RESEARCH ARTICLE

Diabetes Risk Allele of Transcription Factor 7-like 2 (*TCF7L2*) Polymorphisms is Associated with Higher Glucagon-like Peptide 1 (GLP1) and Lower Insulin Secretion

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Received date: Jul 17, 2024; Revised date: Sep 13, 2024; Accepted date: Sep 23, 2024

Abstract

BACKGROUND: The most influential susceptible gene associated with diabetes, transcription factor 7-like 2 (*TCF7L2*), has been observed in diverse populations. *TCF7L2* influences type 2 diabetes risk through glucagon-like peptide 1 (GLP1) production. The presence of risk allele of *TCF7L2* leads to the alteration of gene expression in pancreatic beta cells; however, how the mechanism is related with GLP1 remains unclear. This study was conducted to explore the variations of GLP1 increment and insulin secretion between individuals with and without diabetes risk allele of single nucleotide polymorphisms (SNPs) in *TCF7L2*.

METHODS: A cross-sectional analytic study was conducted involving individuals subjects who harbored known variants of SNPs in the *TCF7L2*: heterozygote or mutant of rs12255372 (GT or TT), rs7903146 (CT or TT), rs10885406 (AG or GG); as well as control subjects with wild type of rs12255372 (GG), rs7903146 (CC), and rs10885406 (AA). Anthropometric parameters, blood glucose, insulin, and GLP1 were measured; and homeostasis model assessment-beta cell (HOMA-%B) index was calculated.

RESULTS: The GLP1 increment response was higher in subjects carrying the diabetes risk allele (0.34±0.80 ng/mL) than those with the wild type (-0.04±0.57 ng/mL) (p=0.041). The HOMA-%B was reduced in subjects carrying the diabetes risk allele (71.64±24.72) than those with the wild type (103.23±68.00) (p=0.011). Among individuals carrying the diabetes risk allele, the likelihood of GLP1 increment with high response was twice as high (p=0.007), while the occurrence of low HOMA-%B was 1.47 more frequent (p=0.011).

CONCLUSION: *TCF7L2* polymorphisms were associated with the GLP1 increment response and reduced HOMA-%B, which might be potentially contributing to GLP1 resistance in patients with diabetes risk factors.

KEYWORDS: diabetes risk, *TCF7L2*, GLP1, HOMA-%B

Indones Biomed J. 2024; 16(5): 442-9

Introduction

The family history of diabetes strongly plays a role in diabetes development, particularly type 2 diabetes mellitus (T2DM), however the risk is still not confirmed to the Mendelian Law. More than 20 diabetes-related genes have been identified in the last decade. Genetic studies in T2DM have revealed substantial information that identified beta cell dysfunction related genes and diabetes risk alleles were common in population, however the those effects in diabetes risk remain relatively low.(1) The main pathophysiology of

T2DM is insulin resistance and progressive loss of insulin. (2) In the earlier states, insulin secretion is increasing to maintain normal blood sugar level. The defect of insulin secretion in early is relatively mild and selectively related to glucose-stimulated insulin secretion. The etiology of insulin secretion defect has not been clearly defined yet, but genetic factors are possibly have a role. Transcription factor 7-like 2 (*TCF7L2*), is one of diabetes susceptible gene that has been identified prior of Genome-wide Association Study (GWAS) (3), and has been explored in various population, including: Americans, Europeans, and Africans (4-12). *TCF7L2* is also associated with T2DM in Asian population, including China (13), Hongkong (14), India (15), and Japan (16,17). Recent study in the Balinese population revealed that the CT genotype of rs7903146 had a significant of high blood sugar level compare to CC and TT genotypes.(18)

The association of *TCF7L2* with T2DM has been consistently replicated in multiple populations with diverse genetic origins, however the mechanisms by which *TCF7L2* exerts its effect on T2DM remains poorly understood. (19) Some evidences showed that *TCF7L2* influences T2DM risk through the influence of glucagon-like peptide 1 (GLP1) production in the intestine. Initial studies indicate an impact on the insulinogenic index, suggesting that this genetic locus operates through insulin secretion mechanisms.(20-22) Indeed, research has demonstrate that the risk variant is linked with increased *TCF7L2* expression at the mRNA level, while simultanously decreasing insulin secretion in pancreatic β-cell.(23) The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and GLP1 take a crucial physiological role in enhancing insulin secretion following their release from the gut in response to nutrients.(24)

The homeostasis model assessment (HOMA) is a method used to quantify insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B). HOMA is derived from a mathematical assessment of the balance between hepatic glucose output and insulin secretion, based on the glucose and insulin fasting levels.(25) It has been proposed as a reliable β-cell function measurement.(26) The HOMA-%B estimates steady state of the beta cell function and correspond well with non-steady state estimates of beta cell function. However it is not necessarily equivalent to those derived from stimulatory models such as the hyperinsulinaemic clamp, the hyperglycaemic clamp, the intravenous glucose tolerance test (acute insulin response, minimal model), and the oral glucose tolerance test (OGTT).(27)

