subjects signed the informed consent. After fasting for 8 hours, 5 mL of blood samples were drawn from veins of the selected subjects. A half of the drawn blood was collected for identification of *CAPN10 SNP-19*, while another half for measuring triglycerides and fasting blood glucose (FBG). The study protocol was approved by Ethics Committee of Faculty of Medicine, Universitas Muhammadiyah Semarang (No. 064/EC/FK/2019).

#### Identification of *CAPN10 SNP-19*

DNA was isolated from the whole blood using GeneJET Genomic DNA Purification Kit (Catalog #K0721, Thermo Fisher Scientific, Waltham, MA, USA). The identification of *CAPN10 SNP-19* was conducted by peqSTAR 2X Thermocyclers (VWR, Lutterworth, UK) with DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific). Based on previous report, forward primer 5'-GGTGTGTCTTCCAGCGTAGG-3' and reverse primer 5'-ATGAACCTGGCAGGGTCTAAG-3' were used.(12) PCR was set as follows: the pre-denaturation condition at 95°C for 4 min, the 35 cycling condition of 95°C for 1 min, 60°C for 30 sec, and 72°C elongation for 1 min, and the final extension at 72°C for 10 min. PCR product was visualized with 2% of agarose gel. The electrophoresis result showed 155 bp of allele 2R (2 repeats of 32 bp/wild type) and 187 bp of allele 3R (3 repeats of 32 bp/variant of *CAPN10 SNP-19*).

#### Triglycerides and FBG Measurements

Serum of the blood was measured using Triglycerides FS (DiaSys Diagnostic Systems, Waterbury, CT, USA) and Glucose GOD FS (DiaSys Diagnostic Systems) based on the colorimetric enzymatic method with glycerol-3-phosphate-oxidase and glucose oxidase. Procedure and calculation were performed according to the kit inserts. Briefly, serum was mixed with each provided reagent, homogenized and incubated at room temperature for 10 min. Then the reaction was read with spectrophotometer at absorbance of 500 nm within 60 min.

#### Statistical Analysis

Data was calculated using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). Independent T and Chi-square tests were applied based on variable scale. The *p*<0.05 was set as significant.

### Results

Subject characteristics of T2DM and control subjects are shown in Table 1. Age, body mass index (BMI), systolic blood pressure (SBP), triglycerides and FBG of T2DM subjects were significantly (*p*<0.05) higher than the control subjects (Table 1).
Table 1. T2DM (n=107) and control (n=107) subject characteristics.

| Variable             | Control  | T2DM     | p-value* |
|----------------------|----------|----------|----------|
| Age (year)           | 30-60    | 40-69    | 0.000    |
| Gender (n)           |          |          |          |
| Male                 | 33       | 35       | -        |
| Female               | 74       | 72       | -        |
| BMI (kg/m²)          | 15.83-35.41 | 18.90-36.52 | 0.017    |
| SBP (mm/Hg)          | 82-158   | 100-200  | 0.000    |
| DBP (mm/Hg)          | 57-114   | 47-128   | 0.113    |
| Triglycerides (mg/dL)| 4.70-264.28 | 97.87-310.09 | 0.000    |
| FBG (mg/dL)          | 62-123   | 60-374   | 0.000    |

*Analyzed with Independent T-test.

PCR electrophoresis results are shown in Figure 1. Each C and D lane showed a 155 bp band, suggesting a wild-type homozygous (2R/2R) genotype. Each M, N, O and P lane showed a 187 bp band, suggesting a variant homozygous (3R/3R) genotype. Meanwhile, each A, B, E, F, G, H, I, J, K and L lane showed 2 bands, 155 bp and 187 bp, suggesting a variant heterozygous (2R/3R) genotype. In the T2DM group, the highest genotype frequency was the 2R/3R genotype with a total of 84 subjects (79%).

The number of T2DM Javanese subjects with 2R/3R and 3R/3R CAPN10 SNP-19 genotypes was significantly higher than the number of T2DM Javanese subjects with 2R/2R genotype (p=0.002) (Table 2). When each number of 2R/3R and 3R/3R T2DM subjects was compared with the number of 2R/2R T2DM subjects, the number of 2R/3R T2DM subjects was significantly higher than the number of 2R/2R T2DM subjects (p=0.000) (Table 3). Meanwhile, the number of 3R/3R T2DM subjects was not significantly higher than the number of 2R/2R T2DM subjects.

T2DM and control subjects characteristics as shown in Table 1 were grouped based on the 2R/2R and 2R/3R + 3R/3R genotypes. However, all parameters including age, BMI, SBP, diastolic blood pressure (DBP), triglycerides and FBG, did not show any significant difference between the groups (Table 4). Although not significant, BMI, triglycerides and FBG of 2R/3R + 3R/3R group of T2DM subjects were higher than the BMI, triglycerides and FBG of 2R/2R group of T2DM subjects. In accordance, triglycerides and FBG of 2R/3R + 3R/3R group of control subjects were also higher than the triglycerides and FBG of 2R/2R group of control subjects, although not significant.

