

PAPER • OPEN ACCESS

A Study of *Kir6.2* Gene Sequence in Rat Model of Type 2 Diabetes Mellitus Treated by CSN1S2 Protein of Etawah Crossbred Goat Milk

To cite this article: Hazna Noor Meidinna and Fatchiyah Fatchiyah 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **546** 062016

View the [article online](#) for updates and enhancements.

A Study of *Kir6.2* Gene Sequence in Rat Model of Type 2 Diabetes Mellitus Treated by CSN1S2 Protein of Etawah Crossbred Goat Milk

Hazna Noor Meidinna^{1,2}, Fatchiyah Fatchiyah^{1,2*}

¹Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University Malang, Indonesia

²Research Center of Smart Molecule of Natural Genetics Resources, Brawijaya University Malang

*Corresponding author : fatchiya@ub.ac.id

Abstract. Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by hyperglycemia. High blood glucose levels in T2DM patients are treated by sulfonylurea. However, the long-term use of sulfonylurea can affect the regulation of glucose homeostasis and cause hypoglycemia. The cascade gene associated with the hypoglycemia is *Kir6.2*, a constituent of ATP-sensitive potassium channel (K_{ATP}), in the neuron. *Kir6.2* mutations cause dysregulation of insulin secretion by pancreatic beta cells and glucagon secretion by pancreatic alpha cells. The aim of this study was to analyze the effect of CSN1S2 protein of etawah crossbred goat milk on *Kir6.2* gene sequences in the rat model of T2DM. The experimental animals used were male Wistar rats (*Rattus norvegicus*) which were divided into two major groups, namely control group and T2DM group. Each group was administrated by CSN1S2 protein with the dose of 375 mg/kg BW, 750 mg/kg BW, 1500 mg/kg BW, and without CSN1S2 protein administration. Each group was replicated three times. DNA was isolated from the rat brain. *Kir6.2* gene was amplified by using specific primers. PCR products were purified and sequenced by using ABI 3730xl DNA Sequencer. DNA sequences were analyzed by using MEGA7 software. Amplification of the *Kir6.2* gene produced 1173 bp DNA. There was no change in the *Kir6.2* sequence in all treatments. The 25 mg/kg BW dose of streptozotocin had no effect on *Kir6.2* gene sequence in the rat brain. This study also showed that administration of CSN1S2 protein at the dose of 375 mg/kg BB, 750 mg/kg BW, and 1500 mg/kg BW did not cause mutations in the *Kir6.2* gene in the brain of the rat model of T2DM.

1. Introduction

Diabetes mellitus (DM) tends to increase in number and significance so that it becomes one of the worldwide metabolic disorders. Indonesia has the sixth largest number of diabetic patients (10.3 million) in 2017 [1]. DM can cause complications to premature death [2]. The presence of insulin resistance in type 2 DM (T2DM) is the cause of the high blood glucose levels (hyperglycemia) [3].



The imbalance production of glucose and glucose intake as opposed to insulin-stimulated glucose uptake in target tissues are the factors affecting the development of hyperglycemia in T2DM [4]. It has been known that islet dysfunction and peripheral insulin resistance are present in T2DM [5]. Glucose-stimulated insulin secretion in pancreatic islet is affected by ATP-sensitive potassium channels (K_{ATP}) [6].

When blood sugar levels are high, pancreatic β -cells produce more energy in the form of ATP molecules. Increased levels of ATP will cause to the closure of K_{ATP} channel in the cell membrane. It triggers a cascade that leads to the release of insulin [7]. Sulfonylurea is used to stimulate the insulin secretion in T2DM patient. This drug targets the plasma membrane expressing the K_{ATP} channel of pancreatic β -cells. However, the long term used of sulfonylureas can cause undesirable side effects, such as hypoglycemia. It indicates the existence of impaired glucose homeostasis regulation [8–10].

The T2DM patients undergo impaired regulation of glucagon secretion characterized by an increase in plasma glucagon concentration in fasting conditions [11]. K_{ATP} channels in the hypothalamus play an important role in the glucose homeostasis by regulating the secretion of glucagon hormones and catecholamines through the autonomic nervous system [12]. The *Kir6.2* subunit is a constituent of the K_{ATP} channel in the pancreatic β cells and neurons [13]. Several studies had investigated that mutation of *Kir6.2* cause familial hyperinsulinemia in infancy and impaired counter-regulatory neurohumoral responses. The presence of defects in glucagon secretion in *Kir6.2* null mice is an upstream of alpha cells that secrete glucagon [6,14,15]. Therefore, T2DM treatment does not only target the improvement in insulin secretion abnormalities, but also in dysfunctional glucagon secretion [11].

