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Single Nucleotide Polymorphism at rs7903146 of Transcription Factor 7-like 2 gene Among Subjects with Type 2 Diabetes Mellitus in Myanmar

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Abstract

Objectives. To investigate the association between the single nucleotide polymorphism (SNP) rs7903146 in the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes mellitus (T2DM) and to examine the impact of this variant on pancreatic beta-cell function in the Myanmar population.

Methodology. A case-control study was undertaken in 100 subjects with T2DM and 113 controls. The SNP rs7903146 was genotyped using the allele-specific polymerase chain reaction method. Plasma glucose and serum insulin levels were determined using the enzymatic colorimetric method and ELISA respectively. Beta-cell function was calculated by the HOMA-β formula.

Results. The frequencies of carrier genotypes (CT and TT) were higher in subjects with T2DM than in controls. The minor T alleles of rs7903146 were found to statistically increase type 2 diabetes risk than the C allele with an allelic odds ratio of 2.07 (95% CI 1.39-3.09, p=0.0004). The mean HOMA-β level of the group with non-carrier genotype (CC) was significantly higher than that of the groups with carrier genotypes (CT and TT) in subjects with T2DM and controls with a p-value of 0.0003 and less than 0.0001, respectively.

Conclusion. The rs7903146 variant of the TCF7L2 gene was found to be associated with T2DM and low β-cell function among Myanmar subjects.

Key words: type 2 diabetes, risk variants, bioinformatics, whole exome sequencing, Pashtun population

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex disorder of carbohydrate metabolism and is characterized by abnormal glucose homeostasis leading to hyperglycemia.[1] Environment and lifestyle changes are believed to play a primary role in the current epidemic of T2DM, but the inherent susceptibility to the disease is widely attributed to complex genetic factors. There is compelling evidence that genetic susceptibility to the disease is polygenic, and genome-wide association studies have identified over 400 loci associated with T2DM risk.[2] The TCF7L2 gene was found to be associated with T2DM. Five SNPs in intron 3 of the TCF7L2 gene were identified and the association of each SNP with T2DM was studied in different populations. Frequencies of the risk allele (T) were found to be high among the Japanese, Chinese, and African-American populations but were low in the Finnish population.[3-5] Data among the Myanmar population are yet to be available. In the present study, we investigated the SNP rs7903146 genotypes in 100 subjects with T2DM and 113 controls in Myanmar. The impact of these variants on beta-cell function was also studied in these two groups.

According to the National Center for Biotechnology Information, the TCF7L2 gene is located on the long arm of chromosome 10 and has 14 exons and 13 introns.[6] In 2006, Grant et al. identified that a common microsatellite in the TCF7L2 gene region (DG10S478) in intron 3 of TCF7L2 gene was associated with T2DM in an Icelandic case-control sample. This association was supported in two further populations, a Danish female cohort, and an European-American cohort. There are five SNPs found to have the strongest correlation to DG10S478 in HapMap samples; rs12255372; rs7903146; rs7901695; rs11196205; rs7895340. All five SNPs were shown to be associated in various

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degrees with T2DM. Among all five SNPs, rs12255372 and rs7903146 were found to be in strong linkage disequilibrium with DG 10S478 and have a higher strength of association with T2DM than other SNPs.[7] Peng et al., published a meta-analysis in which significant associations were found between T2DM and rs7903146, rs12255372, rs11196205, rs7901695, and rs7895340 with summary odds ratios (95% CI) of 1.39 (1.34–1.45), 1.33 (1.27–1.40), 1.20 (1.14–1.26), 1.32 (1.25–1.39) and 1.21 (1.13–1.29), respectively.[8] In this study, we focused on the rs7903146 variant of the TCF7L2 gene which showed the highest odds ratio in the study of Peng et al.[8] The risk T allele is associated with increased risk of T2DM due to impaired insulin secretion, impaired incretin effects, as well as enhanced rate of hepatic glucose production.[9] Cropana et al., conducted a study in which genetic variations in the TCF7L2 gene may increase the risk of T2DM in obese adolescents. They found that the rs7903146 variant in the TCF7L2 affects functional beta-cell capacity with impaired proinsulin processing and impaired hepatic insulin sensitivity.[10]

