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TCF7L2 gene polymorphisms rs12255372, rs7903146, rs10885406 and the association with type 2 diabetes in a population of Legian Village, Kuta, Bali.

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ABSTRACT

Background: Polymorphisms in the transcription factor 7 like-2 (TCF7L2) gene have been consistently reported to be associated with increased risk of type 2 diabetes mellitus in various populations, in particular, the rs7903146, rs12255372, and rs10885406 polymorphism. **Objective:** The aim of this study was to investigate whether these polymorphisms in the Balinese population of Legian village. **Methods:** A cross-sectional study enrolling 286 participants (178 male, 108 female), mean age was 46.0±10.0 (range 20–83) years. PCR-RFLP conducted genotyping for rs7903146, rs12255372, and rs10885406 polymorphism, fasting and two hours after meal blood glucose were measured. Student's *t*-test and analysis of variance (ANOVA) and chi-square test were employed. **Results:** The frequencies of the CC and CT genotypes of the rs7903146 polymorphism were 93.4% and 6.6%. The GG and GT genotypes of the rs12255372 polymorphism were 94.8% and 5.2%, while in the rs10885406 they were 87.1%, 12.2%, and 0.7% for the AA, AG, and GG genotypes. The TT genotypes of the rs7903146 and rs12255372 not found. The prevalence of type 2 diabetes in this population were 9.0%. The frequency of the CT genotype of the rs7903146 was higher in diabetes compare to the non-diabetes group (7.6% vs. 6.5%, p=0.822), while GT genotype in rs12255372 was lower (3.8% vs. 5.3%, p=0.737). The AG genotype of the rs10885406 also lower in diabetes group (7.6% vs. 12.6%, p=0.679). In the CT genotype of rs7903146, the two hours after meal blood glucose were significantly higher (141.15 ± 125.06 vs. 107.50 ± 46.94, p=0.012). Interestingly, although not statistically significant, individuals with the GG genotype showed the lowest blood glucose. **Conclusion:** Rs7903146, rs12255372, and rs10885406 polymorphisms in the TCF7L2 genes did not show association with type 2 diabetes in the Balinese population of Legian Village. However, two hours after meal blood glucose level was found to be significantly higher in the CT genotype of the rs7903146.

Keywords: Type 2 diabetes, TCF7L2 genes

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INTRODUCTION

Type 2 diabetes represents a multifactorial disease, and several findings indicate that genetics is an important contributing factor.[1] The investigation of heritable susceptibility to disease is ultimately an effort to associate disease phenotype with underlying genotype. Advance technology for identification of single-nucleotide polymorphisms (SNPs) and use of microarrays have strengthened research methodologies for genetic analysis. Many complex traits are thought to be inherited since they often run in families. However, these complex traits do not show typical Mendelian pedigree patterns. These nonmendelian diseases may depend on several susceptibility loci, with a variable contribution from environmental factors. Different strategies can pursue the identification of susceptibility loci. In the general population, association studies are the most used for discovering susceptibility loci.[2]

Several single nucleotide polymorphisms (SNPs) associated with type 2 diabetes. Transcription factor 7-like 2 (T-cell specific, HMG-box or high mobility group-box) also known as TCF7L2 or TCF4 is a protein acting as a transcription factor. In humans, this protein is encoded by the TCF7L2 gene on chromosome 10q25.3. A variant of the protein is linked to a higher risk to develop type 2 diabetes. It is a member of the Wnt signaling pathway, expressed in several tissues, including the gut and the pancreas, and plays an important role

in β-cell proliferation and insulin secretion influences synthesis of GLP-1 in intestinal L-cells. Other than diabetes, TCF7L2 implicated in a large variety of diseases.[3,4]

Recently, it has suggested that TCF7L2 is more likely associated with impaired insulin secretion, not with increased insulin resistance.[3,5] Insulin secretion stimulated by glucose, GLP-1, and GIP, was impaired in small interfering (si)TCF7L2-treated isolated human islets.[6] With an overall allelic relative risk of 1.56, TCF7L2 currently represents the most convincing diabetes risk gene.[1] TCF7L2 SNPs including rs12255372, rs7903146, rs10885406 replicated in several populations and ethnicities. However, it never explored yet in Balinese population.