TCF7L2 has been shown in various population as the strongest susceptible gene in diabetes. However, the mechanisms involving GLP1 are not yet understood, even it has contributed in insulin synthesis as well as beta cell differentiation and proliferation. The risk *TCF7L2* alleles induces alteration in gene expression of pancreatic beta cells. HOMA-%B could be utilized to estimate the pancreatic beta cell function. Therefore, this study demonstrated whether GLP1 response after an oral glucose load differs between individuals with and without diabetes risk allele of single nucleotide polymorphisms (SNPs) in the *TCF7L2*, and whether the HOMA-%B differ between these two groups.

Methods

Study Design and Subjects

A cross sectional analytic was conducted to explore the difference of GLP1 increment and insulin secretion between subjects with and without diabetes risk allele of *TCF7L2* SNPs. The subjects were taken from Legian study population (18), who has been known to have the variants of *TCF7L2* SNPs. Twenty-eight subjects aged >35 years old were grouped with the matching of age and sex in each group. Subjects with heterozygote or mutant of rs12255372 SNP (GT or TT), rs7903146 (CT or TT), and rs10885406 (AG or GG) were grouped into subjects with diabetes risk allele; while subjects with wild type of rs12255372 (GG), rs7903146 (CC), and rs10885406 (AA) were group into subjects without diabetes risk allele or as controls. Subjects under any medication with any type of incretin-based therapy (GLP1 analogues or dipeptidyl peptidase (DPP4) inhibitors), and gastrointestinal disorders based on history taking and physical examination were excluded from the study. Protocol of this study has been approved by Ethic Commission of Research and Development Department Faculty of Medicine Udayana University Denpasar (No. 1318/UN.14.2/Litbang/20140), and written informed consent was obtained from all participants.

Anthropometric Assessment

A careful history of subjects was taken, and the physical condition was examined to determined whether the subjects were excluded due to gastrointestinal disorders or not. Simple anthropometric measurements were applied, including body weight, height, and waist circumference (WC) while the subject stood upright. Waist circumference was measured midway between the lower costal margin and the iliac crest. Body mass index (BMI) was calculated as the weight measured in kilograms divided by the height in meters after squaring it (kg/m^2).

Evaluation of Blood Glucose, Insulin, and HOMA-%B

An oral glucose tolerance test was done for both groups. Blood samples were taken during fasting, 1-hour- and 2-hours-after oral glucose load, for blood glucose measurement. A 75 g glucose anhydrous dissolved in 300 mL of water as standard oral glucose load was used. Fasting and 1-hour-after glucose load of blood sample were collected for insulin measurement. Serum separator tube (SST) BD Vacutainer SSTTM II Advance (Ref.: 367955; BD, Plymouth, UK) was used for blood sample collection for the measurement of glucose and insulin. Later, the blood glucose and insulin level measurement were performed in accordance to the standard protocol. Beta cell function determined basal insulin secretion, was later calculated based on HOMA-%B index.

GLP1 Analysis with Enzyme Linked Immunosorbent Assay (ELISA)

Along with blood sampling for insulin measurement, fasting and 1-hour-after glucose load of blood sample were also collected for GLP1 measurement. EDTA tube BD Vacutainer (Ref.: 367856, BD, Franklin Lakes, NJ, USA), was used for this sample collection. GLP1 examination was performed using a human GLP1 ELISA (multispecies specificity) kit (Cat. No.: RSCYK160R; Biovendor, Asheville, NC, USA). The principle of examination was based on a competitive enzyme immunoassay using a combination of specific antibodies to GLP1(7-36) with the biotin-avidine affinity system. The plate with 96 wells was coated with anti-rabbit IgG antibodies from goats. Standard GLP1 or samples, labeled antigens, and GLP1 antibodies were added to the wells, resulting in competitive immunoreactions. After incubation and washing of the dishes, HRP-labeled streptoavidine (SA-HRP) was added to form an HRP-labeled streptoavidin-biotinylated GLP1-antibody complex on the surface of the well wall. Finally, the activity of the HRP enzyme was determined by o-Phenylenediamine dihydrochloride (OPD) and the GLP1 concentration was calculated.

Determination of TCF7L2 SNPs

DNA samples were isolated from the blood sample in the EDTA tube using Puregene Kit (Qiagen, Hilden, Germany) method with modification, as described in the previous study.(28) SNPs of the *TCF7L2*: rs7903146, rs12255372, and rs10885406 were identified using the polymerase chain reaction restricted fragment length polymorphism (PCR-RFLP) method. The first step was DNA isolation from the peripheral blood sample, and DNA content was measured by NanoDrop-1000® (Thermo Fisher Scientific, Waltham, MA, USA), followed by PCR using a specific primer for each SNPs. The PCR product was visualized using electrophoresis gel. The next step was RFLP using a specific enzyme for each SNPs. RFLP product was visualized using electrophoresis gel. Detection of rs7903146 was performed using a previously published PCR-RFLP method (29), while genotyping assays of rs12255372 and rs10885406 were performed using novel PCR-RFLP methods (30).