The TCF7L2 gene has recently been implicated in the pathogenesis of T2DM through the regulation of pancreatic beta-cell insulin secretion.[11] It encodes for an enteroendocrine transcription factor that has a role in the wingless-type integration site family, member 1(WNT) signaling pathway, which seems to be critical to pancreatic islet development and adipogenesis.[12] WNT signaling is also required for islet beta-cell proliferation.[13] TCF7L2 forms heterodimers with β-catenin to induce the expression of various genes such as glucagon-like peptide 1, the insulin gene and genes that encode proteins involved in processing and exocytosis of insulin granules.[14]

GLP-1 produced in intestinal endocrine L cells lowers the blood glucose levels through stimulation of insulin secretion and biosynthesis in pancreatic cells. Variants of the TCF7L2 gene could influence the susceptibility to T2DM by altering the level of GLP-1.[15] However, some studies have shown that risk allele carriers of TCF7L2 have normal concentrations of GLP-1 but have impaired insulin secretion in response to GLP-1 infusion, which indicated that the effect lies at the level of GLP-1 action on pancreatic beta-cells rather than secretion of GLP-1.[16] Ferreira et al., found that after treatment with exenatide, only carriers of the T allele (CT/TT) showed significantly decreased postprandial plasma insulin levels compared to the non-carrier CC genotype groups.[17] In concert with TCF7L2, the WNT signaling pathway and GLP-1 exert a critical effect on blood glucose homeostasis by stimulating insulin production by pancreatic beta-cells.

METHODOLOGY

A case-control study was conducted in the present study. After exclusion of patients with malignancy, chronic liver diseases, chronic renal failure and those who were taking insulin, 100 subjects with T2DM were recruited from the out-patient department and in-patient care of the Medical Ward of North Okkalapa General Hospital by simple random sampling method. According to WHO criteria 2006, individuals with fasting plasma glucose concentration more than or equal to 7.0 mmol/l or 126 mg/dl or 2-hour postprandial glucose level more than or equal to 11.1 mmol/l or 200 mg/dl were considered as having T2DM.[18] Inclusion criteria were subjects diagnosed with T2DM, above 35 years of age regardless of gender. One hundred and thirteen healthy persons without T2DM from the population of Quarter B, North Okkalapa Township were selected by simple random sampling to serve as non-diabetic controls. The control subjects had fasting plasma glucose levels less than 6.1 mmol/l or less than 110 mg/dl (based on WHO 2006 criteria).[18]

The estimated sample size for each study group was calculated using the following formula:

P_1 = Proportion of T allele in type 2 diabetes = 0.13 (13%)[19]
P_2 = Proportion of T allele in controls = 0.06 (6%)[19]
$N_1 = N_2$ = Number of each group

$$N_1 = N_2 = \frac{2Z\alpha^2\, P(1-P)}{(P_1-P_2)^2}$$ [20]

$$P = \frac{P_1+P_2}{2} = \frac{0.13+0.06}{2} = 0.095$$

Zα = 1.64 (standard normal deviation for α = 0.1 = Zα = 1.64)

$$N1 = N2 = \frac{2\times(1.64)^2\times 0.095(1-0.095)}{(0.07)^2}$$

$$= 94.38$$

= (Number for each group - T2DM and controls)

So, a total of 200 subjects were selected for this study.

A total of 5 ml of fasting venous blood was collected for the determination of fasting plasma glucose, fasting serum insulin, and for genotyping. Fasting insulin and glucose levels were used to estimate insulin secretion using homeostatic model assessment (HOMA-β). Plasma glucose level was determined in duplicate by a glucose oxidase method adapted to an autoanalyzer (Human, Germany). Serum insulin concentrations were determined by Enzyme-linked Immunosorbent Assay (DRG International, Inc, USA).

Genomic DNA was extracted by the salting-out method and rs7903146 genotype assays were conducted by allele-specific polymerase chain reaction method and agarose gel electrophoresis.

