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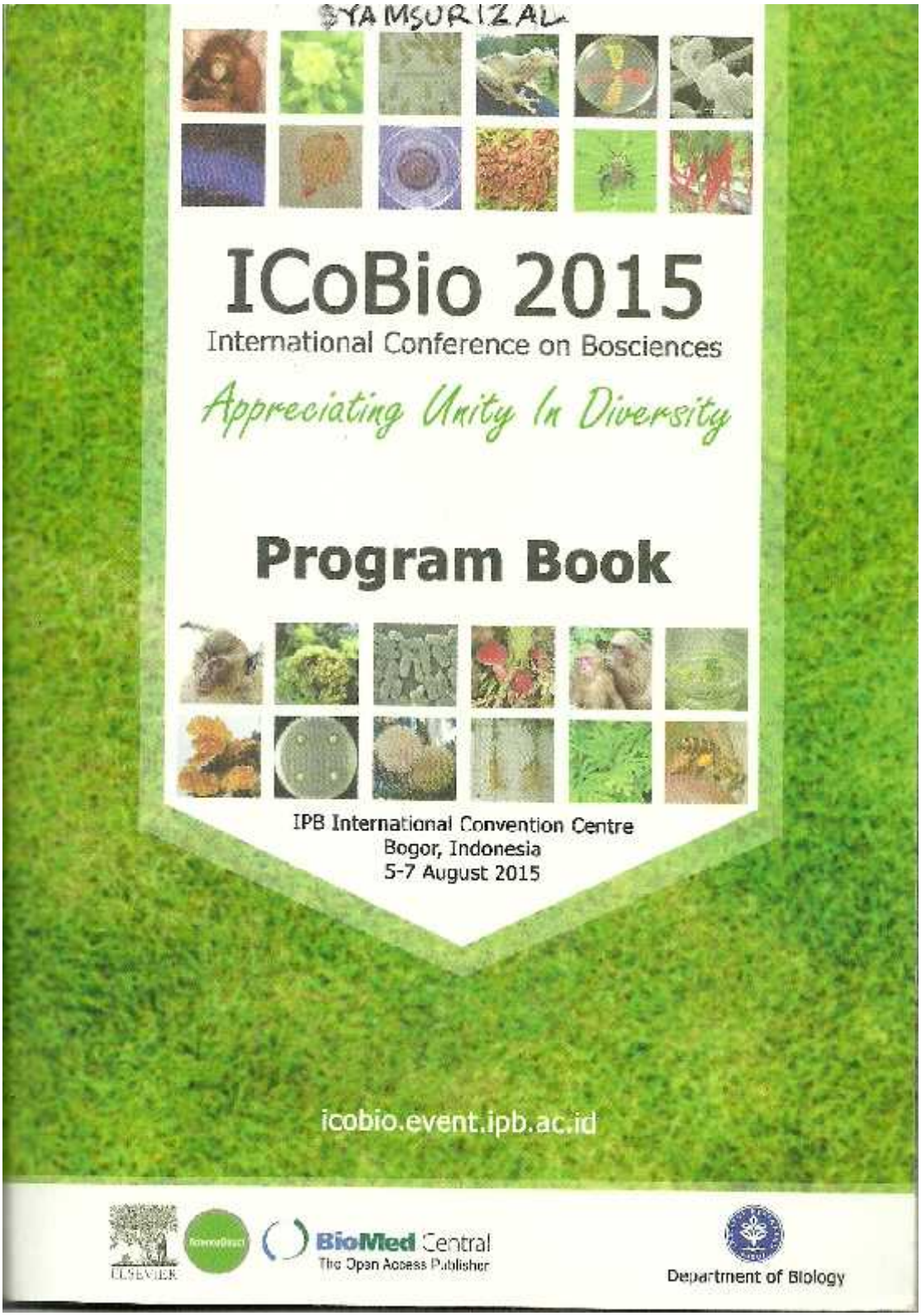
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Panitia Pelaksana