Results

Characteristics of the Subject

A total of 56 subjects were involved in this study (Table 1), which consist of 28 subjects with diabetes risk allele and 28 subjects without diabetes risk allele. The mean of age of subjects with diabetes risk allele was 50.50±10.44 years old, and subjects without diabetes risk allele was 52.46±9.31 years; which were not significantly different (p=0.462). Out of 56 subjects, 36 (64.3%) were male, while the other 20 (35.7%) were female.

Based on diabetes and prediabetes diagnosis criteria, the OGTT result revealed that 7 of 56 subjects (12.50%) were diabetes, 13 subjects (23.21%) had prediabetes (including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)), and 36 subjects (64.29%) were in normal condition. Among 7 diabetes subjects, 5 people

Table 1. Characteristic of the subjects (n=56).

Parameter	Mean±SD	Range
Age (years)	51.48±9.87	30 – 74
Body weight (kg)	70.88±14.53	34 – 115.40
Body height (cm)	161.86±9.40	144.50 – 182.00
BMI (kg/m^2)	26.88±4.08	15.11 – 35.34
WC (cm)	90.16±10.85	60.00 – 116.00
Male	94.05±9.63	71.50 –116.00
Female	83.15±9.47	60.00 – 99.00
Fasting glucose (mg/dL)	102.23±38.89	73.00 – 310.00
1-hour glucose (mg/dL)	173.96±80.71	81.00 – 511.00
2-hours glucose (mg/dL)	138.98±83.02	76.00 – 500.00
Fasting insulin (µIU/mL)	7.22±3.86	2.00 – 18.90
1 hour insulin (µIU/mL)	78.33±41.94	6.00 – 174.00
Delta insulin (µIU/mL)	71.11±40.58	2.50 – 164.70
Fasting GLP1 (ng/mL)	3.28±1.02	1.74 – 3.28
1-hour GLP1 (ng/mL)	3.43±1.06	1.94 – 7.89
Delta GLP1 (ng/mL)	0.148±0.718	(-1.70) – 1.98
HOMA-%B	86.81±52.31	6.20 – 404.30

belonged to those with diabetes risk allele of *TCF7L2* SNPs (Table 2). Fasting blood glucose levels, 1-hour- and 2-hours-after OGTT, had similar patterns in both groups and tended to be higher in the subject with diabetes risk alleles, but not significantly different (Table 3, Figure 1).

The HOMA-%B was calculated in 50 subjects, 26 subjects with diabetes risk allele, and 24 subjects without diabetes risk allele of *TCF7L2* SNPs. HOMA-%B was not estimated in 6 subjects due to very low insulin level, which was below 2.9 µU/L as the minimum standard of insulin level for HOMA-%B calculation.

Different Response of GLP1 Increment after Oral Glucose Load and Insulin Secretion between Subjects with and without Diabetes Risk Allele of *TCF7L2* SNPs

The blood fasting glucose level of GLP1 subjects with diabetes risk allele was 3.29±1.19 ng/mL, then one hour after oral glucose load was 3.63±1.22 ng/mL. It showed an increment of GLP1 (delta GLP1 calculated by subtracting GLP1 1 hour after oral glucose load with fasting GLP1 was 0.34±0.80 ng/mL). Meanwhile, the blood fasting glucose level of GLP1 subjects without diabetes risk allele was 3.28±0.85 ng/mL, then glucose level of 1-hour-after oral glucose load was 3.23±0.84 ng/mL. This group's result contradicted the previous one which the increment of GLP1 was negative 0.04±0.57 ng/mL. The GLP1 increment response following oral glucose load was higher in subjects with diabetes risk allele of *TCF7L2* SNPs than those without with mean difference was 0.38 (p=0.041) (Table 3, Figure 2).

HOMA-%B determined the insulin secretion determined and found it was lower in subjects with diabetes risk allele compare to those without diabetes risk allele (p=0.011) (Table 3). High GLP1 increment response was found twice as high among subjects with diabetes risk allele (prevalence ratio (PR)=2.00; 95%CI: 1.15–3.46; p=0.007). Moreover, the likelihood of low HOMA-%B level was 1.47 time more frequent (RP=1.47; 95%CI: 1.06–2.05; p=0.011) compare to those without diabetes risk allele. Based on these finding, this study confirmed that response of GLP1 increment following oral glucose load and insulin secretion in subjects with and without diabetes risk allele were different.