Nutrition is one of the alternatives that can be used in the treatment of T2DM [16]. Bioactive peptides play an important role as signaling molecules in physiological functions and chronic diseases, such as hypertension and diabetes. CSN1S2 protein is a specific protein found in ethawah goat's milk [17,18]. CSN1S2 protein is known to have many function, including anti-inflammatory in rheumatoid arthritis, antimicrobial, and antioxidants [19,20]. The main focus of this study is to analyze the effect of CSN1S2 protein of etawah crossbred goat milk on *Kir6.2* gene sequences in the rat model of T2DM. We predict that the CSN1S2 protein will not lead to *Kir6.2* mutation.

2. Materials and Methods

2.1 Isolation of CSN1S2 Protein from Etawah Crossbred Goat Milk

A 250 ml of Etawah Crossbred Goat Milk was heated at 40°C, then 5 ml of glacial acetic acid was added. Samples were filtered using nylon membranes to obtain protein from the milk. The protein obtained was quantified by the UV-vis NanoDrop spectrophotometer. Long-term storage was carried out at -20°C [18,21].

2.2 Type 2 Diabetes Mellitus Animal Model Establishment

The experimental animals used were male Wistar rats (*Rattus norvegicus*) with an age range of 2-3 months and body weight range of 150-200 grams. Experimental animals were purchased from The Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University. They were acclimatized for 1 week. There were two types of experimental groups, namely the control group and the diabetes mellitus group. Each group was fed for 2 months. The control group was given comfeed pars. The diabetes mellitus group was given comfeed pars and high-fat diet. Cholesterol level measurement was done every 2 weeks during the treatment by using cholesterol sticks. The rats in diabetes mellitus group with cholesterol levels exceeding 200 mg/dl were injected with streptozotocin (STZ). The injection was carried out intraperitoneally with a dose of STZ 25 mg/kg body weight. After the STZ injection, blood glucose levels were monitored. DM is diagnosed when blood sugar exceeds 250 mg/dl [22,23]. Blood sugar levels were measured every 2 weeks.

2.3 CSN1S2 Protein of Etawah Crossbred Goat Milk Treatment

The control (C) and diabetes mellitus (DM) group were not treated with CSN1S2 protein. There were three variations of CSN1S2 protein concentration used in this study. Each CSN1S2 protein concentration was administered to the control group and diabetes mellitus group. The control group was consisted of C+375 mg/kg BW of CSN1S2 protein treatment (CM375), C+750 mg/kg BW of CSN1S2 protein treatment (CM750), and C+1500 mg/kg BW of CSN1S2 protein treatment (CM1500). The diabetes mellitus group was consisted of DM+375 mg/kg BW of CSN1S2 protein treatment (DMM375), DM+750 mg/kg BW of CSN1S2 protein treatment (DMM750), and DM+1500 mg/kg BW of CSN1S2 protein treatment (DMM1500). Each group consists of three replications. The CSN1S2 protein treatment was carried out orally for 28 days [24,25].

2.4 DNA Isolation and Amplification

DNA isolation method of the rat brain samples was according to Sambrook et. al. [26] with some modifications. The quantity and quality of DNA were measured by using NanoDrop spectrophotometer and 0.8% agarose gel electrophoresis then visualized by BioRad Gel Documentation. Amplification of the *Kir6.2* gene was carried out using the Biorad PCR machine. Primer was designed based on *Kir6.2* mRNA sequence of *Rattus norvegicus* obtained from NCBI with gene ID: X97041.1. The forward and reverse primer were 5'-ATGCTGTCCCGAAAGGGCA-3' and 5'-TCAGGACAAGGAATCTGGAG-3', respectively. The PCR program consisted of hot start 95°C for 3 minutes (1 cycle), denaturation 95°C for 30 seconds, annealing 55°C for 30 seconds, and extension 72°C for 2 minutes (34 cycles), and then post extension 72°C for 7 minutes (1 cycle). PCR products were measured qualitatively by using 1.5% agarose gel electrophoresis.

2.5 Sequencing and Sequencing Data Analysis

The 5 µl of purified PCR products were put into a 1.5 mL sterile microtube and added to the forward or reverse primer solution. The sample was homogenized by mixing gently. Sequencing of the DNA samples was carried out by using ABI 3730xl DNA Sequencer (Koeln, Germany). DNA sequencing samples were analyzed using MEGA7 software.

