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# ABCC8 exon 16-3 C>T polymorphism is associated with insulin secretion in patients with type 2 diabetes in Yogyakarta

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**Abstract**

**Background** ABCC8 gene plays a central role in insulin secretion, hence its potential as the candidate genetic risk factor for development of type 2 diabetes mellitus (T2DM). The polymorphism of ABCC8 exon 16 -3 C>T has been investigated in various populations with inconsistent result.
**Objective** The study aimed is to explore the association of ABCC8 exon C>T polymorphism in T2DM among Yogyakarta population
**Method** A number of 40 T2DM patients and 44 non T2DM patients were recruited as case and control subjects respectively. The polymorphism was genotyped using PCR-RFLP. The association between insulin secretion and the polymorphism was analyzed with ANOVA and post hoc test. Chi-square and odds ratio (OR) were calculated to determine whether the polymorphism was a risk factor of T2DM.
**Results** The research data was obtained from 84 patients, divided as 40 individuals as case subjects and 44 individuals as control subjects. There was a significant difference between CC and CT genotypes among the patients with T2DM (p=0,005). Fasting insulin concentration exhibited a significant difference between CC and TT genotypes among T2DM subjects (p=0,013). Allele T frequency was found to be 0,45 among T2DM subjects and 0,48 among non T2DM subjects, there was no significant difference of allele T frequency between T2DM and control subjects (p=0,723).
**Conclusion** ABCC8 exon 16 -3 C>T polymorphism was not associated with T2DM prevalence in Yogyakarta population, however the impact of the polymorphism on diabetes pathogenesis in the disease cannot be excluded because of the association with insulin secretion in T2DM subjects.

**Keywords:** type 2 diabetes mellitus, ABCC8 gene, genetic polymorphism, Homeostasis Model Assessment,

## Introduction

Diabetes mellitus type 2 is defined as a group of metabolic disorder characterized by hyperglycemia caused by defect in insulin secretion, action, or both.[1] It is estimated that 9% of worldwide population of 18 years and above suffered from DM, the disease has caused 1,5 million mortality in 2012 and is a major contributor of cardiovascular disease.[2] It is predicted in Indonesia that the number of people with DM type 2 will increase from 8,4 million in 2000 to 21,3 million in 2030. DM prevalence in urban area is estimated to be 14,7% and 7,2% in rural area during 2003 that will elevate to 12 million in urban and 8,1 in rural area in 2030.3

The Adenosine Triphosphatase (ATP)-sensitive Potassium (K+) channel (KATP) regulates insulin secretion by coupling intracellular metabolic activity to membrane potential.[4] In turn, KATP channel activity is determined by the presence of intracelluler nucleotides (ATP and Magnesium-Adenosine Diphosphate (MgADP)) generated by β-cell metabolism.[5] KATP channel is composed of 4 Kir6.2 subunits forming the calcium canal and 4 SUR1 subunits which enclose the Kir6.2. ATP binding to Kir6.2 closes the KATP channel which will lead to Ca2+ influx, inducing the release of insulin granules from β-cell pancreas. In contrast SUR1 subunit that binds to MgADP will open the KATP channel, therefore preventing Ca2+ accumulation and reducing insulin secretion.[6]

SUR1 subunit consists of 3 transmembrane domains (TMD) connected by nucleotide-binding domains (NBD), the aforementioned subunit is linked to Kir6.2 through an