Table 2. Frequency distribution of normoglycemia, prediabetes (IFG, IGT), and diabetes.

	n (%)		
	Subjects with Diabetes Risk Allele	Subjects without Diabetes Risk Allele	Total
Diabetes	5 (17.86)	2 (7.14)	7 (12.50)
IFG	3 (10.71)	5 (17.86)	8 (14.29)
IGT	2 (7.14)	3 (10.71)	5 (8.92)
Normal	18 (64.29)	18 (64.29)	36 (64.29)
Total	**28 (100)**	**28 (100)**	**56 (100)**

Discussion

In this study, two anthropometric parameters of obesity (BMI and WC) were evaluated. There was no mean difference in the BMI between subjects with and without diabetes risk allele of the *TCF7L2* SNPs (p=0.795). The mean BMI of both groups were between 25-29.9 kg/m^2, which was categorized as obese I with moderate risk of co-morbidities, based on obesity classification for the Asia Pacific population.(31) The results of this study showed that, WC among male and female subjects, both in with and without diabetes risk allele group, exceeded the risk co-morbidities cut-off, which are ≥90 cm in males and ≥80cm in females.(31) Since both groups were overweight or obese, suggesting that obesity may not have played a role in the difference in GLP1 increment and insulin secretion, and the difference was strongly possible due to genotype, specifically the *TCF7L2* SNPs. In this study, the oral glucose load was carried out to trigger an increase in GLP1 at 1-hour-after glucose loading while also determining the diagnosis of glucose disorders by looking at fasting and 2-hours-after glucose load. Results of OGTT showed 23.20% with prediabetes and 12.50% with diabetes. The percentage of subjects with prediabetes and diabetes was higher in the group with diabetes risk allele of SNPs in the *TCF7L2*. The oral glucose tolerance test is a sensitive examination for diabetes screening, especially in newly diagnosed patients or in groups with diabetes risks such as insulin resistance.(32)

Higher response of GLP1 increment in subjects with diabetes risk allele may be due to two reasons: first, delayed GLP1 secretion so that GLP1 level still detected at high level one hour after the glucose load. Second, the GLP1 requirement to stimulate insulin secretion in subjects with diabetes risk allele is higher than in those without. A study in healthy Japanese with short duration of T2DM showed that the level of intact GLP1 was low, as well as the increment of GLP1.(33) The GLP1 measurement methods and measurement units in each study were different, which caused difficulties while comparing one to another study results. That study also measured the intact GLP1 and total GLP1 (intact and DPP4 processed). They found

Table 3. Mean difference of blood glucose, insulin, and GLP1 levels in subjects with and without diabetes risk allele of the SNPs in the *TCF7L2*.

Parameter	Mean±SD		Mean difference (95% CI)	*p*-value
	Subjects with Diabetes Risk Allele (n= 28)	Subjects without Diabetes Risk Allele (n= 28)		
Fasting glucose (mg/dL)	111.57±53.48	92.89±7.78	18.67 (-1.72 – 39.08)	0.221[a)]
1-hour glucose (mg/dL)	191.64±104.53	156.28±41.17	35.35 (-7.73 – 78.45)	0.451[a)]
2-hours glucose (mg/dL)	155.39±108.75	124.57±41,49	30.82 (-13.85 – 75.49)	0.793[a)]
Fasting insulin (µIU/mL)	7.14±4.03	7.30±3.76	-0.16 (-2.25 – 1.92)	0.652[a)]
1-hour insulin (µIU/mL)	79.89±38.82	76.78±45.51	3.10 (-19.55 – 25.77)	0.784[b)]
Delta insulin (µIU/mL)	72.74±38.51	69.47±43.19	3.26 (-18.64 – 25.19)	0.766[b)]
Fasting GLP1 (ng/mL)	3.29±1.19	3.28±0.85	-0.007 (-0.54 – 0.56)	0.706[a)]
1-hour GLP1 (ng/mL)	3.63±1.22	3.23±0.84	0.39 (-0.96 – 0.16)	0.279[a)]
Delta GLP1 (ng/mL)	0.34±0.80	-0.04±0.57	0.38 (0.01 – 0.76)	0.041[b)]*
HOMA-%B	71.64±24.72	103.23±68.00	-31.59 (-60.24 – [-2.93])	0.011[a)]*

*Significant if *p*-value<0.05. [a)]*p*-value for Mann Whitney U test; [b)]*p*-value for independent t test.

fasting level of total GLP1 was 15.5±1.7 pM in T2DM and 15.7±1.0 pM in control group. However, the fasting level of intact GLP1 was 0.2±0.1 pM in T2DM and 0.7±0.2 pM in the control group. Meanwhile in this study, the total GLP1 level observed was covering intact GLP1 (7-36 ng/mL) and the first metabolite of GLP1 (9-36 ng/mL).

