

A Pilot Study on the Prevalence of Transcription Factor 7-Like 2 Gene (*TCFL2*), Rs290487 in Ethnic Groups with Type 2 Diabetes Mellitus in Bayelsa State of Nigeria

C.H. Madukosiri¹, O.O. Ezomoh¹, B.M. Amos-Tautua^{2*}, Y.M. Taffeng³, D. Mishack¹, N. Akpeki¹,
C.E. Echendu³, S.P. Songca⁴ and E.T. Omu⁵

Page | 4559

¹Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

²Department of Chemical Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

³Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

⁴School of Chemistry and Physics, University of KwaZulu Natal, Durban, South Africa.

⁵Medical Centre, Federal University Otuoke, Bayelsa State, Nigeria

Date Received: 17-07-2022

Date Accepted: 07-12-2022

DOI: <https://doi.org/10.48198/NJPAS/22.B09>

ABSTRACT

The negative impact of type 2 diabetes mellitus (T2DM) on individuals, families, health system and the economic development as a whole, is an important justification for research in this field. The present study is a hospital-based case-control type to ascertain the genetic susceptibility of T2DM among a sample population of various ethnic groups resident in Bayelsa State, Nigeria. Also evaluated was the relationship between transcription factor 7-like 2 (*TCFL2*) rs12255372 and rs290487 genetic polymorphisms with development of T2DM. Genotyping of *TCFL2* rs12255372 and rs290487 were carried out using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. Among the indigenous groups, the allelic frequencies determined for the abnormal (CC) and the normal (TT) genotypes were 94.4 and 5.6 (%), respectively; whereas, the values 91.7 and 8.3 (%), in that order, were determined for the non-indigenous groups. A convincing significant difference was found between subjects with T2DM and their counterpart controls within indigenous people of Bayelsa State with respect to confounders, including age ($t=8.046$, $p<0.00001$), BMI ($t=2.628$, $p<0.0190$), waist circumference ($t=2.800$, $p=0.0091$) and fasting blood sugar ($t=3.212$, $p<0.0006$). This study verified the association of *TCFL2* rs290487 with the development of T2DM in indigenous and non-indigenous people in Bayelsa State. The risk conferred by the homozygous CC genotype was higher than that of the heterozygous TC state – a fact indicative of an additive model of inheritance.

Keywords: Genetic polymorphism, T2DM, *TCFL2*, rs290487, Bayelsa State, Nigeria.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases typified by high blood sugar resulting

from defects of insulin secretion and/or increased cellular resistance to insulin (Stumvoll *et al.*, 2005). Type 2 diabetes mellitus (T2DM) being the

Corresponding Author: B.M. Amos-Tautua

Department of Chemical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

Email: bmamos64@gmail.com Phone: +2347039871566



most common form of DM worldwide, is characterized by hyperglycemia, insulin resistance and relative insulin deficiency (Maitra and Abbas 2005). Although environmental factors and lifestyle changes are considered to play important role in the epidemiology of T2DM, the individual's inherent / genetic disposition is a fundamental predisposing risk determinant (Olokoba *et al.*, 2012). Therefore, identification of genetic polymorphisms influencing the development of T2DM is a main focus of present-day research, as this could enable the elucidation of the precise mechanism(s) underlying the pathogenesis of T2DM (Tong *et al.*, 2009).

The region of Africa is said to have the greatest percentage (about 66.7%) of undiagnosed diabetes cases, the greatest number of diabetes mellitus-related mortality and the smallest health expenditure on diabetes (FID, 2015). Among the different types of diabetics, T2DM makes up about 85-90%, (Wild, *et al.*, 2004. The reported high economic slowdowns / downturns due to T2DM and its negative impact on the health system were deemed alarming and justified the need for more research in this field (FID, 2015). Although research on the polymorphisms of transcription factor 7-like 2 (TCFL2) gene susceptibility in development of T2DM is globally ongoing (ADA, 2022; Haghighatdoost *et al.*, 2017; Chang *et al.*, 2007), literatures in this field from the developing countries in Africa are particularly scanty, and in some cases such data are non-existing.

As a polygenic metabolic disorder, T2DM development has been linked to and indeed associated with a considerable number of genes including *ADIPOQ*, *SLC30A8* and *TCFL2* (or *TCF4*) (Gupta *et al.*, 2010; Pirie *et al.*, 2010; Martinez-Gomez *et al.*, 2011). Transcription factor 7-like 2 (*TCFL2*) gene harbours the commonest genetic variant which confers the highest risk of T2DM reported in literature and is therefore considered as one of the strongest T2DM

susceptible genes among various ethnicities across the globe (Wang *et al.*, 2013a; Acharya *et al.*, 2015). The exact mechanism whereby *TCFL2* mediates in glucose homeostasis is not yet clearly delineated but research is active in exploring the involvement of *TCFL2* gene in induction of T2DM which was posited to involve genetic mutation, the Wnt signaling pathway and β -cell dysfunction (Maschio *et al.*, 2016; Wang *et al.*, 2017).

There are up to 13,076 single nucleotide polymorphisms (SNPs) of *TCFL2* gene listed in the National Center for Biotechnology Information SNP database (<http://www.ncbi.nlm.nih.gov/SNP>), many of which are identified to have shown strong evidence of association with T2DM, and some of which are rs 7895340, rs 3814570; rs 7094463, rs 7901695, rs 11196205, rs 7903446, rs 7903146, rs 12255372 and rs290487 among others (Wang *et al.*, 2013b; Wang *et al.*, 2016; Jainandunsing *et al.*, 2018). The associations between a number of single nucleotide polymorphisms (SNPs) of the *TCFL2* with T2DM have been confirmed in different populations in many countries (Chang *et al.*, 2007; Sale *et al.*, 2007; Turki *et al.*, 2013). Regrettably, some African countries, including Nigeria, are yet to make a concerted and coordinated effort in determining their genetic disposition to T2DM. The present study is the first of its kind to the best of our knowledge and therefore, aims to investigate (within the limits of our facilities) the association between two of the commonest SNPs, rs290487 and rs12255372 polymorphisms of *TCFL2* gene with T2DM in various ethnic groups resident in Bayelsa State in Nigeria.

