sktp-30-05-2023 06_38_44-215511.pdf

by Chylen Setiyo Rini

Submission date: 05-Jun-2023 08:35AM (UTC+0700)
Submission ID: 2108967943
File name: sktp-30-05-2023 06_38_44-215511.pdf (1.58M)
Word count: 2456
Character count: 12572

Contents Lists Available at: https://journal2.unusa.ac.id/

Medical Technology and Public Health Journal

Journal Home Page: https://journal2.unusa.ac.id/index.php/MTPHJ

Research Article

Specific Primer Design of Leucine Rich Repeats and Guanylate Kinase Domain Containing (LRGUK) Genes in Type II Diabetes Mellitus Patients

Miftahul Mushlih [1] |Siti Nur Maghfiroh Tis'iyyah [2] |Chylen Setiyo Rini [3] |Azizah Krismonita Sari [4]*

[1,2,3,4] *Medical Laboratory Technology Program, University of Muhammadiyah Sidoarjo, Sidoarjo Regency, East Java, Indonesia*

Corresponding Author:
Miftahul Mushlih, Medical Laboratory Technology Program, University of Muhammadiyah Sidoarjo, Sidoarjo Regency, East Java, Indonesia
Email: mif.mushlih@umsida.ac.id

DOI: *10.33086/mtphj.v6i2.3476*

Article History:
Received, September 13th, 2022
Revised, November 07th, 2022
Accepted, December 15th, 2022
Available Online: December 30th, 2022

Please cite this article as:
Mushlih, M. et al., "Specific Primer Design of Leucine Rich Repeats and Guanylate Kinase Domain Containing (LRGUK) Genes in Type II Diabetes Mellitus Patients" Register: Medical Technology and Public Health Journal, Vol. 6, No. 2, pp. 195-200, 2022

ABSTRACT

Diabetes Mellitus type II (DT2) is a disorder of insulin function (insulin resistance) caused by 2 factors, i.e. environmental and genetic factors. Previous studies have identified the presence of specific alleles that differentiate between DT2 and non-DT2 sufferers. Identification of the allele indicated leucine rich repeats and guanylate kinase domain containing (LRGUK) gene. The aim of this research was to design a specific primer to amplify LRGUK gene. The primer design was based on a 576 bp nucleotide base and added 100 bp in the 5' and 100 bp in the 3' direction using NCBI-Primer BLAST. The primers produced were selected based on eight criteria's. The results were validated with 6 samples of DT2 patients and visualized using agarose gel. The results of the analysis showed that the primers Forward 5'-TCCTACTCTGTGTCCTTCCTTG-3' and Reverse 5'-GTGGTGACAAGGAGG TTTGC-3' were able to amplify specifically with a length of 687 bp.

Keywords: *Metabolism abnormalities, type II diabetes mellitus, LRGUK gene, molecular diagnosis*

INTRODUCTION

Changes in community lifestyle allow the emergence of new problems in health sector. These forms of the case can be increasing the kind of disease, which is a combination of environmental influences (lifestyle) and the potential person (genetic) (Kido, 2017) (Siewert et al., 2015). One of the diseases that always increases from year to year caused by these two effects is DT2. DT2 is a disorder characterized by the presence of excess blood sugar, a condition called hyperglycemia. Hyperglycemia is caused by a mechanism of insulin resistance and followed by a lack of insulin production by the pancreas (Guariguata, 2013). DT2 also causes abnormal physiological state of the body(Mushlih, 2020).

Currently, Indonesia occupies the 5th position with DT2 sufferers in the world with a total of 19.5 million people affected by DT2 and is predicted to increase by 28.6 people in 2045 (Webber, 2013). DT2 is closely correlated with an unhealthy lifestyle and family history, there are at least 70 genes involved in DT2 (Barroso et al., 2003). The genes that are strongly indicated to be involved in DT2 are TCF7L2, CDKAL1, KCNJ11, ABCC8, TCF7L2, PPARγ, UCP2, CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8, & FTO (Han et al., 2010)(McCarthy, 2010).

The results of the screening analysis using the PCR-RAPD method using Primer A18 based on Zahid's research (2011) showed a significant difference between DT2 and non-DT2 patients. The sequence identification indicates identification with the LRGUK gene (Kartikasari, 2021). Identification of specific sequences DT2 markers is urgent to minimize the occurrence of DT2 through molecular examination (Mushlih et al., 2020). Molecular analysis requires specific primer for amplification. The purpose of this study was to design a primer that can be used to amplify LRGUK gene in DT2 patients (Mushlih, 2021).

