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Distribution of E23K Genotypes in Diabetic and Non-Diabetic Subjects in Port Harcourt metropolis, Nigeria.

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ABSTRACT

This study examined the distribution of the E23K allele variant of the KCNJ11 gene in type 2 diabetes mellitus and non- diabetics in a Nigerian population. The E23K polymorphism of the KCNJ11 gene results from a substitution of the amino acid lysine to glutamate at codon 23. This alteration causes a critical inhibition of glucose-induced insulin secretion thereby resulting in hyperglycaemia. Hundred consenting Nigerian adults (73 diabetics and 27 non-diabetic subjects) aged at least 40 participated in this study. Genotyping was carried out with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique using BanII restriction digestion enzyme. The restriction fragments were then electrophoresed on DNA grade agarose gel and the bands visualised using a UV transilluminator. The genotypes identified are the EE (150bp band), EK (150bp+178bp bands) and KK (178bp band) genotypes. The KK genotype was preponderant in the diabetic participants (52%) and was followed by the EK genotype (25.9%) while the EE genotype was more in the non-diabetic participants (66.7%). The risks conferred by the different genotypes/allele are as follows: EK (p value = 0.5639; OR = 1.32), EE (p value = 0.000; OR = 0.21), KK (p value = 0.0037; OR = 7.03), K (p value = 0.211; OR = 2.59) and E (p value =

0.0552; OR = O.52). A carrier of the KK genotype is seven times more likely than a non-carrier to develop type 2 diabetes mellitus (p value = 0.0037; 7.03). Only the KK genotype was found to significantly increase the risk of developing type 2 diabetes complications (p value = 0.02; OR = 12.67). The p values of the selected biochemical variables are as follows: leptin = 0.95, fasting blood sugar = 0.15, C-peptide = 0.47, Cystatin C = 0.86, HbA1C = 0.01, insulin = 0.65 and HOMA = 0.65. Of the glycaemic variables analysed, only HbAlc showed a significant difference between the diabetic and control groups (p value = 0.01) but there was no significant difference in its levels in the different genotypes (p value = 0.64). A significant association between the E23K polymorphism and T2DM was found in the Nigerian population that was studied. The KK genotype of the E23K polymorphism of the KCNJ11 gene is an independent predictor of Type 2 diabetes mellitus.

Keywords: E23K, Polymorphism, Diabetes Type2, Genotype, KCNJ11 gene

1. INTRODUCTION

Diabetes mellitus, a polygenic disorder characterized by hyperglycaemia due to pancreatic inability to secrete enough insulin or peripheral insulin resistance, has become a leading health problem worldwide (Khaled *et al*., 2014; Qi *et al*., 2012). Estimates from the International Diabetes Federation show that approximately 425 million adults were living with diabetes and this number will rise to 69 million people by 2045. The proportion of people with type 2 diabetes is increasing in most countries with 79% of adults with diabetes living in low and middle income countries (International Diabetes Federation Atlas, 2017).

Risk factors for T2DM include family history of T2DM, age, obesity, sedentary lifestyle, hypertension and hyperlipidaemia (Taber *et al*., 2015). Apart from rare monogenic diabetes, type 2 diabetes mellitus is mostly a multifactorial disease resulting from the interaction of genetic variation at different chromosomal sites with environmental factors in an orchestrated manner throughout the lifespan (Permutt *et al*., 2005; Assman *et al*., 2014). Environmental factors such as changes in diet and reduction in physical activity are the most likely reasons for the marked prevalence of type 2 diabetes experienced over the last couple of decades (Vimaleswaran *et al.*, 2010).

Although these lifestyle changes may predispose an individual to type 2 diabetes, the disease only occurs in the presence of genetic risk factors for this condition (Vimaleswaran *et al*., 2010). For this reason, several genetic association studies and genome-wide association scans (GWAS) have been carried out in a bid to identify susceptibility genes and loci associated with this disease (Assman *et al*., 2014).

Some susceptibility genes identified by these genetic studies include TCF7L2 (Taiser *et al*., 2014; Nanet *et al*., 2015), PPARG (Mori *et al*.,1998; Engwa *et al*.,2018), SLC30A8, HHEX/IDE (Saxena *et al.,* 2007), CDKAL1, CDKN2A/B, IGF2BP2 and FTO (Scott *et al*.,2007), IRSI,ADAM TS9 and GCKR (Bossegard *et al*.,2009), WFS1 (Sandhu *et al*.,2007), HNF1B (Sparoso *et al*.,2008) and KCNJ11 (Lasram *et al*.,2014).