Materials and Methods

Study Area

Subjects were those with type 2 diabetes mellitus who attended various out-patient clinics at Federal Medical Center, Ovum, Yenagoa (N 4° 56' 13.9488", E 6° 15' 59.1948"); Diete-Koki

Memorial Hospital, Opolo, Yenagoa (N 4° 56' 42", E 6° 20' 15"); the Niger Delta University Medical Centre, Amassoma (N 4° 58' 29", E 6° 06' 17") on the Wilberforce Island, all in Bayelsa State and the University of Port-Harcourt Medical Centre (N 4° 54' 26", E 6° 55' 01"), Choba, Port-Harcourt, Rivers State, Nigeria

Ethical Approval

Informed consent was obtained from all subjects prior to participation in the study. Ethical clearance (no.2019/001/BCH) was obtained from the Ethical Committee of Basic Medical Sciences, Niger Delta University (NDU). Subjects were admitted into the study after being clinically examined and certified fit by the Consultant Physician (Family Medicine), NDU, Bayelsa State, Nigeria

Sample size and Study population

A total of 80 volunteer subjects participated in the study which includes indigenous (IN) Ijaw, Nembe, Ogbia, and Epie ethnic groups in Bayelsa State; non-indigenous (NIN) Igbo and other ethnic groups resident in Bayelsa State in Nigeria. Screening and diagnosis were based on World Health Organization (WHO 2016) and American Diabetes Association (ADA 2002, 2022) criteria which cover both laboratory and clinical parameters. Fifty three (53) of the subjects were indigenous in Bayelsa State; while 27, though residents, hailed from other parts of the country namely Rivers, Delta, Anambra and Imo States. Forty-seven (47) of the IN and 20 of the NIN subjects were diagnosed with T2DM. Six of the IN and 7 of the NIN subjects served as the controls (Figure 1). Therefore 67 of the subject were diagnosed with T2DM and 13 were control.

Data collection

Patients with signs of pyrexia, goiter, oedema, history of chronic infectious diseases, sickle cell disease, cancers or suffering from end-stage kidney disease were excluded from the study. The control

subjects were sampled from the population of staff and students of NDU who voluntarily participated in the study. Some data on demographic, life style characteristics and some biochemical parameter were collected by use of questionnaires.

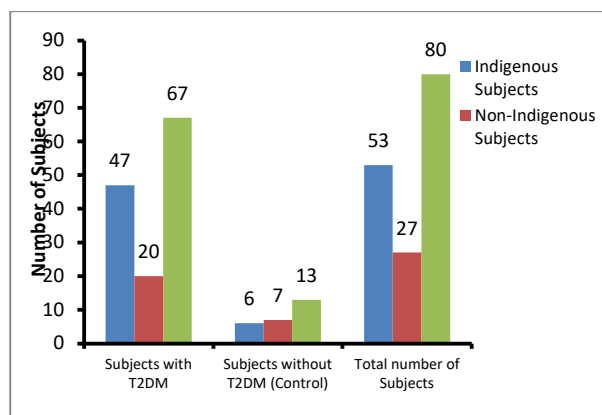


Figure 1: Demographic distribution of subjects

Sample collection

Blood samples were collected from T2DM patients and controls under aseptic conditions and dispensed into EDTA bottles and stored in a refrigerator at -20°C until required for use. The determination of the body mass index (BMI), body height and weight was measured to the nearest 0.1 cm and kg, as the case may be. Waist circumference (in cm) was measured half way between the lowest rib (in the standing position) and the iliac crest (Haghighatdoost *et al.*, 2017).

The sample size of 80 subjects was determined using the formula according to Yamane (1967):

$$n = N / \{1 + N(e)^2\}$$

Where: n=sample size, N= population under study (or respondents to questionnaire) = (100),

e=margin error (0.05).

Master mix composition

The PCR Master mix used for this procedure was Quick – Load Taq 2x Master mix. The composition include: 10 mM Tris-HCl, 50 mM KCl, 1.5mM

MgCl₂, 0.2mM dATP, 0.2mM dCTP, 0.2mM dGTP, 0.2mM dTTP, 5% Glycerol, 0.08% I GEPAL CA-630, 0.05% Tween 20, 0.024% Orange G, 0.003% Xylene Cyanol, 33 units/ml Taq DNA polymerase, pH 5.6 at 25%.

Genomic DNA was extracted from whole blood sample using the Zymo Research Extraction Kits (Inquaba Biotech, South Africa), in accordance with manufacturer instructions. DNA extraction was carried out at room temperature and the extracted DNA was stored in the freezer at 4°C until required for use.

DNA purity and quantification

DNA extract was quantified (checked for purity) using pre-installed NanoDrop software, version 1000. The nanoDrop spectrum was connected by a PC which allowed the archiving of large numbers of measurements in a tabular format – a sample of which was shown in Table 1. The instrument worked on the principle of uv-vis spectrophotometer and measured the light absorbance of DNA samples over a spectral range of 220 -750 nm; and since DNA absorbed with a peak at 260 nm, and protein at 280 nm, the levels of protein or carbohydrates contaminants were designed to be easily assessed by using the absorbance ratios – 260/280 and 260/230 in that order (as shown under their respective columns on table 1). Acceptable purity / quality for DNA samples were considered from 1.60 - 2.0 0, and 2.0 - 2.2 for the above ratios, respectively. While a low 260/280 ratio could be due to a protein contaminant absorbing at 280; low absorbance ratio of 260/230 might indicate carbohydrate contaminant or residual phenols from nucleic acids as given by the manufactures. Sample values outside the given purity range were diluted by 1% using nucleic free water.

