Genomic Profile of rIR and rIRR in Type 2 Diabetes Mellitus Rat Model toward Effect of Goat Milk CSN1S2 Protein

S H Tambunan¹, H N Meidinna¹,², R N Rohmah² and F Fatchiyah¹,²*

¹Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia
²Research Center of Smart Molecule of Natural Genetics Resource, Brawijaya University, Indonesia

*Corresponding author’s email: fatchiya@ub.ac.id

Abstract. Diabetes mellitus is metabolic disorder with hyperglycemia condition. Diabetes mellitus is commonly divided into two types, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). The research focus was to determine the effect of CSN1S2 protein from Etawah Crossbred goat milk on DNA sequence of rIR exon 17 and rIRR exon 2 in T2DM rat model. The experimental rats were divided into four main groups, namely control group, diabetes group, control with CSN1S2 protein treatment group, and diabetes with CSN1S2 protein treatment group. We used 375, 750 and 1500 mg/kg BW of goat milk CSN1S2 protein for the treatment. The DNA was extracted from the rat liver, then amplified with a specific primer of rIR and rIRR genes. The results of the amplification will be sequenced by using ABI 3730xl DNA Sequencer. The CSN1S2 protein treatment in rIR gene exon 17 did not affect a specific DNA sequence alteration. The diabetic rats treated with 375, 750 and 1500 mg/kg BW of CSN1S2 protein had the nucleotide changed to normal (3950A→3950G). Therefore, the CSN1S2 protein may have the function in triggering the DNA repair system.

Keywords: CSN1S2, IR, IRR, T2DM

1. Introduction
Diabetes mellitus is a group of metabolic disorders characterized by increased blood sugar levels that result from insulin deficiency, insulin resistance, or a combination of both. Chronic effects of diabetes can damage several organs, including hearts, eyes, kidneys, nerves, and blood vessels [1]. Diabetes has been reported attacking 10 million people in Indonesia in 2015 and will be elevated in 2030 as many as 21.3 million people. The disease is caused by a lifestyle, ethnic and heredity history [2, 3].

Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) are two of four diabetes categories classified by the America Diabetes Association (ADA). They were previously known as Insulin dependent or juvenile-onset diabetes and non insulin dependent or adult-onset diabetes [4]. T2DM is caused by insulin resistance combined with relative insulin deficiency [5]. Insulin resistance comes from insulin receptor damage and consequently disturbs the regulation of glycogen synthesis and sugar homeostasis in blood [6]. Insulin receptor family (IRF) consists of three members: insulin receptor (IR), insulin-like growth factor receptor (IGFR) and insulin receptor-related receptor (IRR). These receptors can be found in the liver tissue [7]. Insulin receptor has been the main role for activation of tryrosine kinase and leads to glycogen synthesis [8].
Research of food materials targeting the improvement of insulin receptor of T2DM patient becomes important. One of the food materials that can be explored is milk. Milk is the source of macro and micro nutrition, including the bioactive protein for body health [9]. Cow milk is the most common milk consumed by people. The other milk source comes from the goat. This goat milk is rarely to consume. Etawah crossbred goat from Indonesia is a crossing product of etawah goat from India and domestic goat from Indonesia [10]. This goat has a specific protein, namely CSN1S2 with 36 kDa of weight [11]. This protein has the advantage as anti-inflammatory, immunomodulatory, and anti-oxidant [12–14].

The influence of CSN1S2 protein from etawah crossbred goat milk for repairing the abnormality of insulin receptor gene in T2DM is not yet observed. Therefore, this research analyzed the CSN1S2 protein potential effects on the insulin receptor (rIR and rIRR) genes of the Wistar rat (Rattus norvegicus) with T2DM.

2. Materials and Methods
2.1. Animal model
The 2-3 months old adult Wistar rat (Rattus norvegicus) with weight range of 150 gram were collected from The Integrated Research and Testing Laboratory, Gadjah Mada University. These rats were acclimatized for one week. The experimental animals were clustered into four main groups: control (C), control with CSN1S2 protein from Etawah Crossbred goat milk treatment (CM), diabetes (DM), diabetes with CSN1S2 protein from Etawah Crossbred goat milk treatment. The doses of the protein in CM and DM group consisted of 375, 750 and 1500 mg/kg BW of CSN1S2 protein. The CSN1S2 protein administration was carried out for 28 days. These animals treatment was evaluated and recognized by the ethic committee of Brawijaya University (Ethical certificate number: 417 KEP UB).

2.2. The Streptozotocin-induced type 2 diabetes mellitus in rat
The rats in diabetes (DM) group were fed with a high-fat diet for 2 months. The rats with cholesterol levels exceeding 200 mg/dl were injected with 25 mg/kg of streptozotocin (STZ) [15]. The cholesterol level and glucose blood level of the animal were measured with cholesterol stick and glucometer stick, respectively.

2.3. Preparation of CSN1S2 protein from etawah crossbred goat milk
The CSN1S2 protein was isolated from 250 mL of Etawah Crossbred goat milk. The milk was boiled on 40°C and added by 5 mL of glacial acetic acid. Nylon membrane was used for separating the protein precipitation through the mesh of the nylon. Protein concentration was measured by UV-vis NanoDrop spectrophotometer. The protein was stored at -20°C [16].