METHODS

A cross-sectional epidemiologic study conducted in Legian Village, Kuta, Bali. In this study, we investigate the frequency of the TCF7L2 gene polymorphisms rs12255372, rs7903146, rs10885406 and their association with type 2 diabetes in a population of Legian Village, Kuta, Bali.

Subjects recruited from this village aged 18 years old or more by stratified random sampling. There were 3.361 people in this village (1.687 male and 1.674 female) divided into three Banjar(s) (or unit of the village) including Legian Kelod, Legian Tengah, and Legian Kaja. From each Banjar, we randomly choose 100 sample. From 300 participants enrolled, 286 participants were analyzed.

Blood samples for genomic DNA analysis were taken and send by Guthrie card. DNA samples were isolated from Guthrie Cards using Chelex-100 protocol. DNA isolation, genotyping for rs7903146, rs12255372, and PCR-RFLP, fasting conducted rs10885406 polymorphism and two hours after meal blood glucose were measured.

Student t-test and analysis of variance (ANOVA) were used to test the equality of continuous variables. A chi-square test was employed to compare categorical variables. Odd ratios (OR) and 95% confidence intervals (CI) employed for estimating the strength of association of each group of TCF7L2 gene polymorphism with diabetes. The analysis was done using SPSS 15.0.

RESULT

There were 286 participants, male: female was 178:108, mean age was 46.0±10.0 (range 20–83) years. The CC and CT genotypes of the rs7903146 polymorphism found 93.4% and 6.6%, the GG and GT genotypes of the rs12255372 polymorphism were 94.8% and 5.2%, while in the rs10885406 they were 87.1%, 12.2%, and 0.7% for the AA, AG, and GG genotypes. The TT genotypes of the rs7903146 and rs12255372 not found (**Table 1.**).

Table 1. Frequencies of the genotype and allele of the TCF7L2 polymorphism

TCF7L2 polymorphism	Homozygote Wild type n (%)	Heterozygote mutant n (%)	Homozygote mutant n (%)	Allele frequencies n	
rs7903146	CC	CT	TT	C	T
	267 (93.4%)	19 (6.6%)	-	553	19
rs12255372	GG	GT	TT	G	T
	271 (94.8%)	15 (5.2%)	-	557	15
rs10885406	AA	AG	GG	A	G
	249 (87.1%)	35 (12.2%)	2 (0.7%)	533	37

Table 2. Frequencies of diabetes in each group of TCF7L2 polymorphism genotype

TCF7L2 polymorphism	Genotype	Non-diabetes	Diabetes	Total	p-value	OR (CI)
rs7903146	CC	243	24	267	0.686	0.686 (0.259 – 5.468)
	CT	17	2	19		
rs12255372	GG	246	25	271	0.596	0.703 (0.089 – 5.570)
	GT	14	1	15		
rs10885406	AA	225	24	249	0.679	0.536 (0.121 – 2.367)
	AG or GG	35	2	37		
Total		**260**	**26**	**286**		

The prevalence of the type 2 diabetes mellitus in this Balinese population were 9.0%. The CT genotype of rs7903146 was found to be higher in diabetes (2/26 or 7.6% of diabetes) compare to the non-diabetes group (17/260 or 6.5%), although not significant (p=0.822). While the frequency of the GT genotype in rs12255372 was observed lower in the diabetes group (1/26 or 3.8%) compared

with the non-diabetes group (14/260 or 5.3%), p=0.737. The AG genotype of the rs10885406 also lower in diabetes group (2/26 or 7.6% vs. 33/260 or 12.6%, p=0.679), while the GG genotype of the rs10885406 only found in the non-diabetes group (2/260 or 0.7%).

