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Association of *TCF7L2* rs12255372 –G/T polymorphism with type 2 diabetes in a Nigerian population

G.A. Engwa[1], F.N. Nwalo[2], C.O. Ozokonkwo[3], K.N. Agbafor[4], B.N. Nkeh-Chungag[1] and B.E. Ubi[5]

[1] Department of Biological and Environmental Sciences, Faculty of Sciences, Walter Sisulu University, Mthatha 5117, South Africa
[2] Department of Biotechnology, Federal University, Ndufu-Alike Ikwo (FUNAI), P.M.B. 1010 Abakaliki, Nigeria
[3] Department of Microbiology, Godfrey Okoye University, P.M.B 01014, Thinkers Corner, Enugu Nigeria
[4] Department of Biochemistry, Ebonyi State University, P.M.B 53 Abakaliki, Nigeria
[5] Department of Biotechnology and Applied Biology, Ebonyi State University, P.M.B 53 Abakaliki, Nigeria

Corresponding author: G.A. Engwa
E-mail: gengwa@wsu.ac.za

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ABSTRACT. Polymorphisms of the transcription factor 7–like 2 (*TCF7L2*) gene have been associated with susceptibility to type 2 diabetes (T2D) in various ethnic populations, but have not been previously studied in a Nigerian population. We investigated the relationship between the *TCF7L2* rs12255372 (G/T) polymorphism and type 2 diabetes (T2D) in a Nigerian population. This was a preliminary case–control study that included 73 T2D patients and 75 non-diabetic (ND) controls. Following blood collection, fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) were assayed. PCR-Restriction Fragment Length Polymorphism (RFLP) was employed to molecularly genotype for the *TCF7L2* G/T polymorphism using the *MluCI* restriction enzyme. The GG homozygote genotype was more frequent in ND controls

(38.5%) than in T2D patients (23%) while the TT genotype was more frequent in T2D patients (25.7%) than in ND controls (11.5%). Thus, the *TCF7L2* G/T polymorphism was associated ($P < 0.05$) with T2D. The recessive model showed the greatest risk of T2D when the TT genotype was compared to the GX (GG+GT) genotype (odds ratio (OR): 3.91; 95% confidence interval (CI): 1.93-7.96, $P < 0.001$). The FBG and HDL-c were significantly different ($P < 0.05$) in subjects with the mutant (GT and TT) genotypes compared to the GG genotype. In conclusion, the *TCF7L2* G/T polymorphism was associated with increased risk of T2D in a Nigerian population. This variant could affect pathophysiological markers associated with risk of T2D. Further studies are needed in other populations in Nigeria to confirm the effects of this polymorphism on pathophysiological markers of T2D.

Key words: Genetic association; *TCF7L2* G/T polymorphism; Type 2 Diabetes; Obesity; Genetic risk factors

INTRODUCTION

Type 2 diabetes (T2D) is a chronic metabolic disease that is due to impaired secretion of insulin and/or the inability of cells to metabolize glucose, which is commonly known as insulin resistance (Ziegler and Nepom, 2010). Characterized by hyperglycemia, this disease, which was considered more of a Western problem is rapidly becoming a problem of public health concern in Africa with its steadily increasing prevalence (WHO, 2016).