Discussion

CAPN10 polymorphism has been reported to have an association with the vulnerability of T2DM. The retardation of CAPN10 is capable of decreasing insulin secretion in pancreatic cells.(13) A study on pancreatic islet of CAPN10 knockout mice proved that the diminished of CAPN10 altered insulin secretion through microtubule associated protein 1 (MAP1) family.(14) CAPN10 cleavage MAP1 family protein into heavy and light chain so enhances its function to coordinate microtubule and actin filament. Decreased of
Table 2. The differences among the control and T2DM subjects number based on *CAPN10 SNP-19* genotypes.

| Variable          | Subject | Control | T2DM   | \(p\)-value* | OR   | 95% CI      |
|-------------------|---------|---------|--------|--------------|------|-------------|
|                   |         | Lower   | Upper  |              |      |             |
| 2R/3R + 3R/3R     | 81 (75.70%) | 98 (91.59%) | 0.002  | 3.495 | 1.550 | 7.882      |
| 2R/2R             | 26 (24.30%) | 9 (8.41%)    |        |      |      |            |

*Analyzed with Chi-square test. OR: odd ratio, CI: confidence interval.

CAPN10 function effects of actin reorganization and insulin secretion from β cell.

Current results showed that there was a significant difference between the number of T2DM Javanese subjects with 2R/3R and 3R/3R *CAPN10 SNP-19* genotypes than the number of T2DM Javanese subjects with 2R/2R genotype. This results suggested that *CAPN10 SNP-19* genotypes was related to the incidence of T2DM. This finding was in line with a previous study on the Tunisian Arabs which showed that T2DM subjects 2R/3R genotype had significant higher risk than the T2DM subjects with 2R/2R genotype.(12) Besides in Tunisia, another study in Mexico suggested that this polymorphism contributed in the incidence of T2DM as well.(15)

However, there was no association between the *CAPN10 SNP-19* and the T2DM on Minangkabau ethnic group, even though this ethic is a part of Indonesia.(16) Another study in Gaza reported that the most frequent genotype of *CAPN10 SNP-19* was 3R/3R.(10) Similar to Minangkabau, study in Gaza also showed no significant association between the *CAPN10 SNP-19* and T2DM.

T2DM is not only caused by polymorphism factor, but also by lifestyle, gender and obesity.(14) Those suffering from this disease have dyslipidemia causing chronic metabolic disorders characterized by abnormal cytokine production, increased acute phase reactants, mediators, and activation of inflammatory factors.(17) Insulin resistance of T2DM is associated with high triglycerides and low HDL-C level although the relationship were still weak.(18) A study among population in China (Kazak, Ugyur and Han ethnic) also found that there was a relationship between triglycerides level and insulin resistance.(19) Insulin resistance seems to be closely related with inflammation caused by dietary fatty acids. It was hypothesized that there was accumulation of diacylglyceride (DAG) that inhibit insulin signaling and enhance endoplasmic reticulum stress and oxidative stress. (20) High fatty acid (especially Arachidonic acid/AA) produce 12-HETE which is very toxic to beta pancreas and leads to cell destruction.(21)

Current study also analyzed the association between *CAPN10 SNP-19* with the level of FBG and triglycerides. The results showed FBG and triglycerides of both control and T2DM subjects with 2R/3R + 3R/3R genotypes were higher than the subjects with 2R/2R genotype, even though not significant. Another study showed that both FBG and triglycerides level didn't show a significant difference in each *CAPN10 SNP-19* genotype.(22) However, other study noted that subjects with 2R/2R and 2R/3R genotypes had a significantly higher concentration of triglycerides than subjects with 3R/3R genotype.(23)

The variation of *CAPN10* has the ability to decrease the function of β3 adrenoceptor and adipose cells of obese people.(24) The genetic variation of *CAPN10* which was successfully identified to affect triglycerides level in obese people was *SNP-43*. (6) The obese subjects with *SNP-43 G/G* had a significant lower concentration of triglycerides.

Table 3. The differences among the control and T2DM subjects number based on *CAPN10 SNP-19* genotypes.

| Genotype | Subject | Control | T2DM   | \(p\)-value* | OR   | 95% CI      |
|----------|---------|---------|--------|--------------|------|-------------|
|          |         | Lower   | Upper  |              |      |             |
| 2R/2R    | 26 (24.30%) | 9 (8.41%)    |        |      |      |            |
| 2R/3R    | 40 (37.38%) | 84 (78.50%) | 0.000  | 6.067 | 2.602 | 14.142     |
| 3R/3R    | 41 (38.32%) | 14 (13.09%) | 0.978  | 0.986 | 0.374 | 2.605      |

*Analyzed with Chi-square test against 2R/2R genotype. OR: odd ratio, CI: confidence interval..
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