Table 1. Primers used for rs7903146 genotyping by allele-specific PCR.[21]

Primer	Sequence 5'–3'
rs7903146 C	Forward primer specific for allele C detection GAACAATTAGAGAGCTAAGCACTTTTTAGAAAC
rs7903146 T	Forward primer specific for allele T detection GAACAATTAGAGAGCTAAGCACTTTTTAGAGAT
rs7903146 R	Common reverse primer AGATGAAATGTAGCAGTGAAGTGC

Statistical analyses

Descriptive statistics were used to describe the profile of study participants. Quantitative variables were described using the mean and standard deviation. Meanwhile, qualitative variables were summarized as frequencies and percentages. Comparison of mean HOMA-β levels among TCF7L2 gene carriers and non-carriers were performed among diabetic and non-diabetic study participants separately. Hardy-Weinberg equilibrium and the association between disease status and the genetic variants were tested by Pearson's Chi-square test. Odds ratio, 95% confidence intervals, and all statistical tests were carried out using SPSS software version 16.0. A *p-value* <0.05 was considered statistically significant.

Ethical consideration

This research is approved by the Ethical Research Committee of the University of Medicine 2, Yangon.

RESULTS

A total of 250 participants were recruited for this study, 213 subjects had complete data and were completely analyzed for the TCF7L2 gene polymorphism, serum insulin and glucose levels. The results were presented for 213 subjects, 100 with T2DM and 113 without T2DM.

Representative genotyping of rs7903146(C/T) by allele-specific PCR is shown in Figure 1. The clinical and biochemical characteristics of subjects with and without T2DM are presented in Table 2.

Genotype distribution and analysis of the association of rs903146 of the TCF7L2 gene in subjects with and without T2DM are shown in Table 3. The CC, CT, and TT genotype frequencies were 17%, 76%, and 7% in subjects with T2DM and 46%, 51%, and 3%, respectively in the control subjects. The CT and TT genotypes are more frequent in subjects with T2DM than in controls. The CC genotype is more frequent in controls than in subjects with T2DM. The risk of T2DM is higher in the homozygous (TT) genotype group with an odds ratio of 7.14 (95% CI 1.66 – 30.71, p=0.008) than in the heterozygous (CT) genotype group with an odds ratio of 4.00 (95% CI 2.10 - 7.64, p <0.0001).

In Table 4, the C and T allele frequencies are 55% and 45% in subjects with T2DM and 72% and 28% in controls. The T alleles of rs7903146 statistically increase T2DM risk