T2D is a complex disease involving various factors, including environmental, pathophysiological, and genetic factors (Yufang et al., 2012). The latter could predispose people to the disease as several gene variants have been found by genome-wide association studies (GWAS) that show possible associations with the disease (Sladek et al., 2007). The transcription factor 7–like 2 (*TCF7L2*) gene, among others, is a gene that is related to T2D (Cauchi et al., 2007). Previously called TCF-4, the *TCF7L2* is a gene that is located on the 10q25.3 chromosome that codes for a transcription factor that is expressed in several tissues, such as adipocytes, liver, gut and pancreatic tissues (Osmark et al., 2009). This transcription factor is a nuclear receptor for β-catenin (cat); it's bipartite combination (*TCF7L2*/cat) activates several downstream genes of the Wnt signaling cascade (Nelson and Nusse, 2004). Proglucagon, a gene that encodes glucagon-like peptide 1 (GLP-1) is one of the insulinotropic genes that is transcriptionally regulated by *TCF7L2*/cat (Yi et al., 2005). Since *TCF7L2* together with GLP1 are known to be implicated in the homeostasis of blood glucose, it has been suggested that *TCF7L2* gene mutants can influence susceptibility to T2D by impairment of insulin action (Schafer et al., 2007; Shu et al., 2009). Several polymorphic markers of the *TCF7L2* gene have been identified and assessed for association with T2D in various populations (Grant et al., 2006). Among these variants, rs7901695, rs11196205, rs7903146 and rs12255372, have shown to be associated with T2D, of which rs7903146 (C/T) and rs12255372 (G/T) variants have shown the greatest association with T2D in several studies (Chandak et al., 2007; Tong et al., 2009).

Though several studies have shown association of *TCF7L2* gene polymorphisms with T2D in most parts of the globe, very few studies have been conducted in Africa. A study in South Africa showed no association between the rs12255372 (G/T) variant and T2D (Pirie et al., 2010), while another study in Cameroon showed this variant to be associated with T2D (Nanfa et al., 2015). Nigeria presents the greatest burden of T2D in Africa (IDF, 2013), but studies on the prevalent genetic risk factors of the disease are lacking. Hence, we investigated the relationship between the *TCF7L2* rs12255372 (G/T) polymorphism and T2D in a Nigerian population.

MATERIAL AND METHODS

Study participants and Ethical Approval

This preliminary case–control study that involved T2D patients and non-diabetic (ND) controls was part of an ongoing study conducted at Enugu State University Teaching Hospital (ESUTH) in Enugu, Nigeria as previously reported by Engwa et al. (2018a). Prior to the commencement of the study, ethical clearance was obtained from ESUTH Ethics Committee with approval no: ESUTHP/C-MAC/RA/034/174. The study was conducted in accordance with the Helsinki Declaration (2008 revised edition), whereby participants were only recruited when they freely gave their consent to participate in the study in writing (written informed consent). Outpatients visiting the hospital who were above 30 years of age, who had not eaten for the past 12 h (overnight fasting), and without any emergency or critical health conditions or complications were recruited for the study. HIV positive patients as well as pregnant and/or breastfeeding women were excluded from the study. Patients considered as T2D patients were those who had at least one year history of T2D as stipulated by the International Diabetic Federation (IDF) criteria (WHO-IDF, 2014). The non-diabetic patients who served as the control group were those without diabetes or hyperglycemia. The sample size (n) was calculated according to Charan and Biswas (2013) using the formula: $n = \frac{2\mathrm{SD}^2\ (\mathrm{Za}/2+\mathrm{Z_b})^2}{\mathrm{d}^2}$ = 74.19; where, SD = standard deviation, Za/2 = Zscore for type 1 error of 5%, Zb = Zscore at 95% power and d = effect size from previous study (Chen et al., 2012). Thus, 74 participants were expected to be recruited for each of the arms (case and control), making a total of 148 participants.

Data collection and biochemical assay

Patient's data including sex, age and other demographic parameters were obtained using a questionnaire. The diastolic blood pressure (DBP), systolic blood pressure (SBP) and waist circumference (WC) were measured while the weight and height were measured to determine the Body Mass index (BMI). A volume of 2 mL of blood was collected from each participant, transferred into EDTA free tubes and fasting blood glucose (FBG) was assayed from whole blood according to the glucose oxidase enzymatic method using an Accu-Check glucometer (Roche Diabetes Care, Inc., Burgess Hill, UK) as per manufacturer's protocol (Trinder, 1969). Total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-c) were quantified in serum using kits by Randox Laboratories Ltd, United Kingdom. The TC was determined according to the enzymatic method, TG was assayed by the enzymatic method of Esders and Michira (1997) and HDL-

c by the precipitation method of Grove (1979). Low density lipoprotein cholesterol (LDL-c) was calculated using the Freidwald's formula: LDL-c = TC- (TG/5) - HDL-c (Friedwald et al., 1972).