PCR Procedure

The target DNA was amplified using TCF7RS72F 5'AGGAGGCTGCCATATTGTTTACTT3' and

TCF7RS72R:

5'ACACCTTTCTCATTTTTC AATTTCGC 3' primer at 153 bp segment. The amplification (PCR) process lasted for about two hours of 35 cycles in a PCR Thermal Cycler (GeneAmp PCR system 9700, UK). The oligonucleotide primers (20-mers) were designed using Primer3- free Online Design Tool. The PCR temperature profile involved 94°C/30 min (DNA denaturation), 51°C/30 sec (primer annealing), 72°C/30 sec - 7min (DNA extension) and finally 4°C for holding temperature.

Exactly 1.5% agarose solution was prepared using Trace Burate (TBE) buffer solution (10% in distilled water); while 0.5 µg/ml of ethidium bromide was used as the dye. The PCR products, alongside the Quick-load DNA molecular ladder were resolved on the already set agarose gel electrophoresis run at 130 volts, 500 mA for 20-25 minutes, the products of which were visualized with a blue light illuminator (Embi Tech. Pi-1002-prepone™ filter, UK)

Restriction fragment length electrophoresis (RFLE)

Restriction Fragment Length Polymorphism (RFLP) was carried out on the amplified products (5.0µL) which were digested with 0.3 µL of the restriction enzyme (RsaI), according to the manufacturers instruction (New England BioLabs Inc.); incubated for 5-15 min at 37°C in the thermocycler (PCR) and analyzed using 3% Agarose Gel Electrophoresis (Elite 300plus, USA). The enzyme-digested fragments were observed for the TT genotype, CT genotype and CC genotype The strength of the association between CC and TT genotypes of variant rs290487 and risk of T2DM was measured between cases and controls, using odds ratio (OR) determination (Table 3) (Szumilas, 2010).

Analysis of Results

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS 25.0, Chicago,

USA). All continuous variables were expressed as the mean \pm standard deviation (SD). Independent sample t-test was used to compare the baseline information and clinical data between the patients and controls. Differences were considered statistically significant if the p-value was < 0.05 . All p-values in this study were two-tailed tests. Categorical variables such as allelic and genotype frequencies (as explained by Hardy-Weinberg, 1970) among T2DM cases and controls were compared using the t-test. Association between variables was analyzed using Logistic Regression and Correlation coefficient.

Results

Quantification and purity of DNA extract

The quantification results from nanoDrop software showed that all DNA samples were within the concentration range of 5 ng to 100 ng/mL (Figure 2) as specified by the manufacturers. For the purity test, out of a total of 16 test samples (Figure 2), 5 samples were outside the purity range of 1.60 to 2.00 for absorbance peak of 260/280 while no test was within 2.0 to 2.2 for 260/230 column.

Restriction fragment length electrophoresis (RFLE)

A sample result of RFLE for RsaI-digested DNA is shown on Figure 3. Sample 2 with only one

fragment at 153 bp corresponded to TT genotype, meaning the amplicons were not digested by the restriction enzyme since there was no mutation. On the other hand, sample 4 showed the presence of 2 fragments with 128 bp, 153 bp and labelled as CC genotype which was homozygous for the genetic risk factor to T2DM development. None of the samples 1 to 7 showed the presence of 3 fragment – 153 bp, 128 bp and 25 bp which corresponded to CT heterozygous state as genetic risk carriers of T2DM.