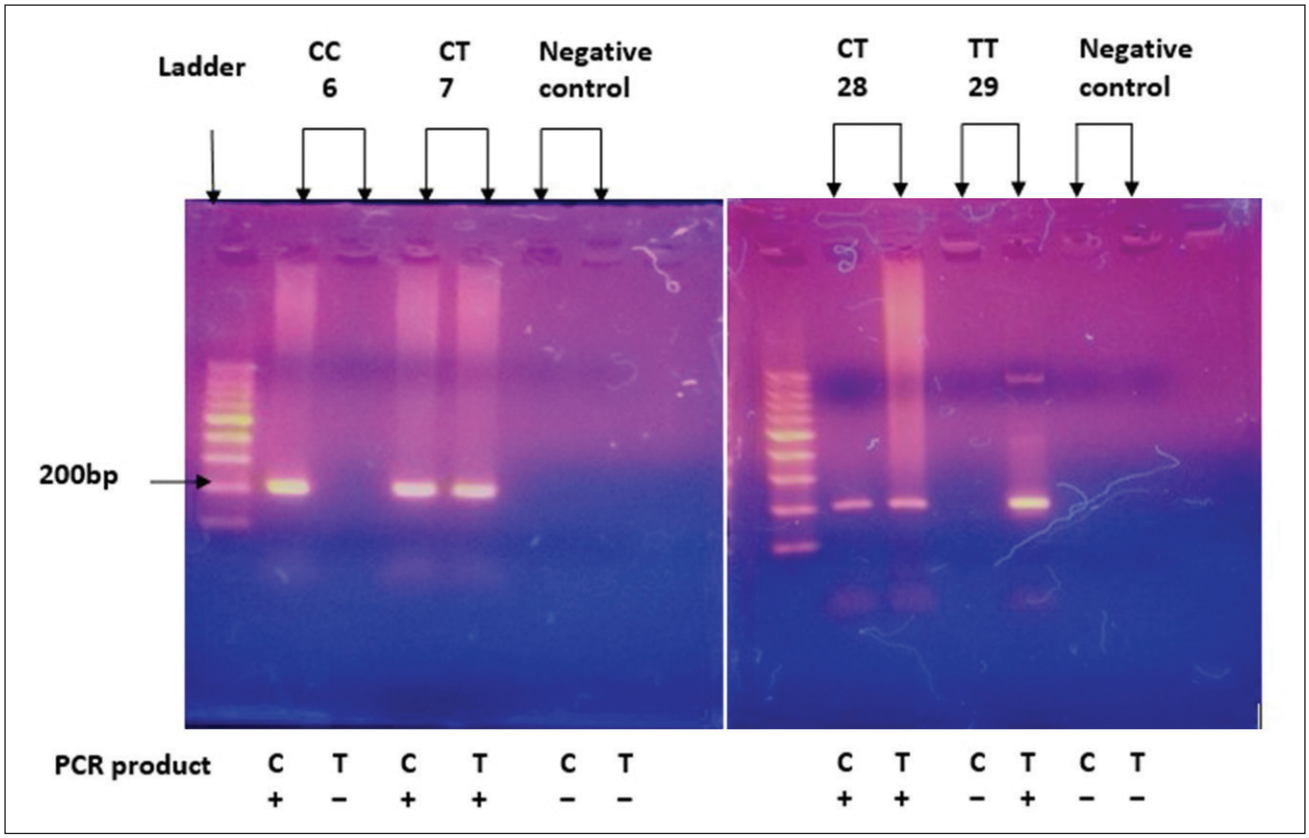

Figure 1. Genotyping of rs7903146 of TCF7L2 gene in agarose gel. This figure showed representative genotyping of rs7903146(C/T) by allele specific PCR. For each sample, two PCR reactions were performed, one with primers rs7903146 C and rs7903146 R (PCR C) and a second with primers rs7903146 T and rs7903146 R (PCR T). PCR products for C and T were identified after separation on an agarose gel. A 205 base pair (bp) band indicated the presence of the allele and amplification failure indicated the absence of the allele. Since products for C and T were placed side by side on the agarose gel, if 205 bp bands were seen on PCR C and PCR T, it indicates CT genotype. In the CC genotype, 205bp band was seen only on PCR C and absent in PCR T. In the TT genotype, 205 bp band was seen only on PCR T and absent in PCR C.

Table 2. Clinical and biochemical characteristics of subjects with and without T2DM

Parameters	Subjects with T2DM (n=100)	Subjects without T2DM (n=113)	*p*-value
Age (years)	57.47±10.71	47.05±8.94	<0.001
BMI (kg/m^2)	26.85±4.71	22.78±4.31	<0.001
Fasting plasma glucose (mg/dl)	136.02±15.76	92.02±11.03	<0.001
Fasting serum insulin (uIU/ml)	16.19±6.18	13.05±5.27	<0.001
HOMA-β	79.69±24.94	174.02±61.39	<0.001

Results were shown in Mean±SD

Table 3. Genotype distribution and analysis of the association of rs7903146 of TCF7L2 gene in subjects with and without T2DM

Genotype	Subjects with T2DM (n=100)	Subjects without T2DM (n=113)	OR	95% CI	*p*-value
CC (%)	17 (17%)	52 (46%)		Reference	
CT (%)	76 (76%)	58 (51%)	4.00	2.10-7.64	<0.0001
TT (%)	7 (7%)	3 (3%)	7.14	1.66-30.71	0.008

$\chi 2 = 21.06$, $p<0.0001$, OR = odds ratio, 95% CI = 95% confidence interval

Table 4. Allele frequencies and analysis of SNP at rs7903146 of TCF7L2 gene in subjects with and without T2DM

Allele(2N)^2	Subjects with T2DM (n=100)	Subjects without T2DM (n=113)	OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
C allele	110 (55%)	162 (72%)	2.07	0.0004	1.98	0.002
T allele	90 (45%)	64 (28 %)	(1.39-3.09)		(1.27– 3.08)	

$\chi 2 = 12.79$, $p<0.0001$, OR = odds ratio, 95% CI = 95% confidence interval, adjusted odds ratio and p-values were obtained from logistic regression analyses adjusting for BMI

Table 5. Comparison of HOMA-β level in non-carrier and carrier risk allele of Subjects with and without T2DM

Groups	Genotypes		*p*-value
	Non-carrier (CC)	Carrier (CT and TT)	
Subjects with T2DM	99.18±13.78 (n=17)	75.70±24.89 (n=83)	0.0003
Subjects without T2DM	210.12±53.77 (n=52)	143.25±49.86 (n=61)	<0.0001

Results were shown in Mean±SD

compared to the C allele with an allelic odds ratio of 2.07 (95% CI 1.39-3.09, p=0.0004, and significantly increase T2DM risk even after adjusting for BMI.

