RELATIONSHIP BETWEEN ABCC8 C/T AND KCNJ11 E23K POLYMORPHISMS WITH TYPE 2 DIABETES IN A NIGERIAN POPULATION

ENGWA A. GODWILL1,3*, NWALO N. FRIDAY2, UBI E. BENJAMIN3

Affiliation:

1Department of Chemical Sciences, Godfrey Okoye University, P.M.B 01014, Thinkers Corner, Enugu Nigeria
2Department of Biotechnology, Federal University, Ndufu-Alike Ikwo (FUNAI), P.M.B. 1010 Abakaliki, Nigeria
3Department of Biotechnology, Ebonyi State University, P.M.B 53 Abakaliki, Nigeria

SUNDAY 5TH – THURSDAY 9TH AUGUST 2018

Introduction

Type 2 diabetes-Most prevalent type of diabetes affecting 150 million people worldwide (90% of diabetes cases globally)

Nigeria: 1.2 million people -Highest disease burden in African (IDF, 2013)

3,85 million people with impaired glucose tolerance (IDF, 2016)

Environmental factors such as;
Poor feeding habit, over feeding, sedentary life style etc. can lead to

- excess fat in the body and **obesity**
- increasing **free fatty acid and cholesterol**

Pathophysiological Changes

➢**Dyslipidaemia-**high cholesterol, high triglyceride, high low density lipoprotein (LDL) and low high density lipoprotein (HDL) levels in blood.

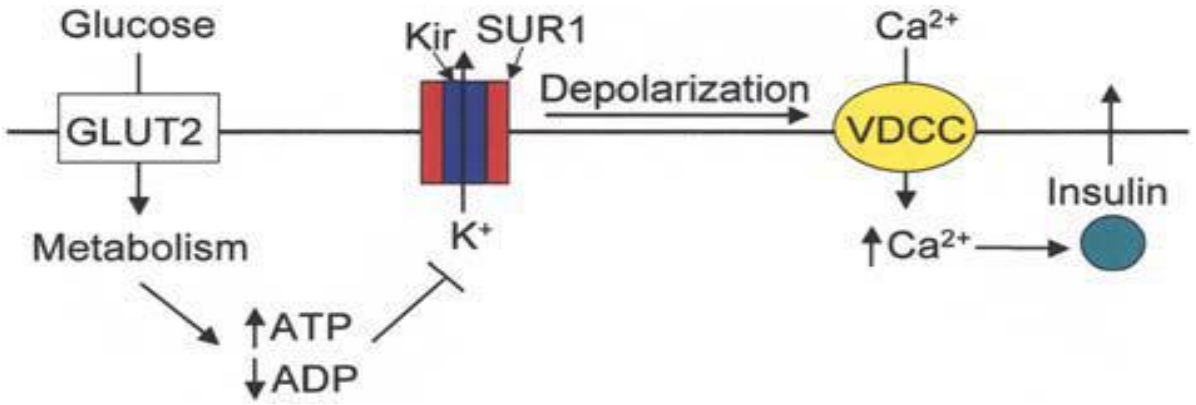
➢**Impaired adipocyte differentiation** - a process of free fatty acid from triglyceride storage into adipose tissue is altered.

These factors (obesity, dyslipidaemia, impaired adipocyte differentiation) greatly contribute to insulin resistance

Insulin resistance may lead to the accumulation of glucose in blood (hyperglycaemia)- type 2 Diabetes (T2D)

ATP binding cassette, subfamily C, member 8 (*ABCC8)* and potassium inwardly-rectifying channel subfamily J, member 11 (*KCNJ11*) genes

- *ABCC8* gene encodes the high-affinity sulfonylurea receptor (SUR1) subunit
- *KCNJ11* encodes Kir6.2 which are part of the ATP-sensitive potassium channel



- Both genes are target for sulfonylureas drug thus mutation may affect drug action
- Studies have shown variant forms of *KCNJ11* (Lys) and *ABCC8* (Ala) genes to be associated with T2D (Gloyn *et al.,* 2003).