The KCNJ11 gene is our primary focus in this work. It is found on chromosome 11 and codes for a 390 amino acid protein that is one of the subunits of the pancreatic K_{ATP} channel (Lasram *et al*., 2014; Rastegari *et al*., 2015).

KCNJ11 and ABCC8 genes which code for Kir6.2 and SUR1 are adjacent to each other on chromosome 11 (Sakamoto *et al*., 2007; Grant *et al*., 2009). Studies have shown that mutations in both genes cause congenital hyperinsulinism (James *et al*., 2009), familial persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI) (Sakamoto *et al*., 2007), permanent neonatal diabetes (PNDM) (Sagen *et al*., 2004). However, these genetic studies have yielded conflicting results for ABCC8 variants and more consistent results for the KCNJ11 variants (Sakamoto *et al*., 2007).

Several single nucleotide polymorphisms (SNP) have been shown to increase susceptibility to type 2 diabetes mellitus. Thus far KCNJ11 has been found to have 219 SNPs, six of which have been linked to type 2 and they are: rs5219 (E23K polymorphism), rs5215, rs5210, rs5218, rs886288, rs2285676 (Haghvirdiazadeh *et al*., 2015). In the E23K polymorphism, there is a substitution of the amino acid lysine to glutamate (AAG –CAG) at codon 23 of the NH_2-terminal tail of the Kir6.2 (Haghvirdiazadeh *et al*., 2015; Rastegari *et al*., 2015). Even though lysine is positively charged and glutamate carries no charge at all, there is no remarkable difference in the function and structure of the Kir6.2 proteins (Haghvirdiazadeh *et al*., 2015). It has been reported that the E23K variant may alter the charge of the ATP-binding region, thereby decreasing the sensitivity of the KATP channel to ATP (Haghvirdiazadeh *et al*., 2015).

Similarly, this locus has been associated with increase in fasting and post prandial glucose levels (Shaat *et al*., 2005; Gonet *et al*., 2012) and higher glycated haemoglobin and blood pressure levels (Koo *et al*., 2007; He *et al*., 2008). Pharmacological studies in this polymorphism have revealed varying drug response with some patients having a better therapeutic response to glimepiride and glibenclamide than gliclazide treatment (Javorsky *et al.*, 2012). E23K variant carriers have also been found to have a reduced response to sulfonylurea therapy (Holstein *et al*, 2009; El-sisi *et al*., 2011).

The relationship between the E23K phenotype and T2DM risk has been studied in various populations such as the European (Florez *et al*., 2004), Arab (Abdelhamid *et al*., 2014), Asian (Zhou *et al.,* 2009) and Tunisian populations (Lasram *et al.,* 2014). Significant association of E23K with T2DM was found in some European descent populations (Florez *et al*., 2004) and East Asian populations (Zhou *et al*., 2009). In the Arabian populations, the reported results were divergent (Abdelhamid *et al*., 2014) suggesting that these findings cannot be extrapolated to other populations. There is paucity of data available on the E23K allele variant in African and Nigerian populations.

There is insufficient knowledge/research in Nigeria of how genetics plays a role in the development of disease and disease progression in the different ethnic groups. Little is known of the prevalence of the E23K allele variant in the Nigerian population.

Although current diagnostic and pharmacologic practices are effective in the diagnosis and management of T2DM, they are lacking in the areas of risk prediction, determination of disease progression and individualized management (such as pharmacologic and lifestyle) therapies.

Determining the significance of E23K allele variants in T2DM patients in Port Harcourt may likely be useful in genetic risk prediction and preventive plan

2. MATERIALS AND METHODS

2.1 Research Design and subject characterization

This is a cross sectional study to identify E23k allele variants and to evaluate A1C, fasting blood sugar, leptin, Cystatin C, C-peptide, insulin and HOMA index in T2DM patients in

Port Harcourt City. A total of 100 consenting individuals were enrolled for this study. This number was based on convenient sampling.

This study received ethical clearance from the Rivers State Health Ethics Research Committee. Participants were informed of the nature of the study and consent was obtained. The participants in this study were between 40-77 years of age: 55 males and 45 females. They were drawn from four Nigerian tribes: Ikwerre, Ijaw, Igbo and Ogoni. Seventy- three of them were diabetic and served as the test subjects while twenty - seven of them were not diabetic and served as the controls.