***Table 1.*** *Baseline subjects characteristics*

| Characteristics | T2DM (n=40) | Non T2DM (n=44) | p-value |
|---|---|---|---|
| Sex (n (%)); male | 28 | 29 | 0.688 |
| Age (year); mean ± SD | 52.03 ± 6.74 | 48.16 ± 5.73 | 0.006* |
| BMI (kg/m2); mean | 24.63 | 24.48 | 0.964 |
| SBP (mmHg); mean | 125.00 | 120.00 | 0.083 |
| DBP(mmHg); mean | 80.00 | 80.00 | 0.495 |
| Total cholesterol (mg/dL); mean | 169.33 | 178.32 | 0.284 |
| HDL (mg/dL); mean | 45.71 | 47.86 | 0.532 |
| LDL (mg/dL); mean ± SD | 125.28 ± 36.89 | 118.00 ± 39.42 | 0.386 |
| Trigliserida (mg/dL); mean | 120.23 | 91.20 | 0.068 |
| FPG (mg/dL); mean | 144.54 | 83.13 | < 0.001 |
| 2-h PP (mg/dL); mean | 186.21 | 93.33 | < 0.001 |
| Fasting insulin (µU/mL); mean | 19.5 | 18.62 | 0.724 |
| HOMA-β; mean | 75.86 | 204.17 | < 0.001 |

**Note:** 2-h-PP: 2 hours post prandial, BMI: body mass index, DBP: diastolic blood pressure, FPG fasting plasma glucose, HDL: high-density lipoprotein, HOMA-β: homeostasis model assessment β, LDL: low-density lipoprotein, SBP: systolic blood pressure, SD: standard deviation.

N-terminal transmembrane domain (TMDO) and a cytosolic linker, hence SUR1 may affect Kir6.2 gating via those structures.[7] SUR1 can modify Kir6.2 gating by several mechanisms such as enhancing opening potential (P0) of Kir6.2, improve Kir6.2 sensitivity to ATP, and altering KATP channel function by binding to sulphonylurea drugs which will hinder opening.[8]

Subunit SUR1 is encoded by ABCC8 gene located in locus 11p15,1 along with KCNJ11 gene which encodes Kir6.2 subunit. ABCC8 is a member of the membrane transporter protein groups of ATP-binding cassette subfamily 8, the gene consists of 39 exons with more than 100kb long genomic DNA.[9] Exon 16 -3C>T polymorphism in ABCC8 gene leads to splicing error due to the site of polymorphism being in the splicing acceptor site.[10] Exon 16 -3 C>T polymorphism in ABCC8 has been explored among DM type 2 in european and asian populations, but results remain inconsistent.[9,11,12,13,14,15]

The purpose of this study is to identify the polymorphism of ABCC8 gene on exon 16- 3 among population with and without T2DM.

## Methods

This research protocol has been approved by the ethical committee of the Medical Faculty of Duta Wacana Christian University (ethical approval no. 225/C.16/FK/2016), informed consent was obtained from all research subjects. This case-control study recruited 40 individuals with T2DM as case subjects and 44 individuals without T2DM as control subjects. Specimen collection was conducted in Bethesda Lempuyangwangi General Hospital, Yogyakarta, during May to July 2016.

### *Participants*

Case subject was selected according to T2DM diagnosis criteria of fasting plasma glucose (FPG) ≥ 126 mg/dL and or 2 hours plasma glucose (2-h PP) ≥ 200 mg/dL3, or had been diagnosed DM type 2 by the hospital. Exclusion criteria were obesity (BMI ≥ 30 kg/m2), other chronic diseases, pregnancy, and or subjects with insulin therapy.

### *Biologic marker measurement*

Insulin secretion was determined using mathematical formula HOMA-β in HOMA 2 Calculator software downloaded from the Oxford Institute (www.dtu.ox.ac.uk/homacalculator/). FPG, 2-h PP, total cholesterol, HDL, LDL, triglyceride analysis sample was taken from EDTA blood plasma. FPG and 2-h PP were determined with GOD-PAP method using Glucose God FS (DiaSys Diagnostic System GmBH, Holzheim, Germany). Fasting insulin level was measured with sandwich ELISA method using DRG Insulin ELISA (DRG Instruments GmBH, Marburg, Germany). Total cholesterol, HDL, and LDL concentrations were measured with CHOD-PAP using Cholesterol FS (DiaSys Diagnostic System GmBH, Holzheim, Germany). Triglyceride was determined with GPO method using Triglyceride FS (DiaSys Diagnostic System GmBH, Holzheim, Germany).