Aim of the study

- This study was undertaken to identify ABCC8 C/T and KCNJ11 E23K polymorphisms and assess their association to the risk and pathophysiology of type 2 diabetes in outpatients attending Enugu State University Teaching Hospital (ESUTH) in Enugu State of Nigeria

Objectives

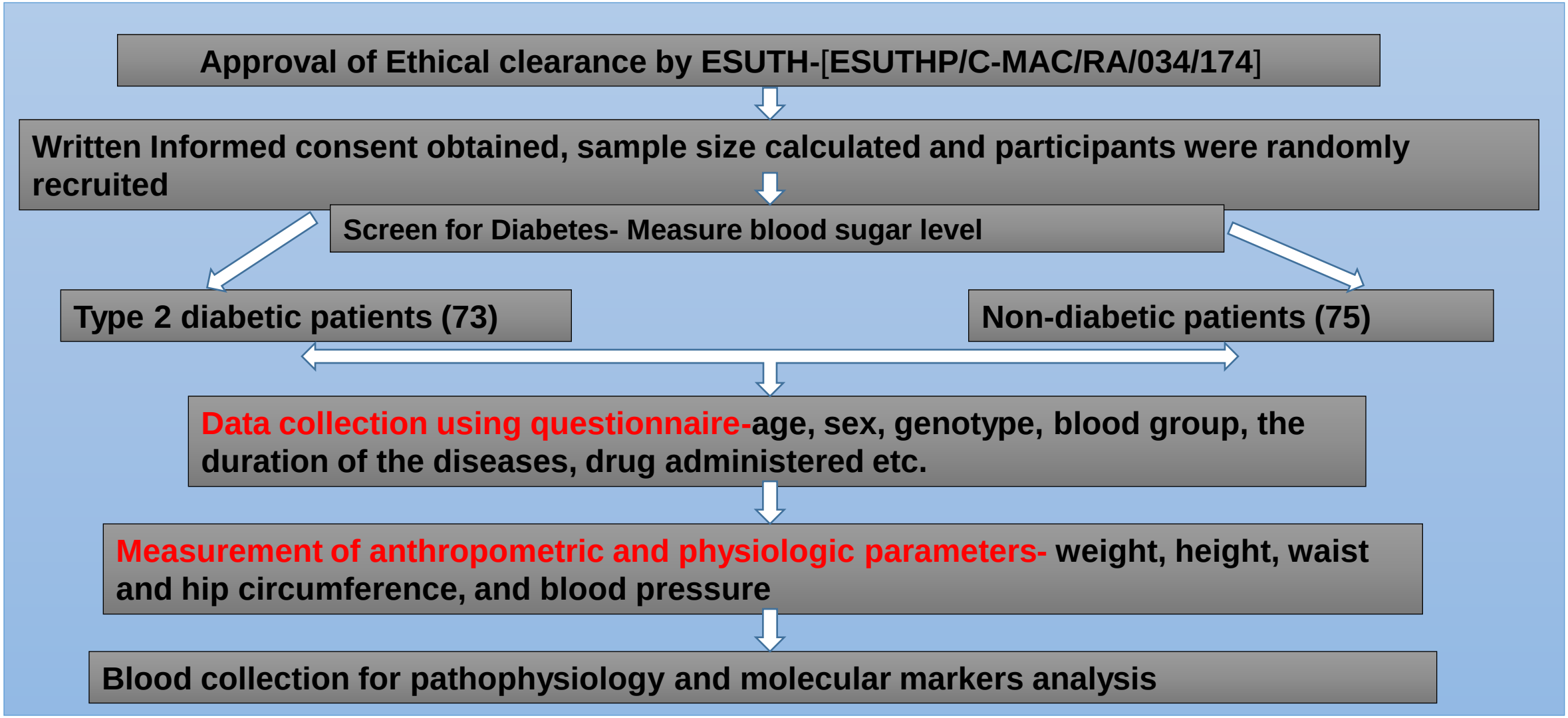
- Assess obesity and lipid profile indices; total cholesterol, triglyceride, HDL, and LDL levels in patients.