We try to look further for the association of each group of TCF7L2 gene polymorphism with diabetes. However, none of the SNPs was statistically significant, as shown in **Table 2.**

Mean fasting blood glucose of the total sample was 98.17±36.73 mg/dL, and mean 2 hours after meal blood glucose was 109.84±56.16 mg/dL. An indication of higher fasting blood glucose was observed in the CT genotype of rs7903146, although not significant (113.84 ± 67.86 vs. 97.05 ± 33.39 mg/dL, p=0.054), while the two hours after meal blood glucose were found to be significantly higher (141.15 ± 125.06 vs. 107.50 ± 46.94, p=0.012). Both fasting and two hours after meal blood glucose were not significantly different in the GT as compared to the GG genotypes of the rs12255372 (fasting 109.53 ± 65.36 vs. 97.54 ± 34.57, p=0.219, two hours after meal 130.93 ± 126.42 vs. 108.62 ± 49.43, p=0.135). As well as the AG compared to AA genotypes of rs10885406 (fasting 102.74 ± 51.12 vs 97.61 ± 34.46, p=0.677 and two hours after meal 120.44 ± 96.38 vs 108.47 ± 48.15, p=0.464). Interestingly, although not statistically significant, individuals with the GG genotype showed the lowest blood glucose (fasting 87.00 ± 5.65, p=0.677 and two hours after meal 92.50 ± 12.02, p=0.464).

We found six combinations of heterozygote and mutant of the TCF7L2 polymorphism including CT –AG (2.8%), CT-AG-GT (3.8%), AG (4.9%), AG-GT (0.7%), GT (0.7%), GG (0.7%). The highest blood glucose level both fasting and after the meal found in the subgroup with a combination of genotype CT – AG – and GT, as shown in **Table 3.** Interestingly the group of CT – AG – GT genotype tend to have highest blood glucose, both fasting and 2 hours after meal (**Fig.1**)

Table 3. Mean fasting and 2 hours after meal blood glucose level of each group TCF7L2 polymorphism

TCF7L2 polymorphism	Blood glucose in mg/dL ± SD	Homozygote Wild type	Heterozygote mutant	Homozygote mutant	p
rs7903146		CC	CT	TT	
	FBG	97.06 ± 33.39	113.84 ± 67.86	-	0.054
	2hPP BG	107.51 ± 46.94	141.16 ± 125.06	-	0.012*
rs12255372		GG	GT	TT	
	FBG	97.54 ± 34.57	109.53 ± 65.36	-	0.219
	2hPP BG	108.62 ± 49.43	130.93 ± 126.42	-	0.135
rs10885406		AA	AG	GG	
	FBG	97.61 ± 34.46	102.74 ± 51.12	87.00 ± 5.65	0.677
	2hPP BG	108.47 ± 48.15	120.44 ± 96.38	92.50 ± 12.02	0.464

FBG = fasting blood glucose, 2hPP BG = 2 hours post prandial (after meal) blood glucose
* statistically significant

Table 4. Frequencies of the combination of TCF7L2 polymorphism and mean fasting and 2 hours after meal blood glucose level of each group

Combination of TCF7L2 polymorphism	n (%)	Diabetes	Non-diabetes	Mean FBG (mg/dL)	Mean 2hPP BG (mg/dL)
CT – AG – GT	11 (3.8%)	1	10	116.27±76.02	148.0±145.24
CT –AG	8 (2.8%)	1	7	110.50±59.70	131.75±99.52
AG – GT	2 (0.7%)	0	2	92.0±11.31	74.0±15.55
AG	14 (4.9%)	0	14	89.21±7.31	97.31±21.12
GT	2 (0.7%)	0	0	90.0±2.82	94.0±11.31
GG	2 (0.7%)	0	0	87.0±5.65	92.50±12.02
Wild type	247 (86.4%)	24	223	97.68±34.59	108.60±48.33
Total	**286 (100%)**	**26 (9.09%)**	**260 (90.91%)**		