Consenting individuals who are of Nigerian descent aged at least 40 years diagnosed with T2DM for at least one year with glycated haemoglobin level ≥ 6.5. Individuals less than 40 years of age, who are not of Nigerian descent and pregnant.

2.2 Sample Collection and Assays

Ten millilitres of venous blood were collected into fluoride oxalate, EDTA and vacuum tubes: 2mls for fasting blood sugar, 5mls for PCR analysis and 3mls for chemistry analysis.

C-peptide, Insulin, Leptin and Cystatin C were analysed using standard ELISA technique.

Fasting blood sugar was analysed using the glucose oxidase-peroxidase method and HbA1C was analysed using the high performance liquid chromatography technique. Polymerase chain reaction was carried out using the PCR-RFLP method described by Souza *et al*., 2017.

The E23K target was first amplified using a set of primers, the amplicon was Restriction Enzyme (RE) digested and visualized by agarose gel electrophoresis. A 3.0 % agarose gel was prepared by adding 3.0 g of agarose DNA grade to 100 ml of 0.5x TBE (Tris Borate EDTA) buffer, swirling to dissolve and melting in a microwave for 3 minutes. While the mixture was cooling outside, 20 µl of Ethidium Bromide was added. The molten agarose was poured into trays with cassettes and allowed to cool. Ten microlitres of RE digested product was loaded into each well. Electrophoresis was done at 100V for 30 minutes. The gel was visualized in the UV transilluminator and pictures taken using the Genomemini Gel Documentation System. The bands observed after electrophoresis were interpreted thus: 178 bp band only represents the KK genotype, 178 bp + 150 bp bands represents the EK genotype and 150 bp band only represents the EE genotype.

2.3 Statistical Analysis

The statistical analysis was carried out using GraphPad Prism Version 8 by GraphPad Software Inc., California.

3. Results

3.1 Demographic Characteristics of Participants in this Study

The details of the demographic characteristics of the participants of this study are shown in table 1. There were 55 males and 45 females drawn from four ethnic groups (Igbo, Ijaw, Ikwerre and Ogoni). The frequencies of these ethnic groups were 30, 26, 18 and 26 respectively. Among the study population, 74 participants were on drugs while 26 were not on drugs, 20 consumed alcohol while 80 did not, 3 were smokers while the remaining 97 were non-smokers, 64 did some form of exercise while 36 did not and 55 had a family history of diabetes while 45 did not.

Table 1: Demographic characteristics of participants in this study

Variable		Number	Frequency
Sex	Male	55	55
	Female	45	45
Tribe	Igbo	30	30
	Ijaw	26	26
	Ikwerre	18	18
	Ogoni	26	26
Drugs	Yes	74	74
	No	26	26
Alcohol	Yes	20	20
	No	80	80
Smoking	Yes	3	3
	No	97	97

Exercise	Yes	64	64
	No	36	36
Family history of diabetes	Yes	55	55
	No	45	45

3.2 Anthropometric Characteristics of Participants

Table 2 shows the mean, median, standard deviation and range values of the anthropometric characteristics of the participants. The age range of the participants was 35-77 years, the mean was 59.4 ± 10.1 years and the median age was 66 years. The range for duration of illness (months) was 0-30 months; the mean was 10.6 ± 9.3 months and median 10.0 months. The participants weighed between 38.0 and 126.0 kg, the mean weight was 76.5kg ± 15.3 and their median weight was 74.0kg. The mean and standard deviation of height was 1.6 ± 0.1, range was 1.1-1.9 and median height was 1.7. Waist circumference was between 28.0 - 53.0cm, the mean and standard deviation was 40.6 ± 4.6 and median weight of 41.0cm. The mean BMI was 23.5 ± 4.3, range was between 5.4 – 37.1 and the median BMI was 23.1. The systolic pressure of the participants ranged between 99.0 - 184.0mmHg, the median value was 130.0mmHg and the mean was 132.3mmHg. The mean for diastolic pressure was 79.0 ± 11.0, the range was 58.0 -107.0mmHg and the median diastolic pressure was 78.0mmHg.