- Molecularly genotype *ABCC8 C/T, and KCNJ11 E23K* polymorphisms by polymorphisms by restriction fragment length polymorphism- polymerase chain reaction (RFLP-PCR)

- Assess the association these genetic polymorphism with T2D and pathophysiological markers.

Materials and Methods

- **Study Design: Case-control study**



Analyses for pathophysiological markers

- **Determination of blood sugar:** It was assayed by the glucose oxidase–peroxidase method using Accucheck glucometer (Tinder,1969)

Determination of Lipid profile:

- Total Cholesterol, triglyceride, and HDL was quantified using the kits from Randox Laboratories, UK according manufacturers' protocol and based on the methods of Allain *et al.* (1974), Esders and Michira (1997) and Grove (1979) respectively.

- LDL was deduced from the Friedwald's formula: TOTAL-Chol – TG/5 – HDL -Chol (mg/dL) (Friedwald's , 1972)

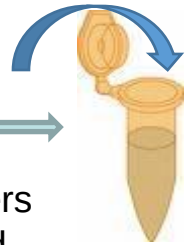
Molecular genotyping by RFLP-PCR

DNA extraction: DNA was extracted using the GeneJET Genomic DNA Purification kit (K0721) by Thermo Fisher Scientific. Inc. , USA according to manufacturers protocol.

extracted **DNA** Added

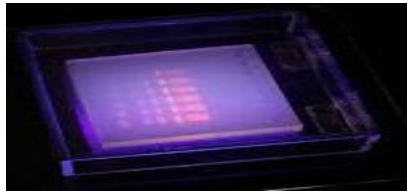
One Tag PCR Master mix with standard buffer mix tube (Contains: Tag Polymerase, dNTPs, MgCl2)

Primers added

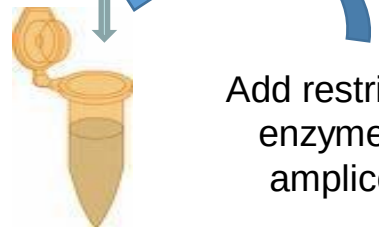


Transfer tube into thermal cycler

Agarose gel (2%) electrophoresis



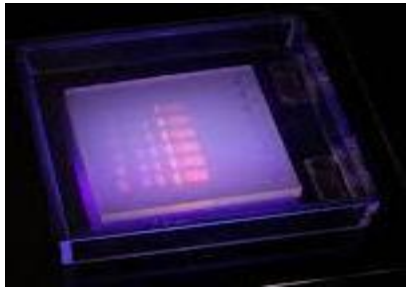
Visualize amplicon in Transilluminator



Add restriction enzyme to amplicon

Incubated at desired temperature for specific period of time

Agarose gel (3-3.5%) electrophoresis of digested DNA



Visualize of digested DNA

Primers and restriction enzymes for molecular genotyping by RFLP-PCR

Gene	Polymorphism	Forward Primer	Backward Primer	Restriction Enzyme	References
KCNJ11	E23K	5′-GACTCTGCAGTGAGGCCCTA-3′	5′-ACGTTGCAGTTG CCTTTCTT-3′	***BanII*** 	Jiang *et al.*, 2014
ABCC8	C49620T (rs1799854)	5’-TTGGGTGCATCTGTCTGTCTGTCTTT-3’	5’-AGCCCACCTGCCCCACGAT-3’	***PstI*** 	He *et al.*, 2008

Results and Discussion

Table 2: Baseline Characteristics of the study participants

Characteristics	T2D (73)	ND (75)	Minimum	Maximum	*p*-value
Age (year)	56.87±1.19	49.03±1.90	30	92	0.001
Height (m)	1.59±0.01	1.61±0.01	1.37	1.90	0.218
WC (cm)	100.25±1.68	89.37±2.5	36.00	149.00	0.000
SBP (mmHg)	132.95±2.62	132.86±3.23	100	213	0.982
DSP (mmHg)	79.12±1.38	81.76±2.24	58	151	0.297
FBS (mg/dl)	166.38±11.23	65.75±3.79	11.00	520.00	0.000