Low HOMA-%B in subject with diabetes risk allele of the *TCF7L2* SNPs variant may reflect the impaired insulin secretion function of pancreatic beta cells. After the population study, it was concluded that TCF7L2 risk allele predisposes individuals to T2DM by its effect on pancreatic beta cells.(1) The risk allele causes over expression of *TCF7L2* in the pancreatic beta cell and decrease insulin secretion in responseto various stimuli.

There are some possibilities that need to be elucidated related to the role of *TCF7L2* in diabetes pathogenesis, more specifically GLP1 and insulin secretion. Firstly, gene expression in subjects with diabetes risk alleles of the variants SNPs in the *TCF7L2* may upregulated or downregulated, which then upregulated or downregulated the protein Tcf7l2 in terms of the level and their function. Secondly, the consequence of different gene expressions, protein levels, and function may affect the GLP1 and insulin secretion. Lastly, the regulation of the *TCF7L2* expression, which changes the GLP1 response and insulin secretion, may contribute a role as diabetes risk factor, or contrary, may play a role as physiologic mechanisms to maintain the blood sugar level during high-calorie intake. *TCF7L2* small interfering RNA in the human pancreatic islet isolate showed that *TCF7L2* depletion increased beta cell apoptosis 5.1 times, decreased beta cell proliferation 2.2 times, and decreased glucose-stimulated insulin secretion 2.6 times, and this similar effect was also found in the rat *TCF7L2* depleted pancreatic islet. On the other hand, *TCF7L2* over

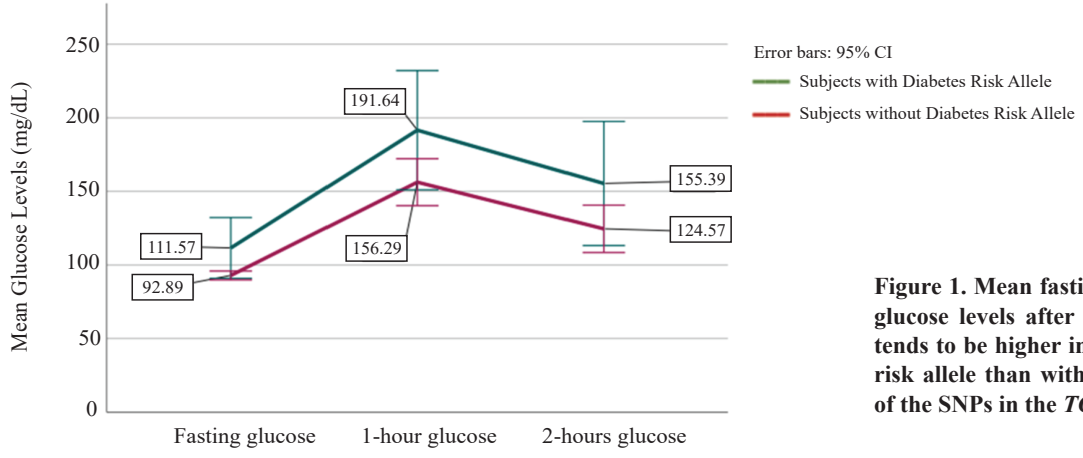

Figure 1. Mean fasting, 1-hour and 2-hours glucose levels after 75 g oral glucose load tends to be higher in subjects with diabetes risk allele than without diabetes risk allele of the SNPs in the *TCF7L2*.