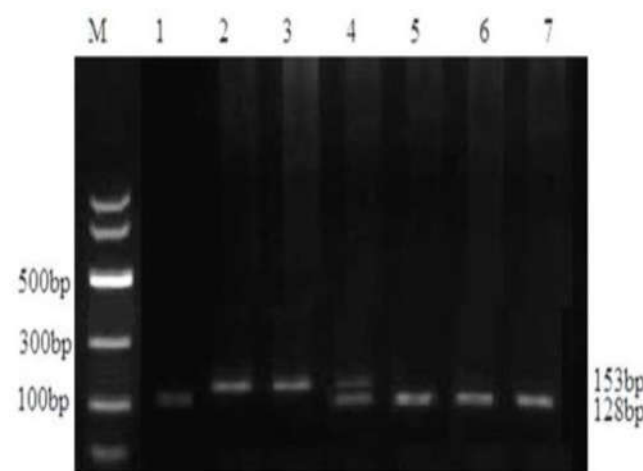


Figure 3. A sample of the agarose gel electrophoresis of PCR products

Plots | Report

Test type: Nucleic Acid

28-Sep-18 3:21 PM

Exit

Report Name

Report Full Mode

Ignore

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos	Cursor abs	340 row
1	Default	28-Sep-18	3:11 PM	6.97	0.139	0.086	1.61	0.25	50.00	230	0.548	-0.033
2	Default	28-Sep-18	3:12 PM	4.89	0.098	0.057	1.73	0.16	50.00	230	0.616	-0.035
3	Default	28-Sep-18	3:12 PM	8.32	0.166	0.070	2.37	0.61	50.00	230	0.272	-0.007
4	Default	28-Sep-18	3:13 PM	36.90	0.738	0.392	1.88	1.78	50.00	230	0.415	0.003
5	Default	28-Sep-18	3:13 PM	4.50	0.090	0.063	1.43	0.66	50.00	230	0.137	-0.001
6	Default	28-Sep-18	3:14 PM	6.13	0.123	0.077	1.59	0.73	50.00	230	0.169	-0.036
7	Default	28-Sep-18	3:14 PM	89.55	1.791	0.597	3.00	-4.97	50.00	230	-0.360	0.521
8	Default	28-Sep-18	3:15 PM	2.54	0.051	0.018	2.85	0.06	50.00	230	0.891	-0.030
9	Default	28-Sep-18	3:16 PM	8.30	0.166	0.083	2.00	0.15	50.00	230	1.086	-0.057
10	Default	28-Sep-18	3:16 PM	4.28	0.086	0.038	2.22	0.32	50.00	230	0.271	0.018
11	Default	28-Sep-18	3:16 PM	6.60	0.132	0.067	1.97	0.52	50.00	230	0.255	-0.045
12	Default	28-Sep-18	3:17 PM	8.16	0.163	0.092	1.76	0.94	50.00	230	0.174	-0.025
13	Default	28-Sep-18	3:17 PM	3.19	0.064	0.030	2.11	0.42	50.00	230	0.153	-0.028
14	Default	28-Sep-18	3:18 PM	12.14	0.243	0.120	2.03	0.23	50.00	230	1.062	-0.041
15	Default	28-Sep-18	3:18 PM	3.69	0.074	0.043	1.72	0.58	50.00	230	0.127	-0.043
16	Default	28-Sep-18	3:19 PM	6.60	0.132	0.086	1.54	0.27	50.00	230	0.488	-0.045
H2O	Default	28-Sep-18	3:19 PM	1.37	0.027	-0.005	-5.50	0.21	50.00	230	0.133	-0.042
BUFFER	Default	28-Sep-18	3:20 PM	0.58	0.012	-0.023	-0.50	-0.21	50.00	230	-0.056	-0.056

Figure 2: Nucleic acid test result of Nanodrop software, version 1000

Homozygous CC and TT genotypes rs290487 TCFL2 and risk of T2DM

Out of 67 T2DM cases sampled, 34 (72.34%) of IN subjects and 11 (55.00%) of the NIN cases expressed TCFL2 rs290487 with CC genotype, (Table 1). For the TT genotype, two (9.09%) of female subjects among IN cases showed positive result. About 16.67% of female IN subjects and 14.29% of male NIN control groups expressed the TCFL2 gene rs290487 with genotype CC (Table 1).

Frequency distribution of genotype rs290487 polymorphism of patients with T2DM showed a significant difference between the CC genotype (for each of IN or NIN cases) and their control counterparts ($t=32.488$, $p=0.00001$; $t=-12.000$, $p=0.00001$ respectively) (Table 2). The results of OR for CC genotype and risk of T2DM were 13.077 and 7.333 for the IN and the NIN groups respectively. This means there are higher odds of having T2DM in IN and the NIN groups who

expressed the CC genotype. An OR value of 0.000 was determined for TT genotypes in both groups which indicated that there were lower odds of these groups having T2DM. Among the IN group, the allelic frequencies for the abnormal (CC) and the normal (TT) genotypes were 94.40 and 5.60 (%), respectively; while for the NIN, the values were 91.70 and 8.30 (%), in that other, (Table 2).

Table 1: Subjects and control cases with T2DM TCFL2 gene rs290487, CC/TT genotypes in various ethnic groups

Ethnic Group	Male/female subjects	Male/female subjects with TCFL2 rs290487 CC genotype (%)	Total number of subjects with TT genotype (%)	T2DM subjects without TCFL2 rs290487 CC/TT/CT expression
Epie	m = 5 f = 6	5(100.00%) 3(50.00%)	- -	3
Ijaw	m = 11 f = 9	7(63.64%) 7(77.78%)	- -	6
Nembe	m = 4 f = 3	3(75.00%) 1(33.00%)	- -	1
Ogbia	m = 5 f = 4	4(80.00%) 4(100.00%)	- -	3
Total IN	47	34(72.34%)	2(9.09%)	11
Total NIN	20	11(55.00%)	1(11.11%)	8
Grand Total	67	45	3	32
Control (NIN)	m = 2 f = 4	0 1(16.67%)	- -	- -
Control (NIN)	m = 2 f = 5	1(14.29%) 0	- -	- -
Control (Total)	13	2(30.96%)	-	-

IN=indigenous volunteer subjects; NIN = non-indigenous volunteer subjects; m = male; f = female.
Out of the 67 cases with T2DM, 45 showed TCFL2 rs290487 CC genotype; three showed TT; while none was positive for CT.
Thirty-two subjects were cases of T2DM with no evidence of TCFL2 rs290487 CC, TT, or CT genotypes.
One subject each, from IN and NIN control groups expressed the TCFL2 rs290487 CC. There were no cases of TT or CT genotypes in the control cases.

Table 2: Genotypic and allelic frequencies of TCFL2 rs290487 polymorphisms in T2DM and controls for Indigenous and Non-indigenous Subjects

Indigenous	Number of Subjects (N=47)	Genotype frequencies (%)	Allelic frequencies (%)	Control (N=6)	G/A	t-test	p-value
TT	2	0.056 (5.6)	0.056 (6%)	0	0		
CC	34	0.944 (94.4)	0.944 (94%)	1	1.00 (100%)	32.488	0.00001
Total	36		1.000 (100%)	1			
Non-indigenous	Number of Subjects (N = 20)	Genotype Frequencies (%)	Allelic frequencies (%)	Control (N=7)	G/A	t-test	p-value
TT	1	0.0833	0.0833 (8.3%)	0	0		
CT	0	0	0	-	-		
CC	11	0.9166	0.9166 (91.7%)	1	1.00 (100%)		
Total	12		1.0000 (100%)	1		-12.000	0.00001

G/A = Genotype/Allelic frequencies. No subject was positive for the variant rs1225372 polymorphism of TCFL2 gene. Genotype/Allelic frequencies of cases (q) were significantly different from those of their controls, ($p < 0.05$). CC, the homozygous mutant Genotype; TT, the reference genotype..