Legend: Results are presented as Mean± SEM; SEM: Standard error of the mean. SBP: Systolic Blood Pressure; DSP: Diastolic Blood Pressure; FBS: Fasting Blood Sugar; BMI: Body mass index; WC: Waist circumference; T2D: type 2 diabetes patients; ND: Non-diabetic patients

Relationship between obesity, lipid profile and T2D

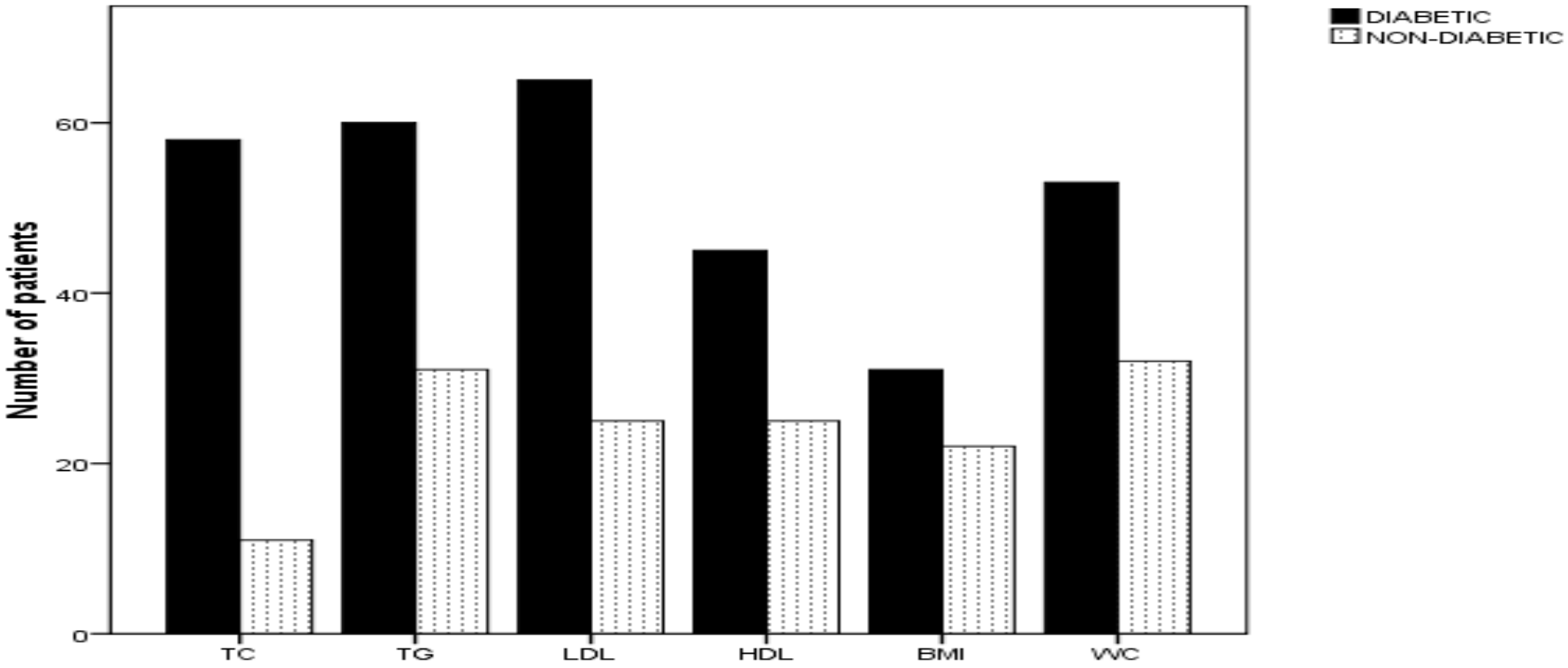
Table 3: Comparison of obesity and lipid profile in patients