3. Daftar Isi

GROUP : MOLECULAR BIOLOGY, BIOTECHNOLOGY AND OMIC TECHNOLOGY

ROOM : MEETING ROOM C

MODERATOR : Dr. Ence D.I. Supena

TIME	CODE	PRESENTER	TITLE
14.00-14.12	MBO-O-01	Seiji Mori	Computational Studies on Mechanistic Insights into Prostaglandin Synthases
14.12-14.24	MBO-O-02	Ayda Trisnawaty Yusuf	Protective Effects of Garlic Oil on Rat Sperm Nuclear DNA Damage Induced by Sarimukti Municipal Landfill Leachates
14.24-14.36	MBO-O-03	SUPAPORN - SEETAHA	Solution structure of p51 or p66 monomer with NNRTIs inhibitor
14.36-14.48	MBO-O-04	Yuni Widyastuti	Gene Action and Combining Ability Studies for Yield and Component Traits in Hybrid Rice (*Oryza Sativa* L.)
14.48-15.00		Discussion	

MODERATOR : Dr. Yuni Widyastuti

15.00-15.12	MBO-O-05	Shuhei Horibe	The role of Ric-8A and Gαi signaling in spindle formation.
15.12-15.24	MBO-O-06	ROZA ELVYRA	The Mitochondrial COX3 Gene of *Kryptopterus apogon* and *Kryptopterus limpok* from Indragiri River Of Riau Province
15.24-15.36	MBO-O-07	Syamsurizal Syam	Genotyping SNP rs290407 TCF7L2 Gene Using Tetra-Primer ARMS-PCR For Detection T2DM In Minangkabau Ethnic
15.36-15.48	MBO-O-08	Syuhbanul Wathon	Stability of Recombinant Human Interferon Alpha-2b in Pichia pastoris
15.48-16.00		Discussion	
16.00-16.15	AFTERNOON TEA BREAK		

GENOTYPING SNP rs290487 TCF7L2 GENE USING T-ARMS-PCR FOR DETECTION T2DM IN MINANGKABAU ETHNIC

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Quickly recognize indication of individual susceptibility to Type-2 Diabetes Mellitus (T2DM) is very important for the treatment efficiency and prevention of the disease. Previous studies showed that polymorphisms in TCF7L2 gene (SNP rs290487) associated with the possibility of T2DM in various ethnic groups in the world. The presence of the T allele in rs290487 can be used as a genetic marker of increased susceptibility to T2DM. The aim of this research was to find an association between SNP rs290487 with T2DM in ethnic Minangkabau. Furthermore, it is also useful for development of an T2DM detection method based on molecular technique are rapid, inexpensive and accurate. Analysis was performed on 132 subjects (66 T2DM patients and 66 controls). DNA was isolated from venous blood samples and amplified using T-ARMS-PCR. The association between rs290487 with the possibility of T2DM known by chi-square test. T-ARMS-PCR analysis show that the frequency of SNP rs290487 in 66 subject with T2DM was 42,4% (7,6% homozygote TT and 34,8% heterozygote TC) and for non-T2DM subject was 37,9% (7,6% homozygote TT and 30,3% heterozygote TC). There was no significant association between rs290487 SNP with the probability of T2DM in ethnic Minangkabau (P^{value}=0.96).

Key words: T-ARMS-PCR, rs290487, genotyping, T2DM, Minangkabau

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA 2010). Indonesian Society of Endocrinology (PERKENI) defines diabetes mellitus as a chronic disease with a set of symptoms in a person who caused an increase in blood sugar levels due to insulin deficiency (PERKENI 2011). Patients with diabetes mellitus in the world each year has increased including in Indonesia and West Sumatra. The prevalence of DM in Indonesia is 4.1%, while in West Sumatra for the year 2000 it was estimated to be 5.1% (Wild et al. 2004; PERKENI 2011)