Molecular genotyping of TCF2L7 rs12255372 –G/T variant

The GeneJET Genomic DNA Purification kit (Thermo Fisher Scientific. Inc., USA) was used to extract DNA from whole blood after which the *TCF7L2* gene was amplified by PCR using the forward primer: 5′-CTG GAA ACT AAG GCG TGA GG-3′ and reverse primer: 5′-GGG TCG ATG TTG TTG AGC TT-3' according to the method of Alami and collaborators (2012). The PCR master mix cocktail contained 12.5 µL of One Taq Quick-Load 2x Master Mix with standard buffer (New England Biolab (NEB), USA), 5 µL (40 µM) of each primer (Inqaba-Biotech, South Africa), and 8 µl (< 10 ng) of genomic DNA to a final volume of 30 µL. The PCR amplification was done using a MiniJet thermal cycler (Biorad, USA) and commenced with a pre-denaturation at 95°C for 2 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 30 s, and a final extension of 72°C for 5 min. The amplicon (346 bp) was separated on 2% agarose gel and then digested with MluCI restriction enzyme (NEB) at 37°C for 3 h. The reaction volume was set to 20 µL, containing 10 µL of amplicons, 1× NEB Smartcut buffer (NEB), 0.5 µL of MluCI (10U per amplicon), and 8 µL of nuclease free water. The digested products were separated by electrophoresis on a 3.5% agarose gel stained with ethidium bromide and visualised under a UV transilluminator. After restriction enzyme digestion, the expected product sizes were: normal homozygous GG genotype: 143 bp, 104 bp, 99 bp,; mutant homozygous TT genotype: 126 bp, 104 bp, 99 bp, 17 bp; and heterozygous GT genotype: 143 bp, 126 bp, 104 bp, 17 bp, respectively. For validation, 10% samples were randomly selected and genotyped in duplicate which showed a concordance rate of 100%.

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 16 and the results were presented in tables and expressed as mean ± standard error of the mean (SEM) and frequencies. Mean differences of the lipid profile indices, FBG and anthropometric parameters between the various *TCF7L2* genotypes (GG, GT and TT) of the study population was compared using analysis of variance (ANOVA) test. Pearson chi-square ($\chi 2$) test was used to compare the allelic and genotypic frequencies between T2D and ND participants as well as to test for the Hardy-Weinberg equilibrium (Elston and Forthofer, 1977). Also, binary and multinomial logistic regression analysis was employed to compare allelic and genotypic frequencies between T2D and ND participants and to determine the odd ratio (OR) for associated risk. A 95% confidence interval was considered and a difference was statistically significant at $P \leq 0.05$.

RESULTS

Baseline characteristics of the study population

A total of 148 individuals participated in the study, of which 73 were T2D patients and 75 were non-diabetic (ND) controls. The clinical and demographic characteristics of the study population are summarized in our previous publication (Engwa et al., 2018b).