Parameter	T2D	ND	Minimum	Maximum	*p-value*
BMI (Kg/m^2)	31.38±1.41	27.81±0.76	18.20	85.13	0.026
WC (cm)	100.15±1.70	89.37±2.25	36.00	149.00	<0.001
TC (mg/dL)	291.78±28.90	159.43±7.77	5.28	1387.07	<0.001
TG (mg/dL)	241.33±15.443	148.82±7.59	22.99	792.16	<0.001
LDL (mg/dL)	212.40±29.42	78.96±7.96	1.26	1302.06	<0.001
HDL (mg/dL)	33.55±2.14	62.74±5.16	1.29	239.16	<0.001

Legend: BMI: Body mass index; WC: Waist circumference; TC: total cholesterol; TG: triglyceride; LDL: Low density lipoprotein; HDL: High density lipoprotein

- Obesity as a powerful predictor of metabolic syndrome, diabetes and its associated complications (Yoon *et al.,* 2006; Despres, 2001).

- Findings corroborate with other studies in Nigeria (Edo and Edo, 2012; Fasanmade and Okubadejo, 2007) and the world at large (Hillier and Pedula, 2001).



- **Figure 1: Frequency of dyslipidaemia and obesity among T2D and ND patients**

•This finding concords with previous studies which have also showed dyslipidaemia characterised by elevated triglyceride, cholesterol, LDL and low HDL level in diabetes (Dixit *et al.,* 2014; Smith and Lall, 2008)

Relationship between *ABCC8* C/T polymorphism and T2D

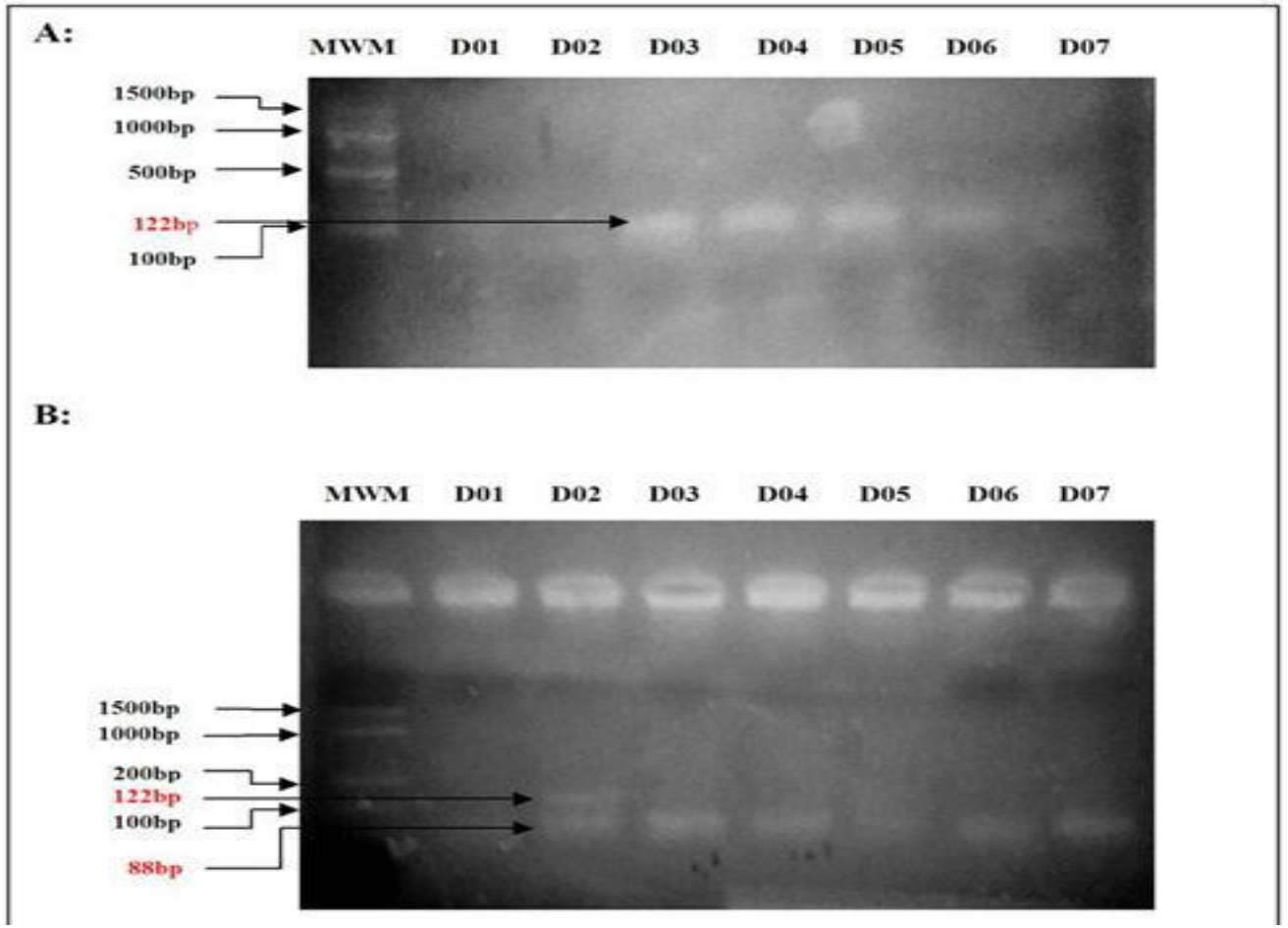
•All samples successfully amplified for *ABCC8* gene with molecular size of 122 bp (Figure 4A)