The diagnostic methods for DM are still being developed, e.g. using DNA analysis. To perform DNA analysis, data are required on genes associated with T2DM. Several ethnic groups in the world have already gene banks for T2DM, e.g. in Denmark, USA, UK, France, and India (Radha and Mohan 2007). Through DNA or gene analysis, patients with T2DM can be more rapidly and accurately diagnosed. Among the genes that are strongly associated with type-2 diabetes mellitus is a "transcription factor 7 like 2 (TCF7L2)" gene on chromosome 10q (Stolerman et al 2009). TCF7L2 gene strongly associated with type-2 diabetes mellitus in ethnic Danish, Caucasian, Indian, and in several ethnic in Asia (Radha and Mohan 2007; Chang et al 2009; HsinWu et al 2009)

The transcription factor 7-like 2 (TCF7L2) gene is a member of the T-cell factor (TCF)/lymphoidenhancing factor family of high mobility group-box containing transcription factors involved in the Wnt signaling pathway. This pathway is a key component to the regulation of cell proliferation and differentiation (Nelson and Nusse 2004). WNT activity is also important for lipid and glucose metabolism, proliferation and affect the function of pancreatic beta cells and for producing the incretin hormones GLP-1 (Jin 2008). Along with TCF7L2 nuclear receptor, Wnt signaling has become an important factor in the secretion of GLP-1 through the intestinal endocrine L-cells. Changes in this pathway can lead to a decrease in the secretion of GLP-1 in which it will affect the secretion of insulin (Smith 2010).

One of the TCF7L2 gene variants were allegedly associated with type-2 diabetes mellitus is a rs290487. Motif sequences of rs290487 is: AGTACAAATCATGGTGACACCA [C/T] GCAAAATTGAAAATGAGAAAGG (NCBI). The presence of the T allele in rs290487 is an indication of increased susceptibility to type-2 diabetes mellitus (Rahan and Mohan 2007; Florez et al 2006; Grant et al 2006).

There has been no genetic data about SNP rs290487 on TCF7L2 genes as genetic markers of type-2 diabetes in ethnic Minangkabau makes it interesting to studied, because of the high prevalence of DM in this ethnic. The aim of this research is to confirm the association between snp

in rs290487 with T2DM in ethnic Minangkabau. In the other hand, it is also useful for development of an early warning system in T2DM based on molecular techniques, rapid and accurate. So that it can be used to help the prevention and treatment of type-2 diabetes mellitus in ethnic Minangkabau.

2. Material and methods

2.1. Subjects of the study

This was a case-control study, by analyzing the SNP rs290487 on 66 patients with T2DM were compared with 66 controls (healthy people without T2DM on ethnic Minangkabau). The study population was ethnic Minangkabau patients with T2DM who attend to the Metabolic Endocrinology Polyclinic at Central General Hospital Dr. M. Djamil.

2.2. Samples

In this study used DNA derived from human peripheral blood (vena cubiti). The isolates are then used as a template in the reaction T-ARMS-PCR to detect the rs290487 SNP in the TCF7L2 gene. For DNA extraction we used PureLink Genomic DNA kits (Invitrogen).

2.3. Primer design

The primer design is done by utilizing the sequence TCF7L2 gene in *Homo sapiens* as reference (NCBI). The process of designing primer performed using an online primer design program created by Ye et al (2001) (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). Primer result of this design will be identify and detects the snp at rs290487.

2.4. Detection of SNP rs290487 (C/T)

To determine the ability of primers to detect SNPs in TCF7L2 gene (rs290487), then performed tests with T-ARMS-PCR method. The PCR reaction was carried out with a PCR master mix using the Invitrogen RTG kit. PCR amplification results were analyzed using the technique of gel electrophoresis on agarose 1.5% (Sambrook and Russel 2001). Through a combination of two outer primer and two inner specific primers we can quickly detect the snp rs290487 and also know the genotype of the samples that have been tested (Zhang et al 2013). The two outer primers amplify a fragment of the gene that contains an SNP. The inner primers are designed to amplify the

two allelic states (e.g., in a C→T transition, one primer will amplify the C allele and the other the T allele). So that the amplicons can be easily resolved by using standard agarose gel electrophoresis through a different size of band (Chiapparino et al 2003). If the results of electrophoresis showing 1 band means that the sample have polymorphism and homozygous (TT); if showing 2 bands means that the sample have not polymorphism (normal) and homozygous (CC); if showing 3 bands means that the sample have polymorphism and heterozygous (CC). Selected samples were sequenced for verification and confirmation of the results of T-ARMS-PCR method. DNA sequences were obtained, analyzed to know the level of similarity with data in GenBank and then characterized using several bioinformatics programs.