Table 3: Calculation of the Odds Ratio (Szumilas, 2015)

	CASES	CONTROL
Gene Expression	a	b
Non Gene Expression	c	d

$$\text{Odds Ratio (OR)} = (a/c)/(b/d) = ad/cb$$

Where

a = Number of exposed cases; b = Number of exposed control

c = Number of unexposed cases; d = Number of unexposed control

An OR of exactly 1 was interpreted to mean that individuals with TCFL2 gene might not necessary have T2DM when exposed to environmental factors; whereas an OR higher than 1 meant that their odds of having T2DM when exposed would be higher, while the reverse was the case for an OR less than 1.

The Physical and Biochemical Characteristics of T2DM with TCFL2 gene rs 290487

The mean (\pm SD) values of age (years), weight (Kg), height (meters), WC (waist circumference, cm), FBS (Fasting Blood Glucose, mmol/L) and BMI (Body Mass Index, kg/m^2) for T2DM cases determined between the various ethnic groups (Epie, Ijaw, Nembe and Ogbia) are represented in

Tables 4 and 5 (for female and male cases respectively). There was only one female volunteer case from Nembe ethnic group, and as such, the mean \pm SD was not computed. There was no significant difference ($p \geq 0.05$) between the ethnic groups in the areas of age, weight, height, and FBS (Tables 4 & 5). However, significant differences ($p < 0.05$) were found for the values of WC and BMI in NIN and IN groups (Epie, Ijaw and Ogbia) as

shown in Tables 4 and 5. Also, there was no significant difference ($p \geq 0.05$) between the values of IN and NIN control groups (in combined male-female groups), with respect to weight, height, FBS, and BMI (Table 5). Contrarily, no statistical difference ($p \geq 0.05$) was found within the groups in respect of all parameters determined for female versus male groups (Table 5).

Student's T-test results showed that IN female group (Table 5) deferred significantly ($p < 0.05$)

from their counterpart control in the areas of age, height, FBS and BMI (Table 6). The correlation coefficient showed significant correlations between age/FBS among IN female cases ($r = -0.717$); weight/FBS among NIN male cases ($r = -0.089$); WC/FBS among IN males ($r = -0.770$); WC/FBS among the NIN female cases ($r = -0.891$); and BMI/FBS for IN males ($r = 0.902$), ($p < 0.05$), (Table 7).

Table 4: Physical and biochemical characteristics of female subjects with T2DM TCFL2 gene rs290487 among ethnic sample groups

Ethnic Group	Age (years)	Weight (kg)	Height (cm)	Waist (cm)	FBS (mmol/L)	BMI (kg/m ²)
Epie	55.00±3.61	62.67±5.51	1.59±0.04	92.60±1.21 ^a	8.50±2.26	24.75±1.10 ^c
Ijaw	52.50± 7.52	68.57±7.28	1.58±0.06	89.35±6.92 ^b	8.96±3.73	27.70±2.58
Nembe	49.01	57.11	1.62	97.04	11.23	22.27
Ogbia	54.75±9.32	68.85±2.70	1.60±0.05	83.53±9.83 ^c	8.05±2.41	27.15±2.49 ^g
Total IN	52.75±6.82	67.03±6.39	1.59±0.05	88.98±7.54	8.79±2.98	26.67±2.67
NIN	59.67±6.47	68.67±13.37	1.55±0.07	103.43±14.23 ^{abc}	5.73±2.29	28.46±4.65 ^{efg}

IN = Indigenous; NIN = Non-Indigenous. *Only one female volunteer subject was sampled from Nemb ethnic group. T-test was done between the ethnic groups and the NIN only. Figures with same superscripts on the vertical axis are significantly different, ($p < 0.05$).

Table 5: Physical and biochemical characteristics of male subjects with T2DM TCFL2 gene rs290487 among ethnic sample groups

Ethnic Group	Age (years)	Weight (kg)	Height (cm)	Waist (cm)	FBS (mm/L)	BMI (kg/m ²)
Epie	51.17±10.96	70.50±8.78	1.64±0.03	74.95±10.80 ^a	6.78±1.87	26.18±3.79 ^c
Ijaw	55.14± 7.78	75.43±5.91	1.65±0.05	77.93±12.05	8.56±2.47	28.05±1.71 ^d
Nembe	55.14±7.78	66.67±4.51	1.63±0.06	80.93±22.88	7.53±3.99	25.23±3.41
Ogbia	67.75 ±12.53	73.3±10.72	1.62±0.07	71.18±14.56 ^b	6.10±2.95	27.79±4.43 ^e
Total IN	57.50±10.31	72.66±7.69	1.64±0.05	76.14±13.24	7.38±2.61	26.87±3.23
NIN	59.71 ±8.16	71.86±5.08	1.59±0.11	97.56±7.63 ^{ab}	7.73±1.53	28.34±5.39 ^{cde}

IN = Indigenous; NIN = Non-Indigenous. T-test was done between the ethnic groups and the NIN only. Figures with same superscripts on the vertical axis are significantly different, ($p < 0.05$).

Table 6: Physical and biochemical characteristics of female and male indigenous and non-indigenous control subjects

Control	Gender (%)	Age (Years)	Weight (kg)	Height (cm)	Waist (cm)	FBS (mmol/L)	BMI (kg/m ²)
IN	Female: 4 (36.36%)	25.00 ±0.00	59.50 ±0.577	1.6575 ±0.026	86.20 ±7.591	4.625 ±0.340	21.998 ±0.494
	Male: 2 (18.18%)	25	61	1.575	72.9	4.6	24.6605
Total	6	24.5 ±0.84	60.00 ±7.01	1.63 ±0.07	81.63 ^a ±10.65	4.62 ±0.27	22.89 ±2.10
	Female:5 (71.43)	29.02 ±8.944	64.00 ±5.523	1.65 ±0.019	89.420 ±6.837	4.46 ±0.404	23.828 ±1.954
NIN	Male: 2 (28.57%)	40	80.5	1.705	93.05	4.8	27.261
	7	30.43 ±11.22	68.71 ±14.56	1.67 ±0.05	90.74 ^a ±8.79	4.57 ±0.42	24.80 ±3.40

IN = Indigenous; NIN = Non-Indigenous. The values for the male groups were means of only two subjects.