Table 2: Anthropometric characteristics of participants

Variable	Mean	SD	Median	Range
Age (years)	59.4	10.1	66.0	40.0 – 77.0
Duration of illness (months)	10.6	9.3	10.0	0.0 – 30.0
Weight (kg)	76.5	15.3	74.0	38.0 – 126.0
Height (m)	1.6	0.1	1.7	1.1 – 1.9
Waist Circumference (cm)	40.6	4.6	41.0	28.0 – 53.0
BMI	23.5	4.3	23.1	15.4 – 37.1
Systolic Pressure (mmHg)	132.3	17.7	130.0	99.0 – 184.0
Diastolic Pressure (mmHg)	79.0	11.0	78.0	58.0 – 107.0

3.3 Inferential Statistics on Anthropometric Data between Diabetics and Non-Diabetics

Table 3 shows the difference in the anthropometric data between diabetic and non-diabetic participants. Mann Whitney U test was performed on anthropometric variables of diabetic and non-diabetic subjects enrolled in the study. Age had a Mann-Whitney U value of 541.0 and a p value of 0.0003. The Mann-Whitney U value of weight was 720.5 and its p value was 0.0589. Height was 554.0 (Mann-Whitney U value) and p value was 0.0032. For waist circumference Mann-Whitney U value was 715.5 and p value was 0.2547. BMI showed a Mann-Whitney U value of 895.5 and a p value of 0.9212. Systolic Pressure had a Mann-Whitney U value of 860.0 and a p value was 0.7604. The Mann-Whitney U value of diastolic pressure was 791.0 and its p value was 0.3772. Only age and height showed statistically significant difference, p value < 0.050.

Table 3: Inferential statistics on anthropometric data between diabetics and non-diabetics

Variable	Mann-Whitney U value	P value
Age (years)	541.0	0.0003
Weight (kg)	720.5	0.0589
Height (m)	554.0	0.0032
Waist circumference (cm)	718.5	0.2547
BMI	897.5	0.9212
Systolic Pressure (mmHg)	860.0	0.7604
Diastolic Pressure (mmHg)	791.0	0.3772

3.4 Descriptive Statistics of Glycaemic Variables in Diabetic and Non-Diabetic subjects

Table 4 is a descriptive statistics of the glycaemic variables between the diabetic and non-diabetic participants. It shows the mean and standard deviation of the glycaemic variables of diabetic and non-diabetic participants as well as the reference range of healthy individuals. The mean and standard deviation of insulin in the diabetic and non-diabetic subjects was 53.8 ± 24.8miu/L and 55.1 ± 22.2miu/L respectively (reference range: <25miu/L). The mean and standard deviation of leptin in the diabetic subjects was 13.9 ± 6.3ng/ml and in the non-diabetic it was 14.1 ± 6.2ng/ml (reference range: < 23.1ng/ml). C-peptide had a value of 3.0 ± 3.8 ng/ml in the diabetic participants and a value of 2.4 ± 2.6 ng/ml in the non-diabetic participants (reference range: 0.5 – 2.7ng/ml). For HbA1C it was7.6 ± 2.3% in the diabetic subjects and 6.5 ± 1.6% in the non-diabetic subjects (reference range < 6.5 %). The HOMA

index had a mean and standard deviation of 1.6 ± 1.2 in the diabetic participants and a mean and standard deviation of 1.4 ± 0.5 in the non-diabetic participants (reference range: < 2.9). Fasting blood sugar was 6.5 ± 2.1mmol/L in the diabetic subjects and in the non-diabetic subjects it was 5.7 ± 1.1mmol/L (reference range: 3.4 – 6.8 mmol/L). Finally, Cystatin C had a value of 0.8 ± 0.4 in the diabetic patients and a value of 0.7 ± 0.4 in the controls (reference range: 0.5 – 1.0 mg/ml).

Table 4: Descriptive statistics of glycaemic variables in diabetic and non-diabetic subjects

Variable	Diabetic Subjects (Mean ± SD)	Non-diabetic subjects (Mean ± SD)	Reference Range in healthy Subjects
Insulin (miu/L)	53.8 ± 24.8	55.1 ± 22.2	< 25
Leptin (ng/ml)	13.9 ± 6.3	14.1 ± 6.2	< 23.1
C-peptide (ng/ml)	3.0 ± 3.8	2.4 ± 2.6	0.5 – 2.7
HbA1c (%)	7.6 ± 2.3	6.5 ± 1.6	< 6.5
HOMA index	1.6 ± 1.2	1.4 ± 0.5	< 2.9
FBS (mmol/L)	6.5 ± 2.1	5.7 ± 1.1	3.4 – 6.8
Cystatin C (mg/ml)	0.8 ± 0.4	0.7 ± 0.4	0.5 – 1.0