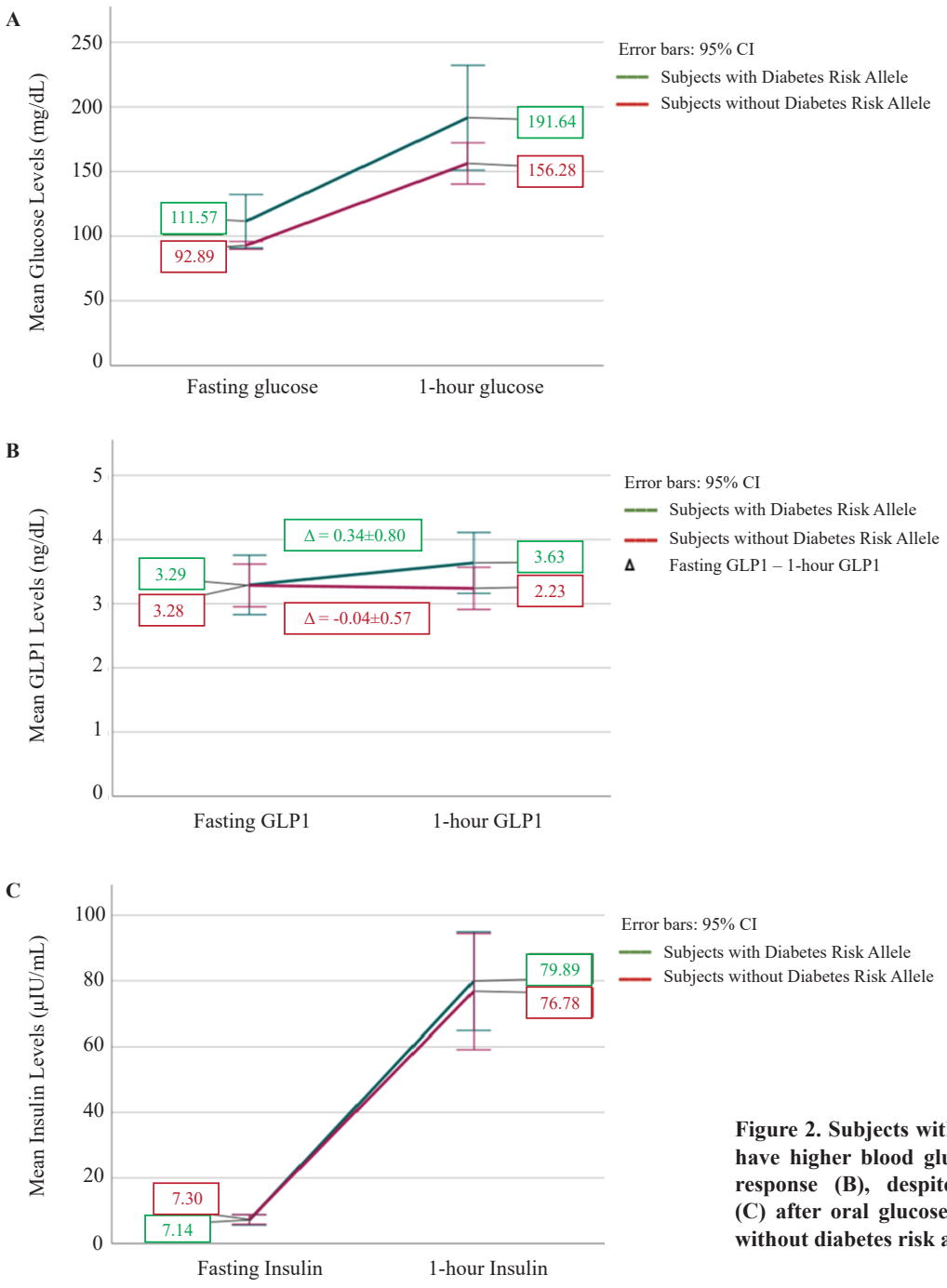

Figure 2. Subjects with diabetes risk allele tend to have higher blood glucose (A) and higher GLP1 response (B), despite similar insulin response (C) after oral glucose load compared to subjects without diabetes risk allele.

expression avoids the pancreatic islet from glucose and cytokine mediated beta cell apoptosis.

In our perspective, the diabetes risk allele of the variant SNPs in the *TCF7L2* is related to persistent overexpression of *TCF7L2* in many tissues. Persistent overexpression of the *TCF7L2* in the pancreatic beta cell increases (or might decrease) the Tcf7l2 protein level, with lower Tcf7l2 protein function, and leads to lower insulin secretion. In early diabetes, GLP1 may increase to induce the Wnt-signaling as a response to low insulin secretion. This perspective of thought explains why HOMA-%B is lower and the response of GLP1 increments higher in subjects with diabetes risk alleles of the variant SNPs in the *TCF7L2*. Subjects with diabetes risk alleles may need a higher increment of GLP1 to increase insulin secretion in order to maintain blood glucose in the normal range, which brings up a new concept of GLP1 resistance of the pancreatic beta cell. The mechanism by which the *TCF7L2* plays a role in diabetes needs further study, in particular, to confirm the new concept of GLP1 resistance of the pancreatic beta cell.

Previous study in diabetes and normal subjects found lower GLP1 levels in both fasting and 1 hour after oral glucose loading in diabetes compared with normal subjects.(34) In this study, patients with diabetes risk allele tend to have higher both fasting and 1-hour post oral glucose load GLP1 while having lower HOMA-%B. The inverse finding of these two studies may relate to different time frames of the diabetes natural history. In the prior study, diabetes subjects were inpatients with diabetes complications, and subjects in this recent study were at risk of diabetes or in a very early stage. Further study is needed to elaborate on whether disease duration affects the GLP1 level as well as GLP1 increment during oral glucose load. Studies on other cytokines and substrates also need to elaborate in diabetes since some cytokines have been found to be associated with HOMA-%B, for example, visfatin.(35) Genetic studies related to diabetes is a broad field to study since it turns out that diabetes is related to various things, such as studies that link the melatonin receptor coding gene to obesity in diabetes.(36)

In this study, blood sampling for blood sugar level examination were only performed 3 times, including fasting, 1-hour- and 2-hours-after oral glucose loading; while blood sampling for GLP1 and insulin level were only examined 2 times, including fasting and 1-hour-after oral glucose loading. Since blood glucose, insulin, and GLP1 levels dynamically changes within minutes, so with only 2 or 3 times blood sampling, the whole dynamic changes of blood glucose, insulin, and GLP1 levels after oral glucose loading can not be assured. This limitation was noticed clearly in some subjects with lower GLP1 levels 1-hour-after oral glucose loading in comparison with their own fasting GLP1 level in which the peak level of GLP1 might have been missed and the GLP1 level has been going down. Therefore, further study with more frequency of sample collections might be needed.