MATERIAL AND METHODS

The study started with a primer design, the reference sequence was the development of the research of Mushlih et al., (2020), which identified alleles using the sequence code NC_000007.14 from https://www.ncbi.nlm.nih.gov/ along 576 bp. To detect the possibility of polymorphism in the RAPD primer attachment, lengthening was carried out at added 100 bp in the 5' and 100 bp in the 3' direction. The primer design used the assistance of NCBI-Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

The results of running produce several primers choices which are then selected based on several criteria, i.e. (1). Primer length between 18-30 bases, (2). Melting Temperature (TM), the optimal TM for primers in the range of 57-63°C, (3). Annealing Temperature (TA) of 55ºC, (4). GC Content, a percentage of G and C bases of 40-60%, (5). GC clamp, 3 G bases or C bases in the last 5 bases of the 3' end need to be avoided, because the 3' end can fold to form a dimer structure which results in the 3' end of the primer not being attached to the template, (6). Does not contain Secondary Structure which causes hairpin or dimer. (7). Self-complementary, it must contain less than 4 GC base pairs at the ends or middle of the primer. (8). Repeats and runs, repetitions must not be more than 3 consecutive bases of the same order so that breathing does not occur, for example the base AGCGGGGGATG has 5 consecutive bases G.

The results of the primer match with the most criteria will be used for in vitro amplification of DT2 patients at Rumah Luka Sidoarjo. Sampling was based on the approval of the ethics committee of the Airlangga University with the number 315/HRECC.FDOM/VI/2022. The sample was previously analyzed for random blood sugar levels and had been diagnosed by a doctor who was positive for DT2.

Blood samples were taken by macro sampling and DNA isolation using the TIANamp Genomic DNA Kit. Running PCR was carried out using a PCR mix composition of 12.5 μl, Forward Primer 0.5 μl, Reverse Primer 0.5 μl, DNA 1.5 μl, and ddH2O 10 μl. running PCR using a Thermocycler T100 (Biorad) with pre-denaturation settings 95oC, 3 minutes, denaturation 95oC, 1 minute, annealing 60oC, 30 seconds, elongation 72oC, 1 minute, with 30 cycles and final elongation 72oC, 5 minutes. The PCR results were then visualized using 1% agarose gel, UV Trans illuminator to determine the target band. Alignment was performed using MEGA X.

RESULTS AND DISCUSSION

DT2 is a disease caused by a combination of environmental factors with genetic factors (Mushlih et al., 2021). The most influential environmental factor is lifestyle, while genetic factors include family history and race (Ramkisson et al., 2017). The LRGUK gene was first reported by Laramie (2008) who stated that there was a close relationship between the presence of mutations in this gene and the occurrence of DT2. After that, however, there were no further reports. RAPD analysis in previous patients showed that the LRGUK gene was reported to be directly involved in

the occurrence of DT2 in Sidoarjo. Analysis of the number of alleles on the observation of RAPD method followed by gene identification showed that 50 percent of alleles played a role in DT2 (Mushlih et al., 2020).

In this study, 100 bp of nucleotide base sequences were added both at the beginning Based on the primer design using primer blast produced 10 primer candidates (see Figure 2) and at the end so that it required a length of 770 bp and attached to the sequence of 108.219 bp -108.789 bp. All candidate primers were located outside the target gene position (Fig. 1.A). Most of the primer produced does not meet the Annealing temperature (AT), and the Secondary Structure. AT is a condition in which the hybridization conditions between the primer and the DNA template. The lowest AT conditions result in miss priming. The highest AT results in the presence of a primer template that reduces AT.