### *DNA isolation procedure*

DNA isolation from whole blood employed DNA Genomic Wizard Kit (ProMega Co, Madison, USA), genotyping PCR-RFLP using PCR thermocycler (Esco Lifesciences, Singapore) and for final reaction using 1 µL DNA, 15 µL mastermix, 12 µL nuclease free water, 1 µL forward primer 5'–GAG-CCA-GAG-GAG-GAT-GTT-GA-3' and 1 µL reverse primer 5'-GGC-TAG-AAG-GAG-CGA-GGA-CT-3' (Integrated DNA Techology, Belgium). PCR was performed under the following condition: 95°C for 5 minutes, then followed by 35 cycles of 95°C for 1 minutes, 60°C for 1 minutes, 72°C for 1 minute, and a final extension for 9 minutes at 72°C. RFLP was conducted with PST1 enzyme Thermo Scientific™ (Thermo Fisher Scientific, Waltham, USA), the product was then electrophoresed on a 3% agarose gel. Visualization was carried out by ethidium bromide staining and transillumination with UV light (GelLogic, Eastman Kodak Company, Rochester, USA). DNA Marker used was DNA Molecular Weight Marker XIII (Roche Diagnostics GmbH, Germany).

### *Statistical analysis*

The association between Exon 16 -3 C>T polymorphism in ABCC8 and insulin secretion was analysed using ANOVA and Post hoc test. Determination risk factor and proportions of genotypes and alleles was determined using chi –square and odds ratio.

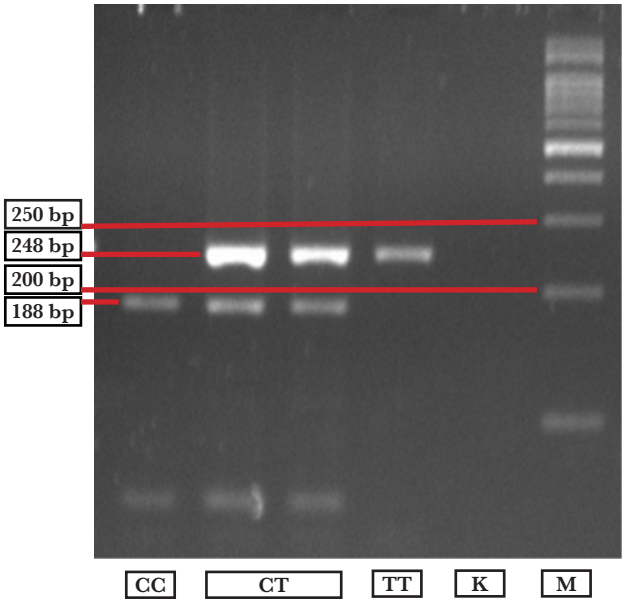


***Figure 1.*** *RFLP gel photograph of CC on 188 bp, CT on 248 and 188 bp, and TT on 248 bp.*

## Results

Genotyping and biochemical analysis was performed on 40 diabetic case subjects and 44 non diabetic control subjects, subjects characteristics was summarized in table 1. There was no significant difference of body mass index (BMI), systole, diastole, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglyceride levels among diabetic and non diabetic subjects. the majority of sex in diabetic and non diabetic subjects was female, age in diabetic subjects was significantly older than non diabetic (p : 0,006). FPG and 2-h PP concentrations was significantly higher in diabetic subjects compared to non diabetic (p < 0,001). Although fasting insulin levels did not differ significanty between diabetic and non diabetic subjects (p : 0,274), HOMA-β was significantly higher in non diabetic subjects (p : 0,000). Figure 1 presents agarose gel photograph of the CC, CT, dan TT genotypes of the exon 16 -3 C>T polymorphism. Table 2 shows the result of genetic analyses of exon 16 -3 C>T polymorphism among subjects.