The mean HOMA-β levels of the non-carrier (CC) genotype group are significantly higher than that of carrier genotype groups (CT and TT) with the $p< 0.0001$ in controls as well as in subjects with T2DM with a $p<0.0003$ as shown in Table 5.

DISCUSSION

This study found a significant association in samples from a Myanmar population between the variants rs7903146 of TCF7L2 and T2DM and this is similar to that previously reported in samples from European-origin populations.[7,22,23] The data from the present study shows that the CT and TT genotypes are more frequent in patients with T2DM with a frequency of 76% and 7% respectively, compared to a frequency of 51% of CT genotype and 3% of TT genotype in controls.

On the other hand, the CC genotype is more frequent in controls with a frequency of 46% compared to a frequency of 17% in subjects with T2DM as shown in Table 3. Genotype distribution of SNP at rs7903146 did not conform to the Hardy-Weinberg equilibrium in both subjects with T2DM (χ^2= 28.66, df=1, $p<0.001$) and in the control group (χ^2=7.89, df=1, p=0.005) (data not shown). It is also noted that deviation from Hardy-Weinberg equilibrium at rs7903146 SNP of TCF7L2 gene was also reported in the study of Marquezine et al.[24]

The risk of T2DM was higher in the risk allele carrier homozygous genotype (TT) group than the heterozygous genotype (CT) group compared to the non-carrier homozygous genotype (CC) group as shown in Table 3. In the present study, the frequency of the T allele was significantly higher in subjects with T2DM (45%) compared to controls (28%). The T allele of rs7903146 increases the risk of type 2 diabetes than the C allele with an allelic odds ratio of 2.07 (95% CI 1.39 - 3.09) with a p=0.004 as shown in Table 4. Chandak et al., showed that the T allele was more frequent in subjects with T2DM (37%) than healthy control subjects (29%) in the Indian population with an odds ratio of 1.46 (95% CI 1.22–1.75, $p=3 \times 10^{-5}$).[25] Moreover, Bahaaeldin et al., also found that the frequency of the T allele was higher in subjects with T2DM (32.9%) compared to controls (26.7%) in an Egyptian study with an odds ratio of 1.35 (95% CI 0.68-2.6), but this was statistically insignificant ($p>0.05$).[26] In agreement with the current work, the T allele was more frequent in subjects with diabetes (45.3%) than healthy controls (34.5%) and is associated with a high risk of diabetes with an odds ratio of 2.13, (95% CI 1.12-7.31, p =0.005) in a Moroccan population.[27]

The odds ratio of rs7903146 of the TCF7L2 polymorphism in this study appears to be higher than that of other studies. However, the odds ratio of rs7903146 of the TCF7L2 polymorphism in the present study was comparable to the studies of Dabelea et al.,[5] and Ren et al.[4] A lack of association between the TCF7L2 rs7903146 variant and T2DM was reported in the study among Hong Kong Chinese[28] and in the Iran study of Pourahmadi et al.[29] Mandour et al., reported that the frequency of the TT genotype was significantly lower among subjects with diabetes compared to healthy controls. Moreover, the study found that the T allele of TCF7L2 rs7903146 was associated with a lower risk of T2DM.[30] These disparate results may be due to differences in ethnicity.

The frequency of risk allele T of rs77903146 was more prevalent in the European, American, and African populations than the Asian population. The higher frequencies of the T allele in the present study suggest that the genetic background and environmental conditions of Myanmar Asians may be different from that of other Asian populations. This difference might be due to many factors such as ethnic stratification, variation in study design, sample size, variation in methods for SNP detection, or gene-gene and gene-environment interaction.