2.5. Statistical analysis

To determine the significance association of the SNP rs290487 TCF7L2 gene with the T2DM in Ethnic Minangkabau performed data analysis. Data analysis was performed include significance alleles using chi-square test and determine the significance of genotypes using logistic regeresi test.

2.6. Ethical clearance

The present study obtained ethical clearance from the Faculty of Medicine, Andalas University number 176/KEP/FK/2013.

3. Result

We conducted a case-control association study consisted of 66 patients with type 2 diabetes and 66 controls, two groups in the range of ± 50 years of age. Both groups are descendants of Minangkabau and representation of ethnic Minangkabau populations. The minimum age selection is important to ensure there is no silent diabetics in the control group. The clinical characteristics of the sample are shown in Table 1

Table 1. The clinical characteristics of sample

variables	Diabetes		Control		p
	Mean	SD	Mean	SD	
BMI (kg/m^2)	24,39	3,42	24,03	4,61	0,62
Weight (kg)	60,38	9,69	60,95	13,91	0,78
Age (year)	51,61	5,85	49,11	4,24	0,06
Waist size (m)	0,87	0,09	0,86	0,12	0,74

Statistical analysis shows that in both test groups either on the case (type 2 diabetes) and control (non diabetic) obtained the p value > 0.05. Therefore at α=5% indicate there was no significant difference in mean body mass index, weight, age and waist size between the cases and controls.

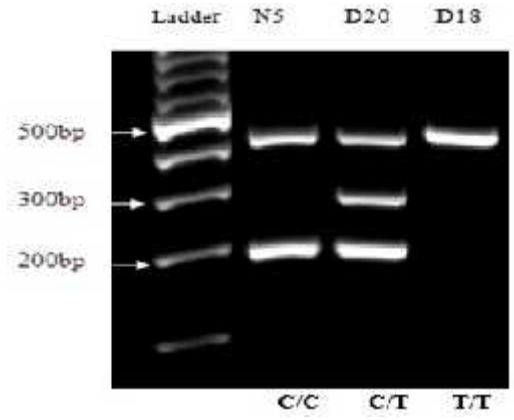
As a key factor in detecting the SNP rs290487 in TCF7L2 gene, we have been design four primers that FOPRS29, ROPRS29, FIPRS29 and RIPRS29. FOPRS29 and ROPRS29 are outer primer will amplify a fragment of the TCF7L2 gene containing SNP rs290487 (fragment size is 443 bp), FIPRS29 used to amplify C allele (fragment size is 208 bp), and RIPRS29 primer used to amplify T allele with fragment size is 293 bp (table 2).

Table 2. The sequences of primers used to genotype SNP rs290487 by T-ARMS PCR

Primer sequence	**Tm (ºC)**
FOPRS29	
160 AAAGAGCAGATTGTTATTCCTTGGATGG 187	66
ROPRS29	
602 GTTTTCTCCTCTCATGCTGCTCATTTTT 575	66
FIPRS29	
396 AACCCAGTACAAATCATGGTGACACAAC 423	66
RIPRS29	
452 TCAAACACCTTTCTCATTTTCAATTTTTCA 423	66

To evaluate the ability of the primers to detect TCF7L2 gene variant rs290487 SNPs, amplification was done using the Tetra Primer Amplification Refractory Mutation System-Polymerase Chain Reaction (T-ARMS-PCR) method. Visualization of the electrophoresis results

(electrophoregram) of the PCR products using a combination of primer FOPRS29, ROPRS29, FIPRS29 and RIPRS29 is presented in Figure 1.