Table 2 indicates that there was no significant difference of CT and TT genotypes distribution between diabetic and non diabetic subjects (p: 0,609 and 0,603). OR of CT genotype was found to be 1,3 (95% CI: 0,48-3,56), while OR TT was 0,75 (95% CI: 0,22-2,60). The T allele frequency did not differ significantly between diabetic and non diabetic subjects (p : 0,723, OR 0,90, 95% CI: 0,49-1,65). Table 3 displays a significant difference of HOMA-β among diabetic subjects. Post hoc test afterwards revealed that HOMA-β of CC and CT genotypes among diabetic type 2 subjects differ significantly (p : 0,005); while HOMA-β value of CC, CT, and TT genotypes did not show any siginificant difference among non diabetic subjects (p : 0,523). There was a significant difference in fasting insulin levels among diabetic subjects (p : 0,013), post hoc test concluded that CC and TT genotypes in DM type 2 displayed a significant difference (p : 0,013). Fasting insulin did not show any significant difference in non diabetics (p : 0256). There were not any significant difference of FPG among CC, CT, and TT genotypes in diabetic subjects (p : 0,053) dan non diabetic subjects (p : 0,857). There were not any significant difference of 2-h PP levels among CC, CT, and TT genotypes in DM type 2 subjects (p : 0,114) and non DM (p : 0,572). There was an indication of insulin resistancy which was represented by HOMA-IR did not differ significantly among the genotype of CC, CT, dan TT in T2DM (p : 0,977) and non T2DM (p : 0,406).

## Discussion

Biologic marker of diabetes (FPG, 2-h-PP, and HOMA-β) except for fasting insulin levels between diabetic and non diabetic subjects were significantly different. These data are consistent with the literature, where plasma glucose concentration is the modulator of plasma insulin. Research subject characteristics did not differ signicicantly among T2DM and control groups, with the exception of age and sex. Body mass index (BMI), lipid profile, and blood pressure varied clinically among subjects but did not show any significant difference between diabetics and non diabetics.

Exon 16 -3 C>T polymorphism is located at 3'-splicing site or splicing acceptor of coding exon 16 ABCC8 gene, hence mutation in the specific point may alter normal splicing process which will generate transcript variant.[10] Splicing process is affected by several factors, accumulation of defective mRNA may be translated into abnormal proteins, however cells have the control mechanisms to prevent generation of the abberant proteins such as nonsense-mediated decay (NMD) and nonstop decay (NSD).[16,17]

The majority of single nucleotide polymorphisms (SNPs) in T2DM are located in noncoding segments, genomic and transcriptomic global analyses of Langerhan islets indicated that gene variants may cause phenotype alterations by expressions of long noncoding RNA (lncRNA).[18,19] Intronic lncRNA is transcripted from intronic segment of the protein coding gene ini sense or antisense strand, however there is little known from intronic lncRNA in this current time.[20] LncRNAs are located in nucleus or cytoplasm and probably affect gene expression through several means, such as recruiting chromatin modification complex to alter DNA methylation or histone modification, functioning as transcription coactivatori, and stimulate protein expression by way of

microRNA isolation.[21,22]

Eventhough the 16-3 C>T polymorphism was found in T2DM, there was no significant difference of allele T frequency between diabetic and non diabetic subjects in this study. This finding is similar with Dutch, Dannish, French, British, Turkish, and South Indian population, but different from Finland population.[9,11,12,13,14] Compared to other mongoloid race, such as Japanese, Chinese Han, and other East Asian population, subjects from Yogyakarta also yield similar findings.[10,15]

In this study, there is also a significant difference of HOMA-β between CC and CT genotypes among T2DM, fasting insulin showed a significant difference between CC and TT genotypes in diabetic subjects; in contrast, there was not a significant difference among CC, CT, and TT genotypes in HOMA-β and fasting insulin among non diabetic subjects.