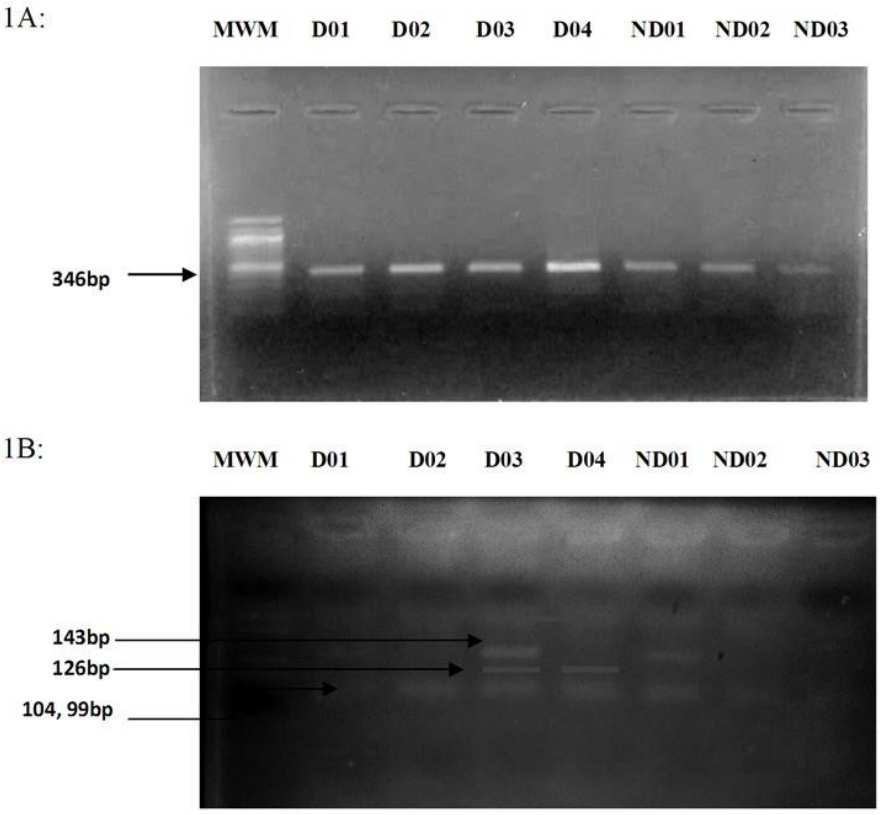

Figure 1. RFLP-PCR of *TCF7L2* rs12255372 (G/T) Polymorphism. Figure 1A shows the presence of the amplified gene for samples D01-D04 and ND1-ND03 at 346bp. Figure 1B shows the restriction digest products of the gene. The homozygous wild GG genotype has 3 restriction sites giving four fragments; 126bp, 104bp, 99bp and 17bp, while the homozygous mutant TT genotype has 2 restriction sites giving 3 fragments; 143bp 104bp, 99bp. Fragments 104bp and 99bp are cluttered together while the 17bp fragment was not seen in the gel. Sample D02, and D04 indicates the presence of the wild-type homozygous GG genotype, D03 show the presence of the heterozygote GT genotype while D01 and ND01 indicates the homozygous wild TT genotype. D01-D04 indicates diabetic samples while ND01-ND03 indicates non-diabetic samples. MWM indicates molecular weight marker.

Genotypes of *TCF7L2* (G/T) gene polymorphism

The genotype distributions of *TCF7L2* gene were shown in Figure 1. The homozygous wild GG genotype was predominant in ND controls (38.5%) than in T2D patients (23%). On the other hand, the homozygous mutant TT genotype was predominant in T2D patients (25.7%) compared to ND controls (11.5%). This accounted for an association ($P < 0.05$) between *TCF7L2* G/T polymorphism and T2D. To determine the likelihood of this polymorphism to promote T2D, the dominant, codominant and recessive

models were evaluated for prediction of risk to T2D by determining the odd ratio (OR) for the respective models. The dominant model (GG vs (GT + TT = XT)) showed T allele carriers (XT genotype) with a significantly higher risk of T2D than those with the GG genotype with an OR of 3.63 (95% CI: 1.80 – 7.33, P < 0.001). Also, based on the recessive model ((GX =GG+GT) vs. TT), a significant greater risk of T2D was observed in those with the TT genotype compared to the GX genotype (OR: 3.91, 95% CI: 1.93-7.96, P < 0.001). Finally, the codominant model (GG vs GT vs TT) showed no significant risk of T2D when the GT genotype was compared with the GG genotype (OR: 1.68, 95% CI: 0.10-27.68, P = 0.718) but a significant higher risk of T2D was observed when the TT genotype was compared with the GG genotype (OR: 3.75, 95% CI: 1.84-7.64, P < 0.001). When the genotype frequencies were adjusted for age, the OR for the codominant, dominant and recessive models decreased but the difference were all significant (P < 0.05). The distribution of the genotype frequencies in the study population violated the Hardy-Weinberg equilibrium ($\chi 2 = 13.80$; P < 0.001). These results are summarized in Table 1.