2.4. DNA extraction
The rat liver tissues were collected and weighed 0.3 gr. The tissues were homogenized by mortar, pestle and extraction buffer. The next procedures referred to Fatchiyah et. al., 2011 with fews modification in buffer of extraction [17,18]. The isolated DNA was quantified by UV-vis NanoDrop spectrophotometer. It was also examined using agarose gel 1% with chemidoc gel imaging.

2.5. DNA amplification
The composition of amplification mix solution referred to Fatchiyah et. al., 2011. The primer was designed from NCBI database sequence. The primer pair for rIR in exon 17 (ID:NC_005111.4) was: rIR-Forward 124954-124974- 5’ GAA AAA ATT GCG GCG GGA GG 3’ and rIR-Reverse 125340-125321-5’ TGG CCC TAG GCA GAA CAA AG 3’. The PCR program was hot start at 94°C for 3 minutes (1 cycle), 32 cycles consisted of denaturation at 95°C for 30 seconds, annealing at 54°C for 45 seconds, and extension at 72°C for 30 seconds. The post extension was at 72°C for seven minutes. The primer for rIRR in exon 2 amplification (ID NC_005101) was rIRR-forward 3510-3539-5’ CCA GTC TTG ACA TCC GCT CG 3’ and rIRR-reverse 4058-4039-5’ ACA GTG GCT GGA GGT CCA AC 3’. The rIRR gene had the PCR program of hot start at 94°C for 3 minutes (1 cycle), 32 cycles consisted of
denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 30 seconds. The post extension was at 72 °C for seven minutes (1 cycle). Then, the amplification results were evaluated by agarose gel 1.5% and stored at -20°C.

2.6. DNA sequencing
The 5 µl of purified DNA amplification product was mixed with 5 µl primer in 1.5 ml microtube. The solution was transferred to ABI 3730xl DNA Sequencer (Koeln, Germany) for DNA sequencing [19]. The sequences data were aligned using multiple alignment program by BioEdit version 7.0 [20].

3. Results and Discussion
The rIR amplification product had the size of 386 bp, while the rIRR gene had base the DNA size of 687 bp. The results of the rIR gene sequencing in each treatment were aligned with the rIR gene sequence from NCBI database (ID: NC_005111.4). The rIR exon 17 alignment was started from the nucleotide sequences number 124820 to 125135. We revealed that all rat groups had similar rIR DNA sequences (Figure 1a). There was no sequence alteration in them. The DM group and all CSN1S2-treated DM group did not experienced insulin resistance in the insulin receptor. Insulin resistance in the rIR gene is indicated by mutations in gene sequences. A factor that might affect the absence of rIR sequence mutations in the diabetic rat is there is no experimental animal model establishment based on the changes in genetic material. The genetically modified T2DM animal model is created mostly using the knockout system. This program can efficiently regulate the metabolic damage in experimental animals so that they experience glucose intolerance [21-22].

![Figure 1](image)

Figure 1. The alignment of (a) rIR and (b) rIRR DNA sequences in all rat groups.

The rIRR exon 2 sequences of all rat groups were aligned started from the nucleotide number 3710 to 4180. The rIRR gene (ID: NC_005101) from NCBI database was used in the alignment. The high cholesterol condition and STZ administration with the dose of 25 mg/kg BW were able to induce T2DM in rat. This is indicated by the mutation in rIRR exon 2 sequence of DM group (Figure 1b). The mutation
occurred was point mutations which changed the nucleotide from 3950G to 3950A. The CM 375 and CM 750 group had the same mutation as in the diabetic rat. However, the 375, 750, and 1500 mg/kg BW of CSN1S2 protein administration in the diabetic rat resulted in the alteration of rIRR sequence to normal. In addition, the control rat receiving 1500 mg/kg BW of CSN1S2 protein had no mutation in its rIRR sequence. This result indicated that the 1500 mg/kg BW of CSN1S2 protein is the safe dose for normal and diabetic rats.

The hypercholesterolemia and hyperglycemia condition in diabetic rat will trigger the accumulation of triglycerides and LDL in cells. Fatty acids and triglycerides cause mitochondrial dysfunction, so free radicals form in the cells. The free radicals can inactivate the action of insulin receptors by inhibiting the phosphorylation process of tyrosine kinase on the IRR [23-24]. Interestingly, the CSN1S2 protein was able to trigger the improvement of rIRR nucleotide sequences in all CSN1S2-treated diabetic rat. It might play a role in counteracting free radicals or as antioxidants in the rIRR exon 2 gene. It was able to suppress the inhibitory activity of fatty acids and triglycerides as well. This inhibition of free radicals will trigger the repair of damaged nucleotide sequences through DNA repair. Base excision repair (BER) is an efficient program in repairing DNA damage through mechanisms of oxidation, deamination and alkylation [25]. BER occurs in women with T2DM is regulated by the activation of enzymes, such as Apyrimidine endonuclease (APEX1) to initiate DNA repair through cutting phosphate sugar, X-ray repair cross-complementing protein 1 (XRCC1) and DNA ligase 3 (LIG3) to insert the correct complementary base the sequence [26].