Gambar 1. DNA electropherogram results of used Tetra-Primers-ARMS PCR for genotyping of SNP rs290487, N = control dan D = DMT2

From the data it can be concluded that the product electrophoresis which produces two bands indicates that the sample have not polymorphism (normal) and homozygous (CC). For the electrophoresis result showing 1 band means that the sample have polymorphism and homozygous (TT), therefore while the electrophoresis product with 3 band means that the sample have polymorphism and heterozygous (CT).

After testing on the entire sample, for subject with T2DM found 5 samples (7,6%) homozygous TT, 20 samples (30,3%) heterozygous CT and 41 samples (62,1%) homozygous CC. For control that non T2DM found 5 samples (7,6%) homozygous TT, 23 samples (34,9%) heterozygous CT and 38 samples (57,6%) homozygous CC (table 3.)

Table 3. Association of SNP rs 290487 (C/T) TCF7L2 wiyh T2DM in ethnic Minangkabau

SNP Motif	Position and gene (kb)	GeneRegion	Minor/ Mayor Alel	Frequencies Minor Alel		*P value*
				Non DMT2	DMT2	
rs290487	91.31	Intron 4	TT	5	5	0,96
			CT	23	20	
			CC	38	41	
			T	33	30	0,65
			C	99	102	

4. Discussion

The success of an ARMS-PCR reaction to detect the SNP is very dependent on the specificity of the primers designed. Selection of appropriate primer will ease in knowing and analyzing the presence/absence of SNPs in a sample. Inaccuracy in choosing the primer will result different regions were amplified so it will affect to the quality test result. If the selected primer is not specific to a particular region or SNP the detection process will cannot be done. Therefore, the primer design is the first step and very crucial in detecting mutations/SNPs in a gene.

Function of the primer as initiation of DNA polymerization in vitro reaction. Appropriate primer will give the correct annealing process and is a necessary condition for the Taq DNA polymerase enzyme to be able to begin the task. Without the proper primer, taq DNA polymerase enzyme is not possible to start the process for the synthesis of complementary strands of the DNA template. In addition the primer also serves to limit the areas which will be amplified in the PCR reaction (Dieffenbach and Dveksler 2003).

Factors to consider in primer design that will be used in the T-ARMS-PCR reaction is a Tm primer because of the primers will be used in multiplex reaction. Variation of Tm between the primers in multiplex PCR reaction should be 1ºC (Fatchiyah et al 2008). The high temperature difference between the primers can effect to absence of amplification proses. Sets of primers with equal Tm but strong secondary structure gave better results than primers with weak or no secondary structure but different Tm (Chiapparino et al 2003). In table 2 it can be seen that the four primers design are ideal for use in a multiplex PCR reaction.

This method includes the use of two sets of primer in one PCR reaction tube. One set prmer designed specifically for polymorphisms (inner primer) and the other used as a control in the PCR reaction (outer primer). Inner primer designed specifically for mutant and wildtype allele, and both designed opposite each other. PCR reaction using outer and inner primers were done in one tube, so the two parts of outer primer and inner-outer primer can react with one another and produce

different products with different length so that it can be distinguished at electrophoresis. In Figure 1 we can see the variation of the band formed from the sample which has a different genotype.

This method use a primer that introduces a mismatch so that it can produce allele-specific reactions. Allele-specific approach relies on the use of allele-specific primer that contains a mismatch in their 3 'end, primer design specifically only for one allele and not react to the other allele (Medrano and Oliveira 2014). Accordingly, DNA polymerase will only perform annealing and elongation primer when the 3 'end it really fits with the template. In this condition PCR product will be produced. Based on the presence or absence of the PCR product, DNA sample can be determined their genotypes.

In the T-ARMS-PCR, high specificity of the reaction depends not only on the mismatch at the 3 'end, but also on deliberate mismatch at position-2 (second to the terminal) from 3' end of the same allele-specific primer (Etlik et al 2011). This extra mismatch destabilizes the base paring between primer with non target template. It is useful to increase the specificity of the reaction and eliminate the possibility of false positives.