Table 1. Association between the *TCF7L2* (G/T) polymorphism and type 2 diabetes (T2D) in a Nigerian Population.

	G/T variant	T2D (%)	ND (%)	OR (95% CI)	P-value	$\chi 2$	P-value
Allele							
	G	69 (23.3)	115 (38.9)	-----			
	T	77 (26.0)	35 (11.8)	3.68 (2.23-6.04)	<0.001		
	Total	146 (49.3)	150 (50.7)				
Genotype							
	GG	34 (23.0)	57 (38.5)	----			
	GT	1 (0.7)	1 (0.7)	1.68 (0.10-27.68) ∗1.52 (0.07-31.88)	0.718 ∗0.787		
Codominant model (TT)	TT	38 (25.7)	17 (11.5)	3.75 (1.84-7.64) ∗3.43 (1.62-7.23)	<0.001 ∗0.001	13.81	0.001
	Total	73 (49.3)	75 (50.7)				
Dominant model (TX)	TT +GT	39 (26.4)	18 (12.2)	3.63 (1.80 – 7.33) ∗3.41 (1.76-7.65)	<0.001 ∗0.001		
Recessive model (GX)	GG +GT	35 (23.7)	58 (39.2)	3.91 (1.93-7.96) ∗3.50 (1.68-7.29)	<0.001 ∗0.001		

T2D indicates type 2 diabetes patients, while ND indicates non-diabetic patients. OR indicates odd ratio, ∗ indicates age adjusted OR, CI: Confidence interval, $\chi 2$: Chi-square. The mutant allele (T) and genotypes (GT and TT) showed significant differences (P < 0.05) between the T2D and ND patients for the codominant, dominant and recessive models.

Relationship between genotypes and pathophysiological markers

Comparison of genotype frequencies of participants for clinical parameters showed no significant differences (P > 0.05) according to age, WC, BMI, TC, TG, and LDL-$_C$. However, FBG was significantly higher P < 0.05) in participants with the mutant TT genotype while HDL-$_C$ was significantly lower (P < 0.05) in participants with the mutant heterozygous GT and homozygous TT genotypes compared to the wild type homozygous GG genotype (Table 2).

Table 2. Relationship between *TCF7L2* (G/T) polymorphism and some study parameters in a Nigerian population.

	GG	GT	TT	P-value
Age (yr)	51.07±1.587	50.00±0.000	55.94±1.687	0.129
WC (cm)	95.44±1.79	104.00±10.00	93.60±2.61	0.631
BMI (Kg/m^2)	29.53±0.99	30.57±2.76	29.67±1.41	0.986
FBG (mg/dl)	97.35±18.54	108.50±65.43	144.69±28.32	0.006
TC (mg/dl)	204.49±13.31	241.07±1.73	256.29±10.79	0.280
TG (mg/dl)	191.14±13.31	252.84±73.02	196.99±10.79	0.728
LDL-c (mg/dl)	123.59±18.50	169.80±47.30	147.77±29.46	0.259
HDL-c (mg/dl)	54.23±4.52	22.12±15.89	39.67±3.04	0.044

Results are expressed as mean ± S.E.M; S.E.M: Standard error of the mean. WC; waist circumference, BMI: body mass index, FBG: fasting blood glucose, TC: total cholesterol, TG: triglyceride, LDL-c: low density lipoprotein cholesterol, HDL-c: high density lipoprotein cholesterol. The FBG was significantly higher ($P < 0.05$) in subjects with the mutant TT genotype while HDL-c was significantly lower ($P < 0.05$) in subjects with the GT and TT mutant genotypes.