Additionaly, exon 16 -3 C>T polymorphism was not associated with T2DM prevalence, but the role of the polymorphism in the development of T2DM can not be excluded due to the association with insulin secretion in T2DM. Association between exon 16-3 C>T polymorphism with other types of diabetes such as gestational diabetes mellitus has been established in Finland population.[11]

There was no significant difference of insulin secretion which was exhibited by HOMA-β and fasting insulin concentration amomng CC, CT, and TT genotypes in non DM subjects. This result contradicts with the previously mentioned result among T2DM subjects, hence this may indicate the impact of the polymorphism toward deterioration of insulin secretion in T2DM.

***Table 2.*** *Distribution of genotypes and alleles frequency in each outcome group*

| Genotype | T2DM n=40 | Non T2DM n=44 | OR (95% CI) | p-value |
|---|---|---|---|---|
| **Genotype** | | | | |
| CT | 22 (55%) | 20 (45%) | 1,3 (0,48-3,56) | 0,609 |
| TT | 7 (17,5%) | 11 (25%) | 0,75 (0,22-2,60) | 0,603 |
| CC | 11 (27,5%) | 13 (30%) | (ref.) | (ref.) |
| **Allele frequency** | | | | |
| T | 36 (45%) | 42 (47,7%) | 0,90 (0,49-1,65) | 0,723 |
| C | 44 (55%) | 46 (52,3%) | (ref.) | (ref.) |

Insulin resistance is influenced by lipid metabolism that commonly develops prior to inadequacy of insulin secretion, however the difference between HOMA-IR among CC, CT, and TT genotypes in T2DM and non DM was not significant.[23] Hence, it was indicated that the decline of insulin secretion in T2DM subjects was probably caused by other genetic mechanism, as there was no ethnic restriction during sampling process.

## Conclusion

Exon 16 -3 C>T ABCC8 polymorphism was associated with insulin secretion in T2DM but does not serve as a sole risk factor of T2DM. Diabetes development is affected by many factors such as genetic difference inter ethnic and gene expression regulation. Expanding sample size in a restricted population ethnically complemented

***Table 3.*** *HOMA-β, fasting insulin concentration, FPG, 2-h PP and HOMA-IR among genotypes of research subjects*

| Variables | Genotype | T2DM (n=40) | | Control (n=44) | |
|---|---|---|---|---|---|
| | | Mean ± SD | p-value | Mean ± SD | p-value |
| HOMA-β | CC | 53, 32 ± 36,64 | 0.005 | 194,92 ± 63,71 | 0.523 |
| | CT | 116,38 ± 90,89 | | 235,78 ± 95,66 | |
| | TT | 98,27 ± 59,69 | | 226,99 ± 134,03 | |
| Fasting Insulin (μU/mL) | CC | 15,81 ± 5,28 | 0.013 | 17,42 ± 8,68 | 0.256 |
| | CT | 21,57 ± 9,15 | | 22,85 ± 10,69 | |
| | TT | 28,22 ± 9,20 | | 22,46 ± 15,49 | |
| FPG (mg/dL) | CC | 192,01 ± 77,28 | 0.053 | 82,70 ± 12,89 | 0.857 |
| | CT | 136,07 ± 50,48 | | 83,46 ± 8,94 | |
| | TT | 162,42 ± 56,27 | | 84,71 ± 9,91 | |
| 2-h PP (mg/dL) | CC | 294,05 ± 98,77 | 0.114 | 98,55 ± 20,16 | 0.572 |
| | CT | 180,57 ± 87,10 | | 91,70 ± 19,96 | |
| | TT | 219,10 ± 100,37 | | 94,88 ± 20,11 | |
| HOMA-IR | CC | 7,66 ± 5,62 | 0.977 | 4,20 ± 2,62 | 0.406 |
| | CT | 8,29 ± 6,26 | | 5,29 ± 3,09 | |
| | TT | 6,74 ± 2,43 | | 4,70 ± 2,98 | |

with exploring other metabolic factors would improve the study in the future. This research would also benefit from expanding genetic analyses into epigenetic area to establish whether the influence of such polymorphism is in gene regulatory juncture.

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