Conclusion

Response of GLP1 increment after oral glucose load was higher while the insulin secretion was lower in subjects with diabetes risk allele compared with subjects without diabetes risk alleles of *TCF7L2* SNPs. Hence, *TCF7L2* polymorphisms were suggested to be associated with the GLP1 increment response and reduced HOMA-%B, potentially contributing to GLP1 resistance in patients with diabetes risk factors.

Acknowledgments

We would like to thank all the subjects who participated in this research, which was coordinated by Mr. I Wayan Budiasa and Mr. Wayan Sunadi. This research would not have been carried out without good cooperation between Legian Village and the Legian Village Precredit Institution (Lembaga Perkreditan Desa/LPD). We acknowledge technical support from laboratory team provided by Prodia Laboratory Denpasar Branch during the study. Part of this study was funded by the Cardiometabolic Research Group of the Faculty of Medicine, Udayana University.

Authors Contribution

MRS, KS, AAGB concepting and planning the research. SO and SGM perform genetic checks. MRS performed the data acquisition/collection, performed the analysis, drafted the manuscript and designed the figures. All authors took part in giving critical revision of the manuscript.

References

1. Gloyn AL, Braun M, Rorsman P. Type 2 diabetes susceptibility gene TCF7L2 and its role in β-cell function. Diabetes. 2009; 58(4): 800-2.
2. Kartika R, Wibowo H. Impaired function of regulatory T cells in type 2 diabetes mellitus. Mol Cell Biomed Sci. 2020; 4(1): 1-9.
3. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, *et al.* Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006; 38(3): 320-3.
4. Zhang C, Qi L, Hunter DJ, Meigs JB, Manson JE, van Dam RM, *et al.* Brief genetics report: Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of U.S. women and den. Diabetes. 2006; 55(9): 2645-8.
5. Goodarzi MO, Rotter JI. Testing the gene or testing a variant? The case of TCF7L2. Diabetes. 2007; 56(10): 2417-9.
6. Helgason A, Palsson S, Thorleifsson G, Grant SF, Emilsson V, Gunnarsdottir S, *et al.* Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet. 2007; 39(2): 218-25.
7. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, *et al*. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007; 445(7130): 881-5.
8. Van Vliet-Ostaptchouk JV, Shiri-Sverdlov R, Zhernakova A, Strengman E, van Haeften TW, Hofker MH, *et al*. Association of variants of transcription factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda Cohort. Diabetologia. 2007; 50(1): 59-62.
9. Meigs JB, Manning AK, Fox CS, Florez JC, Liu C, Cupples LA,

et al. Genome-wide association with diabetes-related traits in the Framingham Heart Study. BMC Med Genetics. 2007; 8(Suppl 1): S16. doi: 10.1186/1471-2350-8-S1-S16.

10. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, *et al.* Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med. 2008; 359(21): 2220-32.
11. Amoli MM, Amiri P, Tavakkoly-Bazzaz J, Charmchi E, Hafeziyeh J, Keramatipour M, *et al.* Replication of TCF7L2 rs7903146 association with type 2 diabetes in an Iranian population. Genet Mol Biol. 2010; 33(3): 449-51.
12. Danquah I, Othmer T, Frank LK, Bedu-Addo G, Schulze MB, Mockenhaupt FP. The TCF7L2 rs7903146 (T) allele is associated with type 2 diabetes in urban Ghana: A hospital-based case–control study. BMC Med Genet. 2013: 14: 96. doi: 10.1186/1471-2350-14-96.
13. Chang YC, Chang TJ, Jiang YD, Kuo SS, Lee KC, Chiu KC, *et al.* Association study of the genetic polymorphisms of the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes in the Chinese population. Diabetes. 2007; 56(10): 2631-7.
14. Ng MC, Tam CH, Lam VK, So WY, Ma RC, Chan JC. Replication and identification of novel variants at TCF7L2 associated with type 2 diabetes in Hong Kong Chinese. J Clin Endocrinol Metab. 2007; 92(9): 3733-7.
15. Chandak GR, Janipalli CS, Bhaskar S, Kulkarni SR, Mohankrishna P, Hattersley AT, *et al.* Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. Diabetologia. 2007; 50(1): 63-7.
16. Hayashi T, Iwamoto Y, Kaku K, Hirose H, Maeda S. Replication study for the association of TCF7L2 with susceptibility to type 2 diabetes in a Japanese population. Diabetologia. 2007; 50(5): 980-4.
17. Horikoshi M, Hara K, Ito C, Nagai R, Froguel P, Kadowaki T. A Genetic Variation of the Transcription Factor 7-like 2 Gene is Associated with Risk of Type 2 Diabetes in the Japanese Population. Diabetologia. 2007; 50(4): 747-51.
18. Saraswati MR, Suastika K, Sudoyo H, Malik SG. Association of TCF7L2 polymorphisms with diabetes, lipid, and obesity in Balinese population. Indones J Biomed Sci. 2017; 11(2): 6-10.
19. Del Bosque-Plata L, Martínez-Martínez E, Espinoza-Camacho MÁ, Gragnoli C. The role of TCF7L2 in type 2 diabetes. Diabetes. 2021; 70(6): 1220-8.
20. Yi F, Brubaker PL, Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. J Biol Chem. 2005; 280(2):1457-64.
21. Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, *et al.* Diabetes prevention program research group. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med. 2006; 355(3): 241-50.
22. Saxena R, Gianniny L, Burtt NP, Lyssenko V, Giuducci C, Sjögren M, *et al.* Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. Diabetes. 2006; 55(10): 2890-5.
23. Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, *et al.* Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest. 2007; 117(8): 2155-63.
24. Nauck MA, Quast DR, Wefers J, Pfeiffer AFH. The evolving story of incretins (GIP and GLP-1) in metabolic and cardiovascular disease: A pathophysiological update. Diabetes Obes Metab. 2021; 23(Suppl 3): 5-29.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7): 412-9.
26. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modelling. Diabetes Care. 2004; 27(6): 1487-95.
27. Diabetes Trial Units [Internet]. HOMA calculator. The Oxford Centre for Diabetes, Endocrinology and Metabolims, University of Oxford [update 2015 Jan 2; cited 2015 Jan 27]. Available from: https://www.dtu.ox.ac.uk/homacalculator/.
28. Malik SG, Saraswati MR, Suastika K, Trimarsanto H, Oktavianthi S, Sudoyo H. Association of beta3-adrenergic receptor (ADRB3) Trp64Arg gene polymorphism with obesity and metabolic syndrome in the Balinese: a pilot study. BMC Res Notes. 2011; 4: 167. doi: 10.1186/1756-0500-4-167.
29. Marquezine GF, Pereira AC, Sousa AGP, J G Mill, W A Hueb, J E Krieger. TCF7L2 variant genotypes and type 2 diabetes risk in Brazil: significant association, but not a significant tool for risk stratification in the general population. BMC Med Genet 2008; 9: 106. doi: doi: 10.1186/1471-2350-9-106.
30. Oktavianthi S, Saraswati MR, Suastika K, Dwipayana P, Sulfianti A, Hayati RF, *et al.* Transcription factor 7-like 2 single nucleotide polymorphisms are associated with lipid profile in the Balinese. Mol Biol Rep. 2018; 45(5): 1135-43.
31. World Health Organization [Internet]. Regional Office for the Western Pacific. 2000. The Asia-Pacific perspective : redefining obesity and its treatment treatment. Sydney: Health Communications Australia. [cited 2024 Jun 1]. Available from: https://iris.who.int/handle/10665/206936.
32. Altemimi MT, Musa AK, Mansour AA. The performance of glycated hemoglobin vs. oral glucose tolerance test in the diagnosis of glycemic disorders among women with polycystic ovary syndrome in Southern Iraq. Indones Biomed J. 2021; 13(2): 178-85.
33. Yabe D, Kuroe A, Lee S, Watanabe K, Hyo T, Hishizawa M, *et al.* Little enhancement of meal-induced glucagon-like peptide 1 secretion in Japanese: Comparison of type 2 diabetes patients and healthy controls. J Diabetes Invest. 2010; 1(1-2): 56-9.
34. Lastya A, Saraswati MR, Suastika K. The low level of glucagon-like peptide-1 (GLP1) is a risk factor of type 2 diabetes mellitus. BMC Res Notes. 2014; 7: 849. doi: 10.1186/1756-0500-7-849.
35. Putera BW, Sartika CR, Wijaya A. The dynamic roles of visfatin and obestatin serum concentration in pancreatic beta cells dysfunction (HOMA-B) and insulin resistance (HOMA-IR) in centrally obese Men. Indones Biomed J. 2012; 4(1): 43-9.
36. Tursinawati Y, Kartikadewi A, Yuniastuti A, Susanti R. Association of rs10830963 MTNR1B and rs841853 SLC2A1 polymorphism with obesity on type 2 diabetes patients: An overview of melatonin receptor and transporter. Indones Biomed J. 2021; 13(2): 106-220.