Figures with the same superscript on the vertical axis are significantly different ($p < 0.05$). There were no significant differences, with respect to weight, height, waist, FBS, and BMI ($p > 0.05$).

There were no significant differences between the values for IN female control and those of counterpart NIN female controls, ($p > 0.05$).

Table 7: Correlation coefficient determined between physical characteristics versus FBS of Males and Females subjects with T2DM TCFL2 gene rs290487

Subject	Age/FBS	Weight/FBS	WC/FBS	BMI/FBS
Male (IN)	-	-	$r = -0.770, p < 0.05$	$r = -0.902, p < 0.05$
Male (NIN)	-	$r = -0.089, p < 0.05$	-	-
Female (IN)	$r = -0.717, p < 0.045$	-	-	-
Female (NIN)	-	-	$r = -0.891, p < 0.05$	-

- =No significant correlation; FBS = Fasting blood sugar; BMI = body mass index; WC = Waist Circumference. Only groups with significant correlation are represented on the table. Correlation is significant at $p = < 0.05$ (2-tailed).

Discussion

The TCFL2 gene is one of the most influential genes implicated in genetic susceptibility to T2DM in humans (Dabelea *et al.*, 2011). The present study has successfully confirmed a significant association between T2DM and TCFL2 rs290487 of the CC genotype, ($p < 0.00001$). A statistically significant difference was found between the frequencies of CC genotype among the cases and control subjects. This finding was further confirmed by the results of the ORs which showed that the CC genotype of rs290487 was

associated with T2DM since it (CC genotype) raised the odds of having T2DM. OR values in this study were in consonant with the values (2.5 to 14) given by Peng *et al.*, (2013). Three subjects (2 females and 1 male), were carriers of TT genotype of rs290487; while none was heterozygous CT. This led to the conclusion that the risk of T2DM increased with homozygous CC genotype of rs290487. This could be interpreted as impairment of β -cell responsiveness to the regulation of glucose homeostasis in the CC genotype individuals (Alibegovic *et al.*, 2010);

and possibly, glucose intolerance through disturbances in glucagon and insulin secretion.

Four well-studied SNP markers in the human *TCFL2* were reported to include rs7903146, rs7901695, rs12255372 and rs11196205 (Alami *et al.*, 2013; Ouhaibi-Djellouli *et al.*, 2014). However, no single subject showed presence of rs12255372 polymorphism in our study. Low association between SNPs 12255372 and T2DM had also been reported in Africa (Wang *et al.*, 2013a) and agrees with the present study. Granted, the risk variant in our polymorphic population is a different type, rs 290487, the possibility that other variants of *TCFL2* exist which might be associated with risk of T2DM, cannot be ruled out. In a similar study, Wang *et al.*, (2013b) found association between CC genotypes of both rs 290487, and rs 7903146 of *TCFL2* with T2DM in Han Chinese population; whereas, in another Chinese population, the Uygur, TT genotype of rs3814570 was posited as the genetic risk factor (Wang *et al.*, 2016). As a result of these variations in genetic susceptibility factors in the development of T2DM, there is need to encourage various human populations to determine the genetic factors that either pose as risks determinant or offer protection against T2DM. This would be necessary for early detection of T2DM, and consequent reduction in mortality/morbidity rates and economic burden of the disease.

Potential effects of interaction of *TCFL2* with T2DM and confounders such as physical characteristics and biochemical data had been indeed the topic of investigations (Wang *et al.*, 2013a). Although the present study found no statistically significant differences ($p < 0.05$) among confounders - age, height, FBS, and BMI between males' (or females') cases versus counterpart controls, (Table 6), it did particularly record a relationship between WC and FBS, thereby suggesting the pair could represent an important risk factor to T2DM for the study

population. In corroboration, other scholars had also recognized abdominal obesity as an important risk factor to T2DM in several populations (Haghighatdoost *et al.*, 2017). Waist circumference (WC) measures visceral adiposity and therefore has served as an abdominal adiposity index among populations (Nymdorj *et al.*, 2008). In fact, scholars agree on the need for every population to determine its own risk confounder(s), since the ability of anyone confounder to act as a DM risk factor is deemed ethnic and race-dependent (Alberty *et al.*, 2006; ,Langenberg *et al.*, 2012; Haghighatdoost *et al.*, 2017). Our WC values, ≥ 90 and 80cm (for males and females respectively), were in consonant with the cut-off points recommended for abdominal obesity by the above cited references.

Conclusion

To the best of our knowledge, this is the first study to investigate the relationship between the *TCFL2* gene and T2DM in various ethnic groups resident in Bayelsa, Nigeria. This study has demonstrated that the genetic variant of *TCFL2*, rs290487 of CC genotype is significantly associated with T2DM among various ethnic groups of Ijaw, Epie, Nembe, Ogbia, and other non-indigenous people from other States who are resident in Bayelsa State, Nigeria. The risk conferred by the homozygous CC genotype was significantly higher than those of homozygous TT when compared to their counterpart controls. No single subject (in cases and control groups) showed evidence of heterozygous state TC genotype rs290487 or of the gene variant rs12255372. This study has contributed to broadening the knowledge in the area of genetic susceptibility to T2DM in Bayelsa in Nigeria.

Conflict of interest

The authors declared that they had no competing interest.

Funding

This study was a self-funded research.

Authors' contributions

CHM conceptualized and designed the research and experiments. CHM, DM, and NA designed questionnaire. ETO, DM and NA collected samples for the study. MT, ECE, DM, NA, CHM, BMAT and OOE participated in data analysis and manuscript preparation. OOE, CHM and GM wrote the first draft of the manuscript. BMAT and SPS carried out the review and final editing of the manuscript; while BMAT acted as correspondence author. CHM, MT, EC and OOE are the guarantors of this work and have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors, particularly, BMAT and CHM critically revised the manuscript and approved the final version.