3. Results and Discussion

The *Kir6.2* gene amplification (Figure 1a) was successfully demonstrated in 1.5% agarose gel electrophoresis. The DNA size of 1173 bp was established. Alignment by using MEGA 7 showed no mutation in the control group and the CSN1S2 protein-treated control group (Figure 1b). It indicated that the administration of CSN1S2 protein at a dose of 375 mg/kg BW, 750 mg/kg BW, and 1500 mg/kg did not cause mutations in the *Kir6.2* gene in the brain.

Several studies of CSN1S2 protein of etawah crossbred goat milk have been established [18,20,21,27]. The CSN1S2 protein will be broken down into several bioactive peptides by the protease enzyme in the small intestine. The rat model of rheumatoid arthritis showed transversion mutation in STAT3 gene. The CSN1S2 milk treatment induced the mutated STAT3 gene to become normal. In addition, the administration of CSN1S2 milk did not cause mutation in STAT3 gene of the normal rat group [20]. The bioactive peptide of goat's milk Etawah can act as an inhibitor of AGES-RAGE interactions that interferes the cascade transduction signals at the cellular level [27].

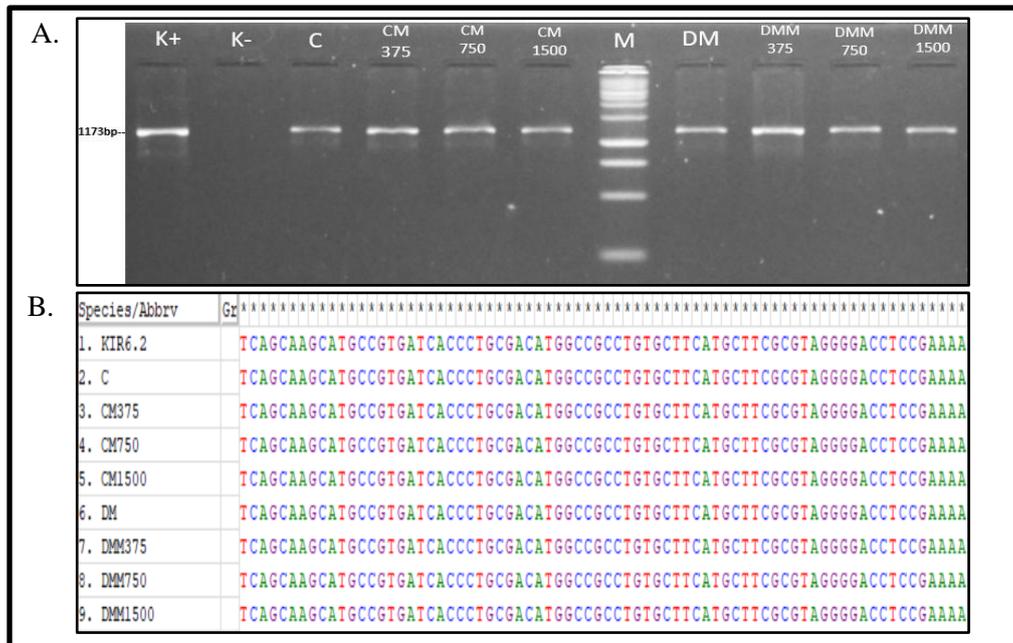


Figure 1. The *Kir6.2* gene in normal rat and T2DM rat model. (A) 1173 bp of *Kir6.2* gene visualized in 1.5% agarose gel. (B) The *Kir6.2* DNA sequence alignment by using MEGA7 software. K+: positive control, K-: negative control; C: control group, CM375: control + 375 mg/kg BW of CSN1S2 protein, CM750: control + 750 mg/kg BW of CSN1S2 protein, CM1500: control + 1500 mg/kg BW of CSN1S2 protein, DM: diabetes mellitus group, DMM375: DM + 375 mg/kg BW of CSN1S2 protein, DMM750: DM + 750 mg/kg BW of CSN1S2 protein, DMM1500: DM + 1500 mg/kg BW of CSN1S2 protein, M: 100 bp DNA ladder (Gene aid)

STZ is widely used in the establishment of animal models of diabetes. STZ, 2-deoxy-2- (N methyl-N-nitrosourea)-1-D-glucopyranose is a strong alkylating agent. STZ can enter the pancreatic β cells through glucose-GLUT2 transporters and induce DNA methylation and DNA damage [22,28]. It induces diabetes in mouse models by inhibiting O-GlcNAcase. GLUT2 in β cells transports STZ into cells and causes irreversible alkylation of β cells and necrosis [29]. In DM group, no mutation was found in the *Kir6.2* gene. The similar result was found in the DMM375, DMM750 and DMM1500 treatment (Figure 1b). This study revealed that the *Kir6.2* gene was not directly affected by hypercholesterol treatment in T2DM rat model establishment.