After testing using T-ARMS-PCR was then obtained data as shown in Table 3. Results of statistical tests to genotype SNPs rs290487 TCF7L2 (TT, CT dan CC) obtained $p^{value} = 0.96$, and the statistical comparisons for the allele TCF7L2 (T and C) obtained the $p^{value} = 0.65$. Statistical analysis shows no significant association between genotype SNP rs290487 TCF7L2 gene with the possibility of T2DM in ethnic Minangkabau. This can be known froms is not too different allele frequency in DNA samples taken from diabetic patients with DNA taken from normal individuals (controls).

Several studies on the association of SNP rs290487 variant in the various ethnicities showed a positive association between TCF7L2 variants with the T2DM. Among them is a study conducted by Luo et al (2009) in East Asian ethnicity, in ethnic Chinese and in northeastern chinese population (Ren et al 2008; Yu et al 2010; Chang et al 2007; Wang et al 2013). However, in some other studies not found a significant association between rs290487 variant with the possibility of T2DM. This

can be seen from the studies conducted in ethnic Japanese, in ethnic Chinese and in the Han Chinese population (Miyake et al 2008; Jinjin et al 2013; Ren et al 2013). Causes of differences in the effects that occur from this variant to T2DM can not be explained in detail. until now there is no fix mechanism that could explain why the SNP variants in the TCF7L2 gene may have different effects on different ethnicities.

This SNP (rs290487) likely not affect to the incidence T2DM in ethnic Minangkabau. Chi-square test results for rs290487 showed the $P^{value} > 0.05$ (not significant). However the effect of the other SNP variant in TCF7L2 gene are not necessarily the same (still have a chance). Therefore, further research is needed on several other SNP variants to determine the possibility of T2DM in ethnic Minangkabau.

Refferences

American Diabetes Association/ADA, 2010. Standards of Medical Care in Diabetes 2010. *Diab Care*: 33

Chang YC, Chang TJ, Jiang YD, Kuo SS, Lee KC, et al. (2007) Association study of the genetic polymorphisms of the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes in the Chinese population. Diabetes 56: 2631–2637.

Chang, YC, Chiu YF, HO LLT, Ting CT, Shih KC, Curb JD, et al, 2009. TCF7L2 Genetic Variants dan Progression To Diabetes In The Chinese Population: Pleiotropic Effects On Insulin Secretion And Insulin Resistance. J Mol Med/ Springer DOI 10.1007/s00109-009-0542-4

Chiapparino E, Lee D and Donini P. 2003. Genotyping single nucleotide polymorphisms in barley by tetra-primer ARMS–PCR. Genome 47: 414–420

Dieffenbach CW and G.S. Dveksler, 2003. PCR primer: a laboratory manual 2nd edition. New York: Cold Spring Harbor Laboratory Press

Etlik O, Koksal V, Arican-Baris ST and Baris I. 2011. Development and validation of a cost-effective in-house method, tetra-primer ARMS PCR assay, in genotyping of seven clinically important point mutations. Molecular and Cellular Probes 25: 177-181

Fatchiyah, et al. 2008. Analisa Biologi Molekuler. Malang: Jurusan Biologi Universitas Brawijaya.

Florez JC, et al., 2006. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N. Engl. J. Med 355: 241–250

Grant S F, et al., 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nature Genet 38: 320–323

HsinWu LS, Hsun Hsieh C, Pei D, Jen Hung Y, Wen Kuo S dan Lin E, 2009. Gen tcf7l2 Association and interaction analyses of genetic variants in ADIPOQ,ENPP1, GHSR, PPARγ and TCF7L2 genes for diabetic nephropathy in a Taiwanese population with type 2 diabetes. Nephrol Dial Transplant 24: 3360–3366

Jin T. 2008. The WNT signalling pathway and diabetes mellitus. Diabetologia 51:1771–1780

Jinjin Wang, Fulan Hu, Tianping Feng, Jingzhi Zhao, Lei Yin, Linlin Li, Yan Wang, Qian Wang and Dongsheng Hu (2013) Meta-analysis of associations between TCF7L2 polymorphisms and risk of type 2 diabetes mellitus in the Chinese population. BMC Medical Genetics 2013, 14:8.