•After restriction digest, the genotypes were:
Wild homozygote CC genotype was a **122bp**

The homozygote **TT** genotype: **88 bp and 34 bp,**

•The CT genotype showed **122bp, 88bp and 34bp**

•The **34bp** which was not seen (Figure 4B).



Legend: MWM: 100 bp molecular weight marker; UD: Undigested sample; bp: base pair

Table 16: Association between the *ABCC8* C/T polymorphism and T2D

	ABCC8 C/T variant	T2D (%)	ND (%)	OD (95% CI)	*P*-value	$\chi 2$	*P*-value
Allele							
	C	48 (16.2)	67 (22.6)	-----			
	T	98 (33.1)	83 (28.0)	1.7 (1.059-2.730)	0.028		
	Total	146 (49.3)	150 (50.7)				
Genotype							
	CC	19 (12.8)	27 (18.2)	------			
Codominant	CT	10 (6.8)	13 (8.8)	1.093 (0.397-3.007)	0.863		
model (TT)				∗1.258 (0.440-3.596)	∗0.668		
	TT	44 (29.7)	35 (23.6)	1.786 (0.856-3.729)	0.122	2.781	0.249
				∗2.576 (1.150-5.771)	∗0.021		
	Total	73 (49.3)	75 (50.7)				
Dominant	TT +CT	54 (36.5)	48 (32.4)	1.599 (0.791-3.232)	0.191		
model (TX)				∗2.126 (1.000-4.519)	∗0.05		
Recessive	CC +CT	29 (19.6)	40 (27.0)	1.734 (0.903-3.329)	0.098		
model (GX)				∗2.388 (1.162-4.909)	∗0.018		

Findings corroborates previous findings which *showed ABCC8* C/T polymorphism to be associated with T2D (Yokoi *et al.*, 2006; Hart *et al.*, 1999), gestational diabetes (Rissanen *et al.*, 2000) and been shown to alter insulin secretion (Goksel *et al.*, 1998; Reis *et al.*, 2000

Table 17: Relationship between *ABCC8 (C/T)* polymorphism and some obesity and pathophysiological markers

	CC	CT	TT	*p-value*
Age (yr)	55.98±2.16	53.57±2.56	50.84±1.62	0.148
WC (cm)	94.19±2.66	97.00±4.02	94.75±1.99	0.836
BMI (Kg/m^2)	29.24±1.51	28.73±1.25	30.05±1.15	0.818
FBS (mg/dl)	119.69±15.59	97.43±12.28	118.92±9.36	0.545
TC (mg/dl)	185.46±20.52	217.42±19.75	247.78±25.75	0.206
TG (mg/dl)	192.12±11.49	220.36±18.23	187.64±15.09	0.470
LDL (mg/dl)	118.61±20.64	132.02±20.71	161.90±26.39	0.460
HDL (mg/dl)	36.11±2.47	41.88±3.98	57.38±5.23	0.005

This confirms the findings of previous studies which also showed no association of this polymorphism with anthropometric parameters (Pietrzak-Nowacka *et al.,* 2012), obesity and body fat (Laukkanen *et al.,* 2004).