The condition of hypercholesterol is known to induce damage to insulin receptors in cells through the formation of reactive oxygen species (ROS). This condition is also able to induce damage to the pancreatic β cells [30]. To make T2DM rat model, a high-fat diet and streptozotocin (STZ) were given. This model involves a combination of a high-fat diet to produce animal model with conditions of insulin resistance, hyperinsulinemia and/or glucose intolerance followed by the administration of STZ, which causes a reduction in the mass of functional β cells. These two factors are designed to mimic the T2DM pathology [31]. This condition targets the damage of pancreatic β cell directly so that this could be the reason for the *Kir6.2* gene of T2DM rat brain not to mutate. In addition to pancreatic beta cells, STZ damages other organs that express GLUT2, such as the kidneys and liver. The brain is not directly affected because the blood brain barrier lacks of GLUT2 protein. There are

cells that express GLUT2 in the brain, especially in circumventricular and hypothalamic organs. Some of these cells may be involved in glucose sensing [32].

4. Conclusion

The administration of CSN1S2 protein at a dose of 375 mg/kg BW, 750 mg/kg BW, and 1500 mg/kg BW did not cause changes in the *Kir6.2* gene sequence in the brain of T2DM rat model.

Acknowledgments

We are sending our regards to our research group (SMONAGENES UB) and Bioscience Institute UB for supporting this research.

References

- [1] International Diabetes Federation. 2017. **IDF diabetes atlas, eight edition**. International Diabetes Federation. Brussel.
- [2] Piero M N, Nzaro G M, Njagi J M. 2014. Diabetes melitus – a devastating metabolic disorder. *AJBPS* **4(40)** 1-7
- [3] Deepthi B, Sowjanya K, Lidiya B, Bhargavi R S, Babu P S 2017 A modern review of diabetes melitus: an annihilatory metabolic disorder *J. Silico Vitro. Pharmacol.* **3(1)** 1-5
- [4] Lee PG, Halter JB. 2017 The pathophysiology of hyperglycemia in older adults: Clinical considerations. *Diabetes Care.* **40** 444-452.
- [5] Giugliano D, Ceriello A, Esposito K. 2008 Glucose metabolism and hyperglycemia *Am. J. Clin. Nutr.* **87** 217-222.
- [6] Jiang Y-D, Chuang L-M, Pei D, Lee Y-J, Wei J-N, Sung F-C, Chang T-J 2014 Genetic variations in the *Kir6.2* subunit (*kcnj11*) of pancreatic atp-sensitive potassium channel gene are associated with insulin response to glucose loading and early onset of type 2 diabetes in childhood and adolescence in taiwan *Int. J. Endocrinol.* **2014** 1-7.
- [7] Martin G M, Kandasamy B, DiMaio F, Yoshioka C, Shyng S-L 2017 Anti-diabetic drug binding site in a mammalian KATP channel revealed by Cryo-EM *eLife* **6** 1-27
- [8] Melander A 2004 Kinetics-effect relations of insulin-releasing drugs in patients with type 2 diabetes:brief overview *Diabetes* **53(3)** 151–155
- [9] Sola D, Rossi L, Schianca G P C, Maffioli P, Bigliocca M, Mella R, Corliano F, Fra G P, Bartoli E, Derosa G 2015 Sulfonylureas and their use in clinical practice *Arch. Med. Sci.* **11(4)** 840–848
- [10] Rubaiy H N 2016 The therapeutic agents that target ATP-sensitive potassium channels. *Acta Pharm.* **66** 23-34
- [11] Hædersdal S, Lund A, Knop FK, Vilsbøll T 2018 The Role of Glucagon in the Pathophysiology and Treatment of Type 2 Diabetes. *Mayo. Clin. Proc.* **93(2)** 217-239.
- [12] Sun H S, Feng Z P 2013 Neuroprotective role of ATP-sensitive potassium channels in cerebral ischemia. *Acta Pharmacol Sin.* **34** 24-32.
- [13] Simard JM, Woo SK, Schwartzbauer GT, Gerzanich V 2012 Sulfonylurea receptor 1 in central nervous system injury: A focused review. *J. Cereb. Blood Flow Metab.* **32(9)** 1699-1717.
- [14] Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M, Ashcroft F, Minokoshi Y, Roeper J, Seino S 2001 ATP-sensitive K⁺channels in the hypothalamus are essential for the maintenance of glucose homeostasis. *Nat. Neurosci.* **4(5)** 507-512.
- [15] Minami K, Miki T, Kadowaki T, Seino S 2004 Roles of ATP-Sensitive K⁺ Channels as Metabolic Sensors. *Diabetes* **53** 176-180
- [16] Kaput J, Noble J, Hatipoglu B, Kohrs K, Dawson K, Bartholomew A 2007 Application of nutrigenomic concepts to Type 2 diabetes mellitus. *Nutr. Metab. Cardiovasc. Dis.* **17** 89-103