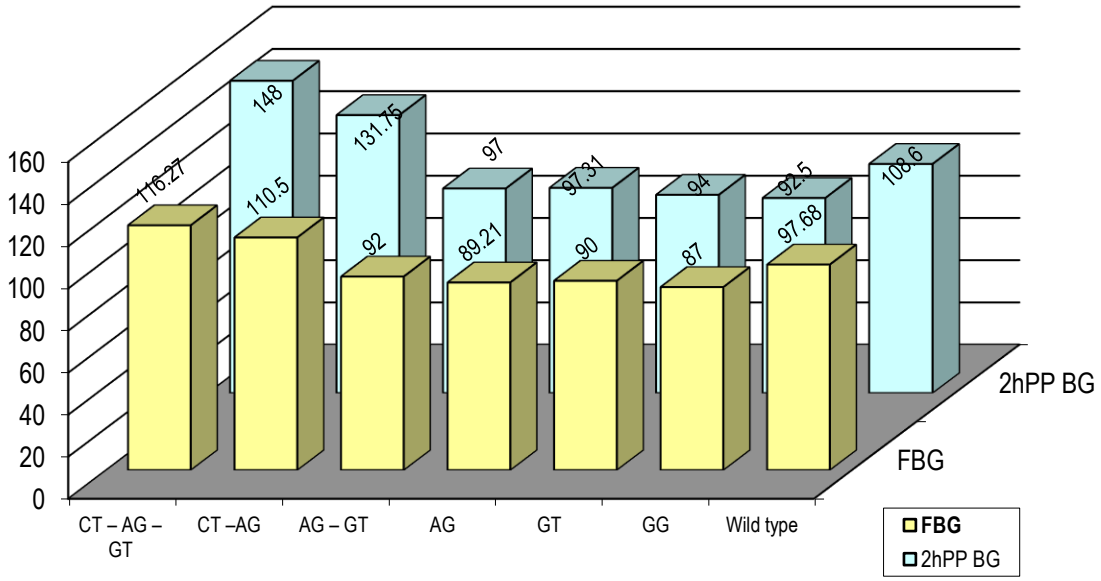

Figure 1. Trend of fasting blood glucose (FBG) and 2 hours after meal blood glucose (2hPP BG)

DISCUSSION

In Iranian population, association study of rs7903146 T allele and type 2 diabetes found that genotype frequencies were significantly different between type 2 diabetes patients (N = 258) and controls (N = 168) TT vs. CT + CC [p 0.0081 OR 3.4 95%CI (1.27-11.9)].[7] Zhang et al. 2006 in a prospective, nested, case-control study (*n* = 3,520) found the frequencies of the T-allele of the *TCF7L2* gene (rs12255372 [T/G]) were significantly higher in case than control subjects; each copy of the T-allele associated with a 1.32-fold (*p* = 0.0002) and 1.53-fold (*P* < 0.0001) increased type 2 diabetes risk in women and men, respectively.[8] Florez et al, 2006 in 3548 participants shown genotype at rs7903146 were more likely to have progression from impaired glucose tolerance to diabetes than were CC homozygotes (hazard ratio, 1.55; 95 percent confidence interval, 1.20 to 2.01; P<0.001).[9] Study in 8.310 individuals in Family-based and case-control designs from Scandinavia, Poland, and the U.S., which genotyped 13 single nucleotide polymorphisms (SNPs) across *TCF7L2* in confirmed the previous association of *TCF7L2* SNPs with the risk of type 2 diabetes (rs7903146T odds ratio 1.40 [95% CI 1.30–1.50], $P = 6.74 \times 10^{20}$).[10]

In our study, TCF7L2 risk genotype rs12255372, rs7903146, rs10885406, are not associated with diabetes in a population of Legian Village, Kuta, Bali. It is the first study of TCF7L2 polymorphisms in our population, and the low frequencies of the heterozygote and mutant genotype may explain why the association not found.

TCF7L2 associated with impaired insulin secretion stimulated by glucose, GLP-1, and GIP,[3,5,6,11] this reason may explain why blood sugar level tends to be higher in the heterozygote of rs12255372, rs7903146, rs10885406.

Lyssenko et al., 2008 has genotyped 16 single nucleotide polymorphisms (SNPs) and examined clinical factors in 16.061 Swedish and 2.770 Finnish subjects, and 2.201 (11.7%) of these subjects developed type 2 diabetes in a follow-up period of 23.5 years. This study showed that common genetic variants associated with the risk of diabetes had a small effect on the ability to predict the future development of type 2 diabetes and the power of genetic risk factors improved with an increasing duration of follow-up, whereas that of clinical risk factors decreased.[12] For the next study, to learn the genetic risk of these SNPs in our population, we need a cohort study with a larger sample size.

CONCLUSION

Polymorphisms rs12255372, rs7903146, rs10885406, in the transcription factor 7 like-2 (TCF7L2) genes not associated with diabetes in a population of Legian Village, Kuta, Bali. However, blood sugar level tends to be higher in the heterozygote rs12255372, rs7903146, rs10885406.

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