A

B

Primer Sequence	Primer Length	MT	AT	GC Content	GC Clamp	Secondary Structure Self-	Complementary Repeats end Run	number of matches
Primer 1 F 5'-TCCTACTCTGTGTCCTTCCTTG-3' R 5'-GTGGTGACAAGGAGGTTTGC-3'	√	√	√	√	√	√	√	8
Primer 2 F 5'-CTACTCTGTGTCCTTCCTTGG-3' R 5'-AACTTGGGGAGTGGTGACAAG	√	√	√	√	√	√		6
Primer 3 F 5'-TCCTACTCTGTGTCCTTCCTTGG-3' R 5'-CAACTTGGGGAGTGGTGACAA-3'	√	√		√	√	√		5
Primer 4 F 5'-ATCTCCTACTCTGTGTCCTTCCTT-3' R 5'-AGTGGTGACAAGGAGGTTTGC-3'	√	√		√	√		√	6
Primer 5 F 5'-GCAACCTGTATCTCCTACTCTGTG-3' R 5'-GGTGACAAGGAGGTTTGCCTT-3'	√	√		√	√		√	5
Primer 6 F 5'-TATCTCCTACTCTGTGTCCTTCCT-3' R 5'-ATGGAAGGGCTTCTGGAGTGA-3'	√	√		√	√		√	6
Primer 7 F 5'-ACCTGTATCTCCTACTCTGTGTC-3' R 5'-TCATGGAAGGGCTTCTGGAGT-3'	√	√		√	√		√	6
Primer 8 F 5'-ACTGCAACCTGTATCTCCTACTCT-3' R 5'-ACTGGAAAAGTTCCATGTCCCA-3'	√	√		√	√		√	5
Primer 9 F 5'-ACCTGTATCTCCTACTCTGTGT-3' R 5'-AGTGGTGACAAGGAGGTTTG-3'	√	√	√	√	√		√	6
Primer 10 F 5'-CAACCTGTATCTCCTACTCTGTGT-3' R 5'-GGTGACAAGGAGGTTTGCCTTA-3'	√	√		√	√		√	5

C

Sample	Gender	Age	Random blood sugar level (mg/dL)	DT2
S1	Male	43	230	Positive
S2	Male	44	260	Positive
S3	Male	50	208	Positive
S4	Male	51	244	Positive
S5	Male	55	267	Positive
S6	Male	58	287	Positive

D

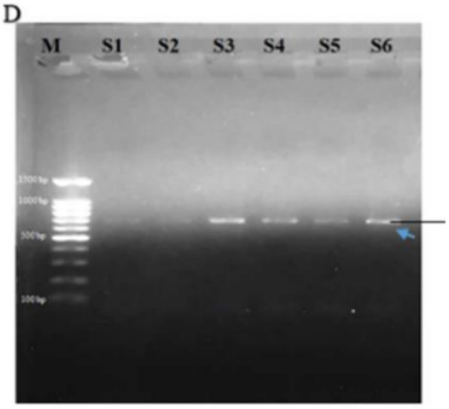

Figure 1. Analysis of the LRGUK primer design, A. Map of the primer position to the target gene, blue blocks showing the target gene, B. Several candidate primers and their conformity with the criteria, C. The criteria for the sample in the study, D. The results of LRGUK gene amplification.

Secondary Structure conditions are found in 7 out of 10 primers. The form of secondary structure can be in Hairpins, Self-Dimer, and Cross Dimer form. Hairpins form can occur as a

result of sticking one site to the other site. Hairpin at 3' end with Gm (energy required to break the hairpin structure) = -2 kcal/MOL and internal hairpin with G = -3 kcal/mol is still tolerable. Meanwhile, self-hairpin at the 3' end with G = -5 kcal/mol and self-dimer at the internal part with G = -6 kcal/mol can still be tolerated (Green et al., 2015).