- [17] Fatchiyah F, Raharjo S J, Dewi F R P 2015. Virtual selectivity peptides of CSN1S2 protein of local goat ethawah breeds milk modulate biological mechanism of calmodulin. *Int. J. Pharm. Bio. Sci.* **6(2)** 707 – 718
- [18] Fatchiyah F, Setiawan B, Suharjo S, Noor Z 2015 The anti-osteoporosis effects of CSN1S2 protein of goat milk and yoghurt on a complete Freund's adjuvant-induced rheumatoid arthritis model in rats. *Biomarkers Genomic Med.* **7(4)**139-146.
- [19] Rohmah R N, Widjajanto E, Fatchiyah F 2015 Protective effect of CSN1S2 protein of goat milk on ileum microstructure and inflammation in rat-CFA-induced rheumatoid arthritis. *Asian Pac. J Trop Dis.* **5(7)** 564-568.
- [20] Rohmah R N, Hardiyanti F, Fatchiyah F 2015 Inhibition on JAK-STAT3 Signaling Transduction Cascade Is Taken by Bioactive Peptide Alpha-S2 Casein Protein from Goat Ethawah Breed Milk **23(4)** 233-238.
- [21] Bia RR, Virgini RP, Setiawan B, Soewondo A, Fatchiyah F 2015 Goat milk CSN1S2 is able to decrease the severity scoring, TNF- α , and RAGE expression in complete Freund's adjuvant-induced rheumatoid arthritis model of rats. *Biomarkers Genomic Med.* **7(2)** 64-71.
- [22] Deeds MC, Anderson JM, Armstrong AS, Gastineau D A, Hiddinga H J, Jahangir A, Eberhardt N L, Kudva Y C 2011 Single dose streptozotocin-induced diabetes: Considerations for study design in islet transplantation models. *Lab Anim.* **45(3)** 131-140.
- [23] Moree S S, Kavishankar G B, Rajesha J 2013 Antidiabetic effect of secoisolariciresinol diglucoside in streptozotocin-induced diabetic rats. *Phytomedicine* **20(3-4)** 237-245.
- [24] Rika M, Fatchiyah 2017 Influence of CSN1S2 protein from Caprine milk Etawah Breed (EB) on histology of microglial cells in rat (*Rattus norvegicus*) Type-2 diabetes mellitus (T2DM). *AIP Conference Proceedings* **1908(060001)** 1-4
- [25] Rachmad YT, Wihastuti TA, Miyajima K, Fatchiyah F 2018 Activity of Caprine CSN1S2 Protein Reducing the COX-2 and IL-17 Expression of Aorta Tissue in Type 2 Diabetes Mellitus Rat *J. Math. Fund. Sci.* **50(3)** 332-345
- [26] Sambrook J, Russell DW 2001 Molecular Cloning A Laboratory Manual Third Edition. Cold Spring Harbor Laboratory Press New York
- [27] Fatchiyah F, Hardiyanti F, Widodo N 2015 Selective Inhibition on RAGE-binding AGEs Required by Bioactive Peptide Alpha-S2 Casein Protein from Goat Ethawah Breed Milk : Study of Biological Modeling. *Acta Inform. Med.* **23(2)** 90-96
- [28] Grady WM, Ulrich CM 2007 DNA alkylation and DNA methylation: cooperating mechanisms driving the formation of colorectal adenomas and adenocarcinomas? *Gut* **56** 318–320
- [29] Goud B J, Dwarakanath V, Chikka B K 2015 Streptozotocin - A Diabetogenic Agent in Animal Models *Ijppr.Human* **3(1)** 253-269
- [30] Kay A M, Simpson C L, Jr J A S 2016 The Role of AGE / RAGE Signaling in Diabetes-Mediated Vascular Calcification. *J. Diabetes Res.* **2016** 1-8
- [31] Skovsø S. 2014 Modeling type 2 diabetes in rats using high fat diet and streptozotocin *J. Diabetes Invest.* **5(4)** 349-358.
- [32] Grieb P 2016 Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's Disease : in Search of a Relevant Mechanism *Mol. Neurobiol.* **53** 1741–1752