Relationship between *KCNJ11* E23K (G/A) polymorphism and T2D

Amplified samples for *KCNJ11* E23K (G/A) fragment gave a band of **209bp** (Figure 5).

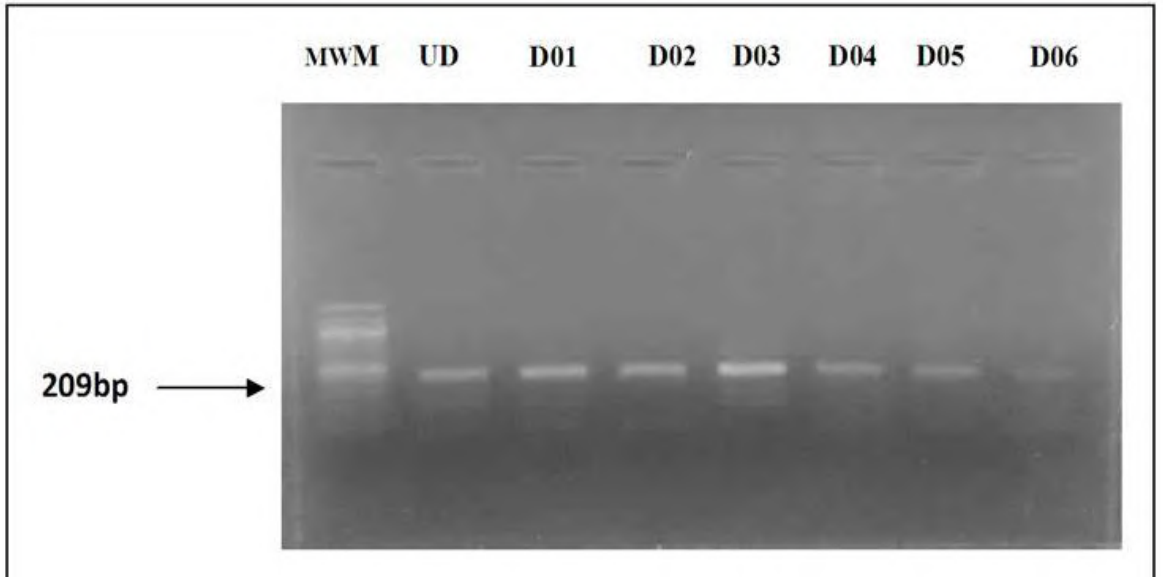
After restriction enzyme digest, the expected product sizes were :

•The normal homozygote **GG** genotype was **150bp and 59bp**

•Mutant homozygote **AA** genotype showed only a **209bp**

•heterozygote **GA** genotype **(209, 150bp, 59 bp) was absent**

•The **59 bp** was not visualized.



Legend: MWM: 100 bp molecular weight marker; UD: Undigested sample; bp: base pair; E: Glutamate, K: Lysine

Table 18: Association between the *KCNJ11 E23K* (G/A) polymorphism and T2D

KCNJ11 G/A variant	T2D (%)	ND (%)	OD(95% CI)	*p*-value	$\chi 2$	*p*-value
Allele						
G	10 (3.4)	12 (4.1)	-------			
A	136 (45.9)	138 (46.6)	1.183 (0.494-2.828)	0.706		
Total	146 (49.3)	150 (50.7)				
Genotype						
GG	5 (3.4)	6 (4.1)	------			
GA	0 (0.0)	0 (0.0)	------			
AA	68 (45.9)	69 (46.6)	1.183 (0.345-4.059)	0.790	0.071	0.790
			∗1.421 (0.394-5.119	∗0.591		
Total	73 (49.3)	75 (50.7)				