In this study, risks of T2DM were consistently higher for the homozygous (TT) genotype (odds ratio 7.14, 95% CI 1.66 –30.71, p=0.008) than for the heterozygous (CT) genotype of rs7903146 SNP, (odds ratio 4.00, 95% CI 2.10-7.64, p<0.0001). It may indicate that subjects carrying both risk alleles (homozygous carrier, TT) have about a sevenfold higher risk of T2DM compared to those without the risk allele (homozygous non-carrier, CC). Subjects carrying one risk allele (heterozygous carrier, CT) may have a fourfold risk of developing T2DM compared to those without any risk allele (homozygous non-carrier, CC). Barra and colleagues found that the odds ratio for the homozygous (TT) genotype was 4.04 (95% CI 1.48-11.0, p=0.004) and that of the heterozygous (CT) genotype was 1.06 (95% CI 0.63-1.80, p=0.81).[14] Our study showed the odds ratios for the homozygous and heterozygous genotypes were higher than other studies. This may be due to genetic variation among different ethnicities in the different studies. Based on the data from the present study, it shows that the polymorphism rs7903146 of the TCF7L2 gene is associated with increased susceptibility to T2DM in the population.

Although insulin resistance is the leading factor in the pathogenesis of T2DM, there is evidence that pancreatic beta-cells also play an important role in the development of T2DM. Many genes associated with T2DM have been linked to beta-cell mass and function. Decreased beta-cell mass and impaired insulin secretion have been reported in patients with T2DM in numerous studies. Gene variants of beta-cell loss which are associated with decreased beta-cell function are also regulated as risk factors for T2DM.[31]

In this study, the mean HOMA-β levels of non-carrier genotypes (CC) was significantly higher than carrier genotypes (CT, TT) in control subjects as well as subjects with T2DM as shown in Table 5. This indicates that risk allele T carrier genotype groups have impaired beta-cell function compared to non-carrier groups. The trend of reduced HOMA-β between different genotypes was similar to the study of Loos et al.[32] Therefore, the risk allele T of rs7903146 is strongly associated with reduced HOMA-β levels and results in diabetes by affecting the function of pancreatic beta-cells. The impaired beta-cell function of risk allele T carrier groups could be due to reduced beta-cell mass through defects in cell differentiation and maturation. This finding was supported by the findings of Papadopoulou and Edlund, in which TCF7L2 activates many genes downstream of the WNT signaling pathway, which is required for the development of the pancreas and islets during embryonic growth.[33]

Similarly, Takamoto et al., demonstrated that newborn mice expression of TCF7L2 dominant-negative form showed a reduction in the area of beta-cells and pancreatic insulin content leading to impaired glucose tolerance with decreased insulin secretion. They also reported that TCF7L2 in pancreatic beta-cells plays a crucial role in the metabolism of glucose through the regulation of beta-cell mass during development.[34] These findings suggest that individuals with at-risk T allele carrier (CT, TT) genotypes of rs7903146 of the TCF7L2 gene exhibit a reduction in HOMA–β levels compared to the non-carrier genotype (CC) group and this may be due to reduced beta-cell mass and subsequent impaired beta-cell function.

In addition, TCF7L2 has been involved in the expression of GLP-1 and gastric inhibitory peptide receptors in beta-cells which mediate the effects of the corresponding incretin hormones to promote beta-cell proliferation.[7] Shu and coworkers found that there was a decrease in TCF7L2 protein level and decreased expression of GLP-1 receptors on pancreatic islet cells of subjects with diabetes. This downregulation of GLP-1 receptors may be the underlying etiology for impaired GLP-1-induced insulin secretion by pancreatic beta-cells in T2DM.[35] Moreover, TCF7L2 is also involved in the expression of beta-cell genes which are required for insulin secretory granule fusion. The number of morphologically docked vesicles was unchanged by TCF7L2 suppression, however, secretory granule movement and capacitance decreased, indicating defective vesicle fusion. This defect in insulin exocytosis may increase diabetes incidence in the carrier of TCF7L2 allele.[36]

CONCLUSION

This study found a significant association between the rs7903146 TCF7L2 variant and T2DM. It also showed higher frequencies of T allele carrier genotypes compared to other studies. Moreover, it provides evidence that variants in TCF7L2 rs7903146 may play a crucial role in the pathogenesis of T2DM by reducing insulin secretion.

Therefore, this study revealed that TCF7L2 is an important gene for determining susceptibility to T2DM in the studied population in Myanmar.

Limitations of study

The limitations of this study are that GLP-1 level was not measured and only a small sample size was included.

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