DISCUSSION

Though Nigeria harbors the greatest burden of T2D in Africa, limited epidemiological data on the genetic predisposing factors of the disease is available. As such, this study was conducted to assess the relationship between the rs12255372 (G/T) polymorphism of the *TCF7L2* gene with T2D in a Nigerian population. Findings from this study revealed the presence of the mutant T allele in participants with a frequency of 37.8% and was significantly ($P < 0.001$) predominant in T2D patients (26%) than ND controls (11.8%). This frequency of 37.8% was similar to that of a finding in sub-Saharan Africa, precisely in Cameroon with a frequency above 30% (Nanfa et al., 2015), suggesting a high prevalence of the T allele in Sub-Sahara Africa. Similarly, a high prevalence of the T allele has been observed in other regions across the globe, such as in the Czech (Včelák et al., 2012) and Arab populations (Alsmadi et al., 2008). This allele was found to be significantly associated with risk of T2D (OR: 3.68, 95% CI: 2.23-6.04, $P < 0.001$). When the genotypes were compared, the wild type homozygous GG genotype was predominant in ND controls, the heterozygous GT genotype was similar in T2D and ND participants while the mutant TT homozygous genotype was more frequent in T2D patients and the difference was significant ($P < 0.05$). To assess the associated risk between rs12255372 (G/T) genotypes of *TCF7L2* gene and T2D, the dominant, recessive and codominant models were employed. All the three models showed the TT and GT genotypes to present a significant risk of T2D susceptibility with an OR above 3.4 suggesting that T2D patients with the mutant genotypes (GT and TT) are 3 or more times at risk than ND individuals. However, the recessive model showed the greatest risk of T2D with an OR of 3.91 (95% CI: 1.93-7.96, $P = 0.001$) and 3.50 (95% CI: 1.680-7.293, $P < 0.001$) when adjusted for age. These results suggest that the GT genotype may not significantly contribute to the risk of T2D. In order words, the risk of T2D susceptibility is mostly dependent on the TT mutant genotype. Overall, this study showed strong association between the rs12255372 (G/T) polymorphism of *TCF7L2* gene and T2D and the finding is consistent with previous reports in Cameroon (Nanfa et al., 2015) which is of the same sub-region as Nigeria and in other different ethnic and geographical populations across the world (Alami et al., 2012; Saadi et al., 2008; Wangs et al., 2007; Chang et al., 2007). However, a weak association was observed in West-Africa (Helgason et al., 2007) and Afro-Americans (Sale et al., 2007) with OR less than 2, while

no association was found in a South-African population (Pirie et al., 2010), a Tunisian population (Kifagi et al. 2011) or in a Brazilian population (Barros et al. (2014).

Since insulin plays a critical role in lipid metabolism and the *TCF7L2* gene product is a transcription factor which is known to be implicated in insulin action, it was necessary to evaluate the relationship between clinic parameters and rs12255372 (G/T) polymorphism. Our findings showed FBG to be significantly higher ($P < 0.05$) in patients with the mutant TT genotype and HDL-c to be significantly lower ($P < 0.05$) in patients with the mutant GT and TT genotypes suggesting that rs12255372 (G/T) polymorphism may increase blood sugar and lower HDL-c, two important pathophysiological markers that promote T2D.

As a preliminary study, one of the limitations was the small sample size. The small sample size may have limited the distribution of the genotypes in the population and may have accounted for the violation of Hardy-Weinberg equilibrium ($P < 0.05$) as small sample size has been reported as one of the factors that can cause deviation from the Hardy-Weinberg equilibrium (Elston and Forthofer, 1977). However, despite the relative small sample size of this study, the high level of statistical significance and odd ratio observed are clear indications that there is most probably an association between *TCF7L2* G/T polymorphism and T2D in this population. Also, this study was conducted in Enugu, a location in the South Eastern region of Nigeria dominated by the Igbo ethnic population and thus did not involve other ethnic populations of Nigeria. Thus, this finding may not be suitable to make generalization to the entire Nigerian population. Further studies need to be replicated in other regions and ethnic populations across Nigeria with larger sample size to confirm this finding.

CONCLUSIONS

The *TCF7L2* rs12255372 (G/T) polymorphism was associated with increased risk for T2D in a Nigerian population. This variant could have certain pathophysiological that affected increase the risk of T2D. Larger studies in other ethnic populations across Nigeria and with accurate genotyping techniques are needed to confirm this finding.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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