Luo, Y. Y., Wang, H. Y., Han, X. Y., Ren, Q., Wang, F., Zhang, X. Y., Sun, X. Q., Zhou, X. H. and Ji, L. N. (2009) Meta-analysis of the association between SNPs in TCF7L2 and type 2 diabetes in East Asian population. Diabetes Res. Clin. Pract., 85, 139–146.

Medrano RFV and Oliveira CA. 2014. Guidelines for the Tetra-Primer ARMS–PCR Technique Development. Mol Biotechnol 56:599–608

Miyake, K., Horikawa, Y., Hara, K. et al. (2008) Association of TCF7L2 polymorphisms with susceptibility to type 2 diabetes in 4,087 Japanese subjects. J. Hum. Genet., 53, 174–180.

National Center for Biotechnology Information, U.S. National Library of Medicine. Transcription factor 7 like 2 gen T-cell specific, HMG-box [Homo sapiens (human)]; 2014. Available at: http://www.ncbi.nlm.nih.gov/gene/6934. Accessed June 6, 2014.

Nelson WJ and Nusse R. 2004. Convergence of Wnt, β-Catenin, and Cadherin Pathways. Science 303: 1483-1487

PERKENI, 2011. *Konsesnsus Pengelolaan dan pencegahan Diabetes Melitus Tipe 2 di Indonesia*. Jakarta: PB PERKENI.

Radha V dan Mohan V, 2007. Genetic Predisposition To Type 2 Diabetes Among Asian Indians. Mellitus. Indian J Med Res 117: 259-274

Ren Q, Xiao J, Han X, Luo Y, Yang W, Ji L. (2013) Rs290487 of TCF7L2 gene is not associated with type 2 diabetes in Chinese Han population: a case control study and meta-analysis. Exp Clin Endocrinol Diabetes. 2013 Oct;121(9):526-30.

Ren, Q., Han, X. Y., Wang, F., Zhang, X. Y., Han, L. C., Luo, Y. Y., Zhou, X. H. and Ji, L. N. (2008) Exon sequencing and association analysis of polymorphisms in TCF7L2 with type 2 diabetes in a Chinese population. Diabetologia, 51, 1146–1152.

Sambrook J. & Russell. D.W. (2001). Molecular Cloning, 3rd Edition. Cold Spring Harbor, NY: Cold Spring Harbor LaboratoryPress.

Smith U. 2007. TCF7L2 and type 2 diabetes-we WNT to know. Diabetologia 50:5–7

Stolerman ES,Manning AK, McAteer JB, Fox CS, Dupuis J, Meigs JB danFlorez JC, 2009. TCF7L2 Variants Are Associated With Increased Proinsulin/ Insulin Ratios But Not Obesity Traits In The Framingham Heart Study. Diabetologia 52: 614–620

Wang J, Li L, Zhang J, Xie J, Luo X, Yu D, Zhao J, Feng T, Pang C, Yin L, Hu F, Zhang J, Wang Y, Wang Q, Zhai Y, You H, Zhu T, Hu D. (2013) Association of rs7903146

(IVS3C/T) and rs290487 (IVS3C/T) polymorphisms in TCF7L2 with type 2 diabetes in 9,619 Han Chinese population. PLoS One. 2013;8(3):e59053.