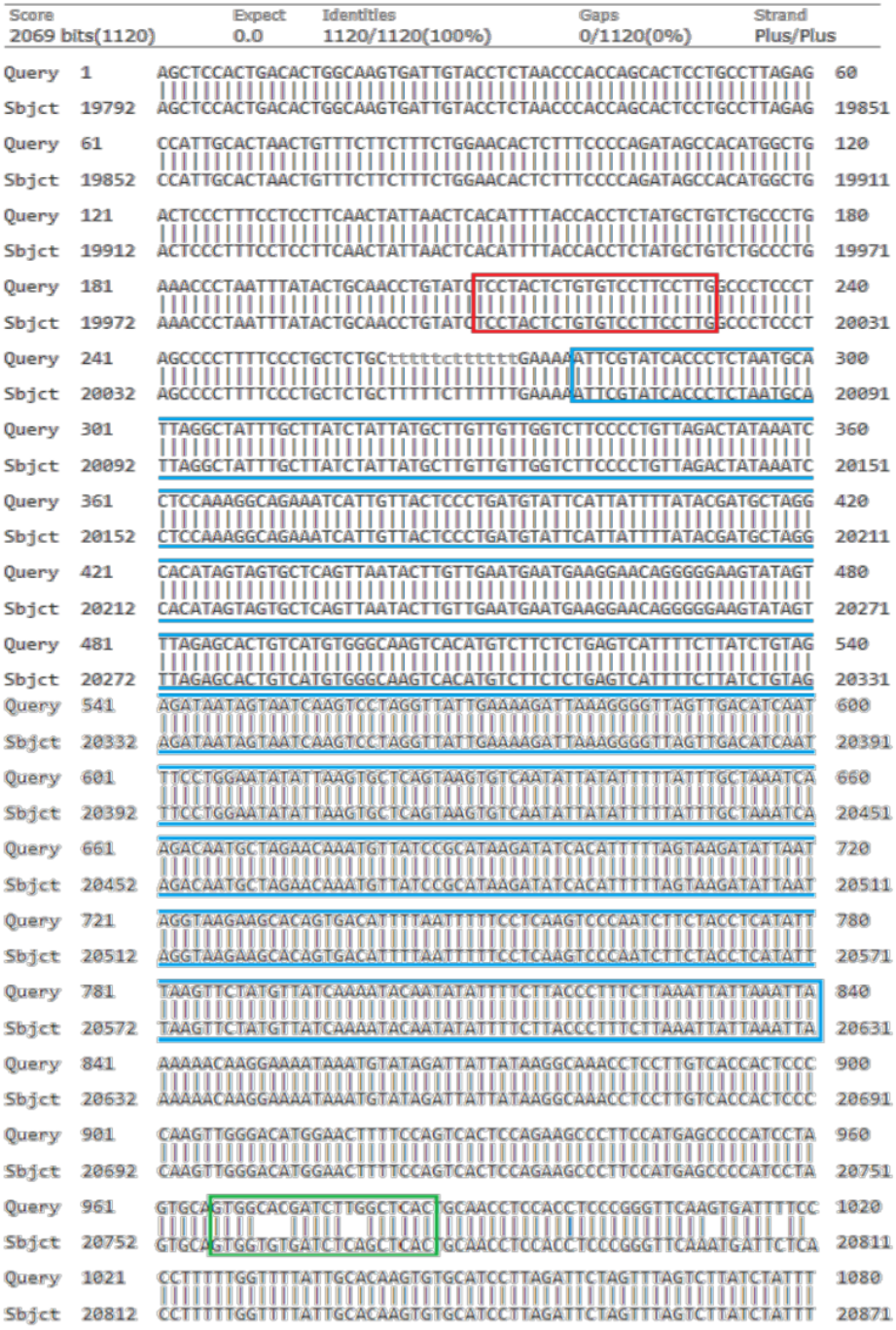

Figure 2. LRGUK Primer Design Map, Blue: target gene, Red: Primer forward, Green: Primer Reverse

Self-Dimer occurs due to the attachment of the same primer, either forward with forward or reverse with reverse. Cross Dimer occurs due to hybridization between the forward primer and reverse primer or vice versa. cross-dimer at the 3' end with G = -5 kcal/mol and the cross-dimer at the internal with G = - 6 kcal/mol is still tolerable (Chen et al., 2003).

Molecular analysis of mutations or SNPs in genes requires primers as amplification starters. The right primer design will get the specific part of sequence. The non-specificity of the primer will result in the failure of the amplification process or there are the other targets also amplified, mismatch or incorrect design of the primer with the DNA template will cause the amplification to fail (Asif et al., 2021).

The primer's ability to amplify then tested using 6 samples. all samples used positive DM (figure 1. C). The sample used is only to validate the primary ability whether it can be used to amplify specifically or not. Results Based on the research the gene was well amplified and showed a long band in accordance with the target (687 bp). Overall the primer works well according to the in silico studies. Furthermore, the results of this primer design can be used to identify the involvement of LRGUK in DT2.

CONCLUSION AND SUGGESTION

Based on this research, it can be concluded that the designed primer can amplify the LRGUK gene specifically with a target band of 687 bp. This can be proven on the electrophoresis visualization of the PCR results that the band length corresponds to the product length.

The recommendation from the results of this study is that the primer design results can be used to analyze the characteristics of the LRGUK gene and its involvement in DT2.