The non-association between *KCNJ11 E23K* polymorphism and T2D is consistent with a finding in Ghana (Danquah *et al.*, 2013) but in contrast to that of Tunisia (Lasram *et al.*, 2014)

Table 19: Relationship between *KCNJ11* (G/A) polymorphism and some study parameters

	GG (E)	AA (K)	*p*-value
Age (yr)	64.45±5.37	51.92±1.16	0.004
WC (cm)	94.27±2.73	94.94±1.58	0.902
BMI (Kg/m^2)	32.57±5.99	29.36±0.733	0.299
FBS (mg/dl)	112.67±20.88	115.92±7.61	0.914
TC (mg/dl)	194.02±14.52	226.22±16.78	0.587
TG (mg/dl)	169.42±63.54	196.13±8.74	0.451
LDL (mg/dl)	103.37±22.29	147.15±16.99	0.467
HDL (mg/dl)	81.16±20.30	45.80±2.80	0.002

Conclusion

- T2D was associated by obesity and dyslipidaemia
- *ABCC8* C/T polymorphisms was associated with high risk of T2D development while the *KCNJ11 E23K* was not associated with T2D.
- These genetic polymorphisms could influenced some of these polymorphic markers such as HDL .

Prospectives

- Since *ABCC8* and *KCNJ11 genes are targets* antidiabetic treatment such as sulphonylurea, further studies are needed to investigate the effect of these polymorphic genes (*ABCC8* and *KCNJ11*) on the efficacy and safety of these drugs

References

- Allain, C.C., Poon, L.S., Chan, C.S.G. and Richmond, W. (1974). Total cholesterol assay. *Clinical Chemistry.* **20**: 470-471.

- Esders, T.N. and Michira, CA (1997). Triglyceride estimation. *Journal of Biological Chemistry*. **254**: 2710-2712.

- Friedwald, W.T. Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma without use of Preparative Ultracenrifugation. *Clinical Chemistry.* **18**: 499-502.

- Grove, T.H. (1979). Grove's method of high density lipiptotein estimation. *Clinical Chemistry.* **25**: 560-562.

- Gloyn, A.L., Weedon, M.N., Owen, K.R., Turner, M.J., Knight, B.A., Hitman, G., Walker, M., Levy, J.C., Sampson, M., Halford, S., McCarthy, M.I., Hattersley, A.T., Frayling, T.M. (2003). Large-scale association studies of variants in genes encoding the pancreatic β-cell KATP channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes*. **52**: 568–572.

- Jiang, Y., Chuang, L., Pei, D., Lee, Y., Wei, J., Sung, F., and Chang T. (2014). Genetic Variations in the Kir6.2 Subunit *(KCNJ11)* of Pancreatic ATP-Sensitive Potassium Channel Gene Are Associated with Insulin Response to Glucose Loading and Early Onset of Type 2 Diabetes in Childhood and Adolescence in Taiwan. *International Journal of Endocrinology*. **2014**: 1-7.

Published Articles

- **Godwill Azeh Engwa,** Friday Nweke Nwalo, Claribel Chidimma Chikezie, Christie Onyia, Opeolu Oyejide Ojo, Wilfred Fon Mbacham, Benjamin Ewa Ubi. Possible association between ABCC8 C49620T polymorphism and Type 2 Diabetes in a Nigerian population. **BMC Medical Genetics**. 2018; 19:78. https://doi.org/10.1186/s12881-018-0601-1
-
- **Godwill Azeh Engwa,** Friday Nweke Nwalo, Chosen E. Obi, Christie Onyia, Opeolu Oyejide Ojo, Wilfred Fon Mbacham, Benjamin Ewa Ubi. Predominance of the A allele but no association of the *KCNJ11* rs5219 E23K polymorphism with Type 2 Diabetes in a Nigerian population. **Genetics and Molecular Research.** 2018; 17 (1):1-8. gmr16039889. DOI http://dx.doi.org/10.4238/gmr16039889.