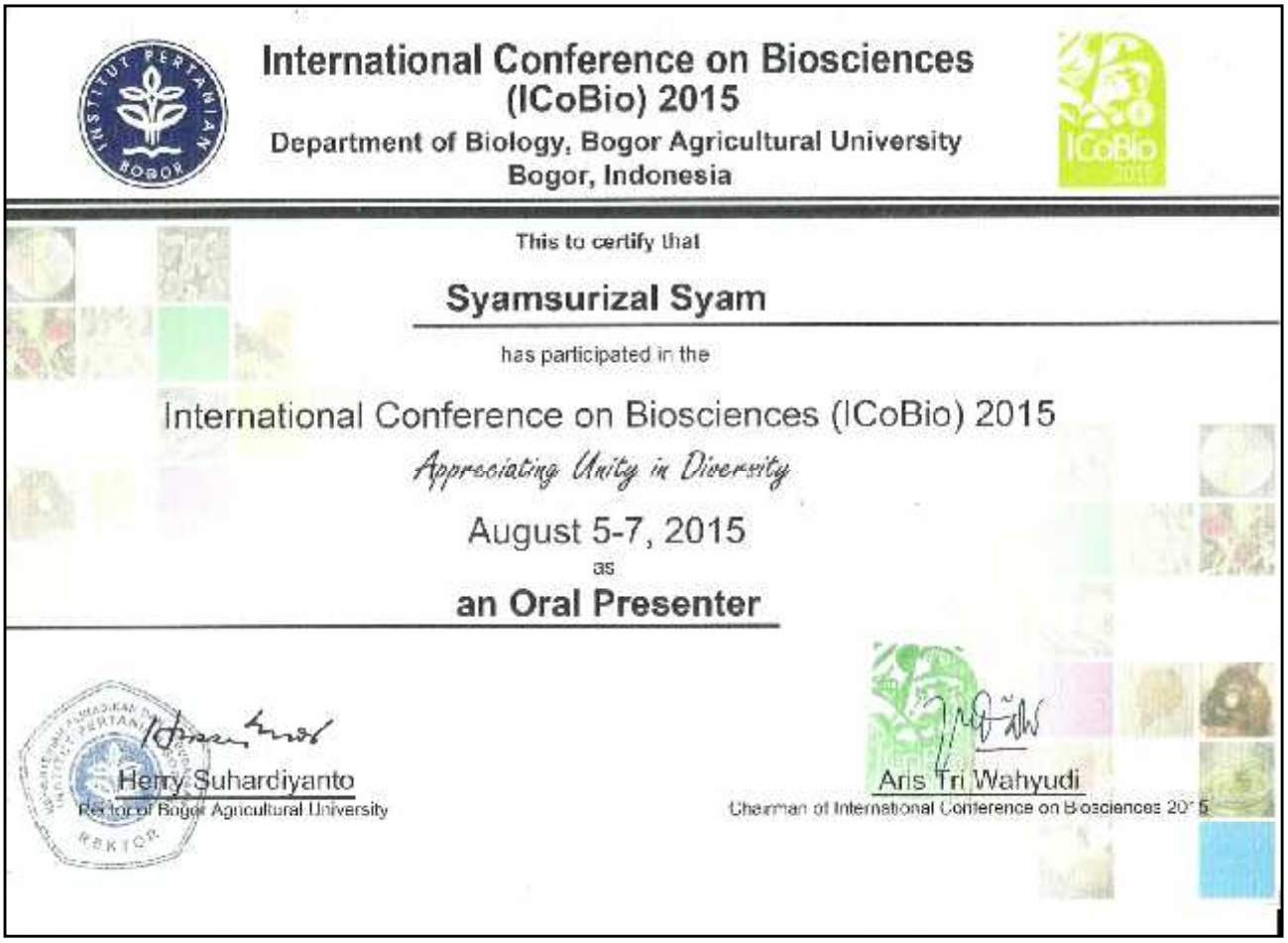
Wild S, Roglic G, Green A, Sicree R, King H, 2004. Global Prevalence of Diabetes, Estimate for the year 2000 and projection for 2030. Diabetes Care 27: 1047-1053

Ye S, Dhillon S, Ke X, Collins AR, Day IN. 2001. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res 29: E88–8.

Yu, M., Xu, X. J., Yin, J. Y. et al. (2010) KCNJ11 Lys23Glu and TCF7L2 rs290487(C/T) polymorphisms affect therapeutic efficacy of repaglinide in Chinese patients with type 2 diabetes. Clin. Pharmacol. Ther., 87, 330–335.

Zhang C, Liu Y, Ring BZ, Nie K, Yang M, Wang M, Shen H, Wu X and Ma X. 2013. A Novel Multiplex Tetra-Primer ARMS-PCR for the Simultaneous Genotyping of Six Single Nucleotide Polymorphisms Associated with Female Cancers. Plosone vol 8: issue 4

4. **Sertifikat**



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