Acknowledgement

We sincerely thank all those who gave us their support during the present study. We particularly extend our appreciation to Dr Y.E Oku, Consultant General Surgeon, General Surgery Unit, Department of Surgery, Federal Medical Centre; the Ethical Committees of the College of Health Sciences, NDU Teaching Hospital Okolobiri, Bayelsa State and those of University of Port Harcourt Teaching Hospital, Rivers State; the subjects who voluntarily participated in the study and all others who by one way or the other contributed to the present study, of whom were Elizabeth Harcourt, Brakoru Enjoyment, Eniyekepemi Fedelis, Sunday Hope, Azuh Chioma Goodness, Obuma Esther, Egapekpar Eno-Anyia, Loveday Ibirubara, Eniyekeyi Tekemene Blessing, Appeal Emmanuella, Orusha Pius, Mark Mercy and Frausas Gloria.

References

- Acharya, S., Al-Elq, A., Al-Nafaie, A., Muzahed, M. and Al-Ali, A. (2015). Type 2 diabetes mellitus susceptibility gene TCF7L2 is strongly associated with hyperglycemia in the Saudi Arabia Population of the eastern province of Saudi Arabia. *European Review for Medical and Pharmacological Sciences*. 19(16):3100-3106.
- Alami, F. M., Ahmadi, M., Bazrafshan, H., Tabarraei, A., Khosravi, A., Tabatabaiefar, M. A. and Samaei, N. M. (2012). Association of the TCF7L2 rs12255372 (G/T) variant with type 2 diabetes mellitus in an Iranian population. *Genetics and Molecular Biology*, 35(2): 413-417.
- Alberty, K.G., Zimmet, P. and Shaw, J. (2006). Metabolic syndrome--a new worldwide definition. A consensus statement from the International Diabetes Federation. *Diabetic Medicine*. 23: 469-480
- Alibegovic, A.C., Sonne, M.P., Højbjørre, L., Hansen, T., Pedersen, O., van Hall, G., Holst, J.J., Stallknecht, B., Dela, F. and Vaag, A. (2010). The T-allele of TCF7L2 rs7903146 associates with a reduced compensation of insulin secretion for insulin resistance induced by 9 days of bed rest. *Diabetes*. 59(4): 836-843.
- American Diabetes Association (2002). Executive summary: *Screening for Diabetes Care*. 25 (Suppl. 1):S21-S24,
- American Diabetes Association (2022). Classification and diagnosis of diabetes: Standard of medical care in diabetes – *Diabetes Care*. 45(Suppl.1): S17-S38.
- Chang, Y., Chang, T., Jiang, Y., Kuo, S., Lee, K. and Chuang, L. (2007). Association study

of the genetic polymorphism of the transcription factor 7-like 2 (TCFL2) gene and type 2 diabetes in the Chinese population. *Diabetes*. 56: 2631-2637.

Page | 4570

Dabelea, D., Dolan, L.M., D'Agostino, R., Hernandez, A.M., McAteer, J. B., Hamman, R. F Mayer-Davis, E.J., Marcovina, S., Lawrence, J.M., Pihoker, C. and Florez, J.C. (2011). Association testing of TCF7L2 polymorphisms with type 2 diabetes in multi-ethnic youth. *Diabetologia*. 54(3): 535-539.

FID (Federation, International Diabetes), (2015). [*IDF diabetes atlas*](#) (Seventh ed.). International Diabetes Federation. [ISBN 9782930229812](#). [OCLC 961366911](#).

Gupta, V., Khadgawat, R., Ng, H.K.T., Kumar, S., Aggarwal, A., Rao, V.R. and Sachdeva, M.P. (2010). A validation study of type 2 diabetes-related variants of the TCF7L2, HHEX, KCNJ11, and ADIPOQ genes in one endogamous ethnic group of North India. *Annals of Human Genetics*. 74(4): 361-368.

Haghighatdoost, F., Amini, M., Feizi, A. and Iraj, B. (2017). Are body mass index and waist circumference significant predictors of diabetes and prediabetes risk? Results from a population based cohort study. *World Journal of Diabetes*. 8(7): 365-373.

Jainandunsing, S., Koole, H.R., van Miert, J.N., Rietveld, T., Wattimena, J.D., Sijbrands, E.J. and de Rooij, F.W. (2018). Transcription factor 7-like 2 gene links increased in vivo insulin synthesis to type 2 diabetes. *EBioMedicine*. 30: 295-302.

Langenberg, C., Sharp, S.J., Schulze, M.B., Rolandsson, O., Overvad, K., Forouhi, N.G., Spranger, J., Drogan, D., Huerta,

J.M., Arriola, L., de Lauzon-Guillan, B., Tormo, M.J., Ardanaz, E., Balkau, B., Beulens, J.W., Boeing, H., Bueno-de-Mesquita, H.B., Clavel-Chapelon, F., Crowe, F.L., Franks, P.W., Gonzalez, C.A., Grioni, S., Halkjaer, J., Hallmans, G., Kaaks, R., Kerrison, N.D., Key, T.J., Khaw, K.T., Mattiello, A., Nilsson, P., Norat, T., Palla, L., Palli, D., Panico, S., Quirós, J.R., Romaguera, D., Romieu, I., Sacerdote, C., Sánchez, M.J., Slimani, N., Sluijs, I., Spijkerman, A.M., Teucher, B., Tjonneland, A., Tumino, R., van der A, D.L., van der Schouw, Y.T., Feskens, E.J., Riboli, E. and Wareham, N.J. (2012). Long-term risk of incident type 2 diabetes and measures of overall and regional obesity: the EPIC-InterAct case-cohort study. *PLoS Medicine*. 9: e1001230

Nyamdorj, R., Qiao, Q., Lam, T.H., Tuomilehto, J., Ho, S.Y., Pitkaniemi, J., Nakagami, T., Mohan, V., Janus, E.D. and Ferreira, S.R. (2008). BMI compared with central obesity indicators in relation to diabetes and hypertension in Asians. *Obesity* (Silver Spring). 16:1622-1635.