4. Conclusion
The CSN1S2 protein of Etawah Crossbred goat milk at a dose of 375 mg/kg BW, 750 mg/kg BW, and 1500 mg/kg BW treatment did not cause mutations in the rIR gene sequence of T2DM rat model. This protein was able to induce the rIRR sequences improvement in T2DM rats.

References
[1] Stein S A, Maloney K A and Pollin T I 2014 Genetic Counseling for Diabetes Mellitus Curr Genet Med Rep 2 56–67
[2] International Diabetes Federation 2015 Executive Summary of Diabetes Atlas 7th Edition IDF Diabetes Atlas
[3] Wild S, Roglic G, Green A, Sicree R and King H 2004 Global Prevalence of Diabetes: Estimates for the year 2000 and projection for 2030 Diabetes Care 27 1047–1053
[4] American Diabetes Asosiation 2014 Diagnosis and classification of diabetes mellitus Diabetes Care 37 S81–S90
[5] Forbes J M and Cooper M E 2013 Mechanisms of diabetic complications Physiol. Rev. 93 137–88
[6] Seino S, Seino M and Bell G I 1990 Human insulin-receptor gene: partial sequence and amplification of exons by polymerase chain reaction Diabetes 39 123–128
[7] Hale L J and Coward R J M 2013 Insulin signalling to the kidney in health and disease Clin. Sci. (Lond) 124 351–70
[8] Fernandez A M and Torres-Alemán I 2012 The many faces of insulin-like peptide signalling in the brain Nat. Rev. Neurosci. 13 225–239
[9] Pereira P C 2014 Milk nutritional composition and its role in human health Nutrition 30 619–627
[10] Moeljanto W T B, Rini D and Wiryanta 2002 Khasiat & manfaat susu Kambing: Susu terbaik dari hewan ruminansia (Jakarta: ArgoMedia)
[11] Budiarti I K, Padaga M and Fatchiyah 2013 Nutritional Composition and Protein Profile of Goat Yogurt PE with Double Culture between Streptococcus thermophilus and Lactobacillus Species Cukurova Med. J. 38 681–686
[12] Chotimah C, Ciptadi G, Setiawan B and Fatchiyah F 2015 CSN1S2 protein of goat milk inhibits the decrease of viability and increases the proliferation of MC3T3E1 pre-osteoblast cell in methyl glyoxal exposure Asian Pacific J. Trop. Dis. 5 219–223
[13] Rohmah R N, Widjajanto E and Fatchiyah F 2015 Protective effect of CSN1S2 protein of goat milk on ileum microstructure and inflammation in rat-CFA-induced rheumatoid arthritis Asian Pacific J. Trop. Dis. 5 564–568

[14] Bia R R, Virgirinia R P, Setiawan B, Soewondo A and Fatchiyah F 2015 Goat milk CSN1S2 is able to decrease the severity scoring, TNF-a, and RAGE expression in complete Freund’s adjuvant-induced rheumatoid arthritis model of rats Biomarkers Genomic Med. 7 64–71

[15] Wang L, Duan G, Lu Y, Pang S, Huang X, Jiang Q and Dang N 2013 The Effect of Simvastatin on Glucose Homeostasis in Streptozotocin Induced Type 2 Diabetic Rats J. Diabetes Res. 2013 1–5

[16] Fatchiyah F, Setiawan B, Suharjono S and 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. 7 139–146

[17] Fatchiyah F, Arumingtyas L E, Widyarti S, Rahayu S 2011 Biologi molekular: Prinsip dasar analisis (Jakarta: Erlangga)

[18] Schnepp B C, Jensen R L, Clark K R and Johnson P R 2009 Infectious molecular clones of adenovirus isolated directly from human tissues J. Virol. 83 1456–64

[19] Pareek C S, Smoczynski R and Tretyn A 2011 Sequencing technologies and genome sequencing J. Appl. Genet. 52 413–435

[20] Hall T 2004 BioEdit Help Contents

[21] Kadowaki T 2000 Insights into insulin resistance and type 2 diabetes from knockout mouse models J. Clin. Invest. 106 459–65

[22] Betram C E and Hanson 2001 Animal models and programming of the metabolic syndrome British Medical Bulletin 60 103-121

[23] Miccoli R, Bianchi C, Penno G and Del Parto S 2008 Insulin resistance and lipid disorders Future lipidol. 3 651-664

[24] Saini V 2010 Molecular Mechanisms of insulin resistance in type 2 diabetes mellitus World J Diabetes 1 68-75

[25] Chiruvella K K, Liang Z and Wilson T E 2013 Repair of double-strand breaks by end joining Cold Spring Harb. Perspect. Biol. 5 a012757

[26] Grindel A, Guggenberger B, Eichberger L, Poppelmeyer C, Gschaider M, Tosevska A, Mare G, Briskey D, Brath H and Wagner K 2016 Oxidative stress, DNA damage and DNA repair in female patients with diabetes mellitus type 2 PLoS ONE 11 e0162082