REFERENCES

Kido Y. (2017). Gene–environment interaction in type 2 diabetes. Diabetol Int. 8(1):7–13.

Siewert S, Márquez F, Mendoza G, Fernández G, González I, Ojeda MS. (2015). Association of TCF7L2 Gene Polymorphisms with T2DM in the Population of Juana Koslay , San Luis Province , Argentina. pp 1–10.

Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract [Internet]. 103(2):137–49. Available from: http://dx.doi.org/10.1016/j.diabres.2013.11.002

Mushlih M. (2020). Difference of Red Blood Cell Count (RBC) Levels in Diabetes Mellitus Type II with Ulcers and without Ulcers. J Ris Biol dan Apl. Vol. 2(1):6–10.

Webber S. (2013). International Diabetes Federation. Vol. 102, Diabetes Research and Clinical Practice. 147–148 p.

Barroso I, Luan J, Middelberg RPS, Harding AH, Franks PW, Jakes RW, et al. (2003). Candidate gene association study in type 2 diabetes indicates a role for genes involved in β-cell function as well as insulin action. PLoS Biol. Vol. 1(1):41–55.

Han X, Luo Y, Ren Q, Zhang X, Wang F, Sun X, et al. (2010). WFS1 in Type 2 Diabetes in a Chinese population. BMC Med Genet. Vol. 10–5.

McCarthy MI. (2010). Genomics, Type 2 Diabetes, and Obesity. N Engl J Med. 363(24):2339–50.

Zahid RA, Sulaiman BK, Abd, Ahmed B. (2011). Molecular Investigation of Genetic Polymorphisms. Iraqi J Cancer Med Genet. Vol. (2):47–54.

Kartikasari EW. (2021). Identifikasi gen pada alel positif penanda Diabetes Mellitus tipe II menggunakan marker primer D 20. Universitas Muhammadiyah Sidoarjo.

Mushlih M, Sari FK, Amin HS, Iknan SA. (2020). Identification of molecular markers for type 2 Diabetes mellitus in Sidoarjo, Indonesia. J Teknol Lab. Vol. 9(2):186–91.

Mushlih M. (2021). BIOLOGI MOLEKULER. "Aplikasi Dasar di Dunia Kesehatan".

Mushlih M, Sari FK, Hadie DA, Ardiyansyah S. (2021). Genetic Polymorphism In Individuals With Type II Diabetes Mellitus Using PCR-RAPD In Sidoarjo District. Vol. 02:153–9.

Ramkisson S, Pillay BJ, Sibanda W. (2017). African Journal of Primary Health Care and Family Medicine. African J Prim Heal Care Fam Med [Internet]. Vol. 9(1):8. Available from: https://phcfm.org/index.php/phcfm/article/view/1405/2160

Laramie JM, Wilk JB, Williamson SL, Nagle MW, Latourelle JC, Tobin JE, et al. (2008). Polymorphisms near EXOC4 and LRGUK on chromosome 7q32 are associated with Type 2 Diabetes and fasting glucose; The NHLBI Family Heart Study. BMC Med Genet. Vol. 9:1–9.

Green SJ, Venkatramanan R, Naqib A. (2015). Deconstructing the polymerase chain reaction: Understanding and correcting bias associated with primer degeneracies and primer-template mismatches. PLoS One. Vol. 10(5):1–21.

Chen SH, Lin CY, Cho CS, Lo CZ, Hsiung CA. (2003). Primer Design Assistant (PDA): A web-based primer design tool. Nucleic Acids Res. Vol. 31(13):3751–4.

Asif S, Khan M, Waqar Arshad M, Shabbir MI. (2021). PCR Optimization for Beginners: A Step by Step Guide. Res Mol Med.Vol. 9(2):81–102.

Naroeni A, Arrasul LNP, Saraswati H, Iskandar AD. (2020). Optimization of in House Polymerase Chain Reaction for High Risk Human Papillomavirus (Hpv 18) E1 Genebased Detection. Vol. 11:1767–75.

sktp-30-05-2023 06_38_44-215511.pdf

ORIGINALITY REPORT

5%	4%	2%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	eudl.eu Internet Source	1%
2	www.ncbi.nlm.nih.gov Internet Source	1%
3	isrc.in Internet Source	1%
4	Submitted to UC, Irvine Student Paper	1%
5	ejournal.iainpalopo.ac.id Internet Source	1%

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		