Maitra, A. and Abbas, A.K. (2005). Endocrine system. In: Kumar V, Fausto N, Abbas AK (eds) 2005. Robbins and Cotran Pathologic basis of disease (7thed), Philadelphia, Saunders. 1156-1226.

Martínez-Gómez, L.E., Cruz, M., Martínez-Nava, G.A., Madrid-Marina, V., Parra, E., García-Mena, J., Espinoza-Rojo, M., Estrada-Velasco, B.I., Piza-Roman, L.F., Aguilera, P. and Burguete-García, A.I. (2011). A replication study of the IRS1, CAPN10, TCF7L2, and PPARG gene polymorphisms associated with type 2 diabetes in two different populations of Mexico. *Annals of Human Genetics*. 75(5): 612-620.

- Maschio, D.A., Oliveira, R.B., Santos, M.R., Carvalho, C.P.D.F., Barbosa-Sampaio, H.C.L. and Collares-Buzato, C.B. (2016). Activation of the Wnt/ β -catenin pathway in pancreatic beta cells during the compensatory islet hyperplasia in prediabetic mice. *Biochemical and Biophysical Research Communications*. 478(4):1534-1540.
- National Center for Biotechnology Information SNP database (<http://www.ncbi.nlm.nih.gov/SNP>)
- Olokoba, A.B., Obateru, O.A. and Olokoba L.B. (2012). Type 2 Diabetic Mellitus: A Review of Current Trends. *Oman Medical Journal*. 27 (4): 269-273.
- Ouhaibi-Djellouli, H., Mediene-Benchekor, S., Lardjam-Hetraf, S.A., Hamani-Medjaoui, I., Meroufel, D.N., Boulouar, H., Hermant, X., Saidi-Mehtar, N., Amouyel, P., Houti, L. and Goumidi, L. (2014). The TCF7L2rs7903146 polymorphism, dietary intakes and type 2 diabetes risk in an Algerian population. *BMC Genetics*. 15(1): 1-8.
- Peng, G., Li, L., Liu, Y., Pu, J., Zhang, S., Yan, J., Zhao, J. and Liu, P. (2011). Oleates blocks palmitate-induced abnormal lipid distribution, endoplasmic reticulum expansion and stress, and insulin resistance in skeletal muscle. *Endocrinology*. 152: 2206-2218.
- Pirie, F.J., Motala, A.A., Pegoraro, R.J., Paruk, I.M., Govender, T. and Rom, L. (2010). Variants in PPARG, KCNJ11, TCFL2, FTO, and HHEX genes in South African subjects of Zulu descents with type 2 diabetes. *African Journal of Diabetes Medicine*. 18(1):12-6.
- Sale, M.M., Smith, S.G., Mychaleckyj, J.C., Keene, K.E., Longfeld, C.D., Leak, T.S., Hicks, P.J., Bowden, D.W., Rich, S.S. and Freedman, B. I. (2007). Variants of the transcription factor 7-like 2 (TCFL2) gene are associated with type 2 diabetes in an African-American population enriched for nephropathy. *Diabetes*. 56: 2638-2642.
- Stumvoll, M., Goldstein, B.L. and van Haeften, T.W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*. 365:1333-1346.
- Szumilas, M. (2010). Explaining odds ratios. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*. 19(3): 227.
- Tong, Y., Lin, Y., Zhang, Y., Yang, J., Zhang, Y., Liu, H. and Zhang, B. (2009). Association between TCF7L2 gene polymorphisms and susceptibility to type 2 diabetes mellitus: a large human genome epidemiology (HuGE) review and meta-analysis. *BMC Medical Genetics*. 10(1):1-25.
- Turki, A., Al-Zaben, G.S., Mtiraoui, N., Marmuoch, H., Mahloub, T. and Almawi, W.Y. (2013). Transcription factor-7-like 2 gene variants are strongly associated with type 2 diabetes in Tunisia Arab subjects. *Gene*. 513: 244-248,
- Yamane, T. (1967). Elementary Sampling Theory, New Jersey: Prentice-Hall, Inc.
- Wang, H., Ren, Y., Hu, X., Ma, M., Wang, X., Liang, H. and Liu, D. (2017). Effect of Wnt signaling on the differentiation of islet β -cells from adipose-derived stem cells. *Biomedical Research International*. 2017.
- Wang, J., Li, L., Zhang, J., Xie, J., Luo, X., Yu, D., Zhao, J., Feng, T., Pang, C., Yin, L. and

- Hu, F. (2013a). Association of rs7903146 (IVS3C/T) and rs290487 (IVS3C/T) polymorphisms in TCF7L2 with type 2 diabetes in 9,619 Han Chinese population. *PLoS One*. 8(3): e59053.
- Wang, J., Zhang, J., Li, L., Wang, Y., Wang, Q., Zhai, Y., You, H. and Hu, D. (2013b). Association of rs12255372 in theTCF7L2 gene with type 2 diabetes mellitus: a meta-analysis. *Brazilian Journal of Medical and Biological Research*. 46: 382-393.
- Wang, Z., Liu, L., Chen, Z., Ma, Q., Li, Y., Su, Y., Wang, L., Ding, L., Wang, S., Zhu, J. and Yao, H. (2016). Association of rs3814570 and rs7094463 polymorphisms in TCF7L2 gene with susceptibility to type 2 diabetes mellitus in a Chinese Uygur population. *International Journal of Clinical and Experimental Pathology*. 9(9): 9508-9519.
- Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 27(5):1047-1053.
- World Health Organization (2016), [*Global Report on Diabetes*](#). Geneva, Accessed 30, August 2016.