3. 5 Statistical Differences in Biomarkers between Diabetic and Non-Diabetic Subjects

Table 5 shows the test for statistical difference between diabetic and non-diabetic patients. Mann-Whitney U test was performed. The Mann-Whitney U value for leptin was 976.5 and its p value was 0.95. For fasting blood sugar Mann-Whitney U value was 799.0 and the p value was 0.15. C-peptide had a Mann-Whitney U value of 891.0 and a p value of 0.47. Mann-Whitney U value of Cystatin C was 962.5 and its p value was 0.86. Mann-Whitney U value of HbA1C was 651.0 and its p value was 0.01. Insulin had a Mann-Whitney U value 926.5 and a p value of 0.65. The Mann-Whitney U value of the HOMA index was 926.0 and its p value was 0.65. Only HbA1C showed a statistically significant difference: the rest were not statistically significant.

Table 5: Statistical differences in biomarkers between diabetic and non-diabetic subjects

Variable	Mann-Whitney U value	P Value	Comment
Leptin	976.5	0.95	Not significant
FBS	799.0	0.15	Not significant

C-peptide	891.0	0.47	Not significant
Cystatin C	962.5	0.86	Not significant
HBA1C	651.0	0.01	Significant
Insulin	926.5	0.65	Not significant
HOMA	926.0	0.65	Not significant

3.6 Distribution of E23K Genotypes in Diabetic and Non-Diabetic Subjects

Table 6 shows the distribution of the E23K genotypes in the diabetic and non-diabetic participants of this study as well as their Chi square and P values. A total of 28 subjects were carriers of the EE genotype (10 diabetics and 18 non-diabetic), 32 subjects had the EK genotype (25 diabetics and 7 non-diabetic) and 40 the KK genotype (38 diabetics and 2 non-diabetic). The Chi square and p values for the diabetic are 5.354 and 0.0688 respectively. The χ^2 and p values for the non-diabetic are 15.86 and 0004 respectively. For all the participants the χ^2 and p values are 30.00 and 0.0001 respectively.

Table 6: Distribution of E23K genotypes in diabetic and non-diabetic subjects

Status	Genotype			χ2 value	P value
	EE	EK	KK		
Diabetic	10	25	38	5.354	0.0688
Non-diabetic	18	7	2	15.86	0.0004
Total	28	32	40	30.00	0.0001

3.7 Association of E23K Genotype to Type 2 Diabetes

Table 7 shows the frequency of the genotypes and alleles in the diabetic and non-diabetic patients as well as the p values and odds ratio using a 95% confidence interval. The EE genotype had a frequency of 10 (13.7%) in the diabetic subjects and a frequency of in the non-diabetic subjects 18 (66.7%) with a p value of 0.0003 an odds ratio of 0.21 (0.08 – 0.50). The frequency of the EK genotype in the diabetic and non-diabetic subjects are 25 (34.2%) and 7 (25.9%) respectively with a p value of 0.5639 and an odds ratio of 1.32 (0.51- 3.41). In the diabetic subjects the KK genotype had a frequency of 38 (52.1%) and a frequency of 2 (7.4%) in the non-diabetic subjects with a p value of 0.037 and an odds ratio of 7.03 (1.59-31.16). The K allele had a frequency of 63 (86.3%) in the diabetics and a frequency of 9

(33.3%) in the non-diabetics with a p value of 0.0211 and an odds ratio of 2.59 (1.13 – 5.92). The E allele had frequency of 35 (47.9%) in the diabetics and a frequency of 25 (92.6%) in the non-diabetics with a p value of 0.552 and an odds ratio of 0.52 (0.26 – 1.02)

Table 7: Association of E23K Genotype to Type 2 Diabetes

Genotype	Diabetic (%)	Non-Diabetic (%)	P value	Odds ratio (95 % CI)
EE	10 (13.7)	18 (66.7)	0.0003	0.21 (0.08-0.50)
EK	25 (34.2)	7 (25.9)	0.5639	1.32 (0.51 – 3.41)
KK	38 (52.1)	2 (7.4)	0.0037	7.03 (1.59 -31.16)
K allele	63 (86.3)	9 (33.3)	0.0211	2.59 (1.13 – 5.92)
E allele	35 (47.9)	25 (92.6)	0.0552	0.52 (0.26 – 1.02)

3.8 Gel bands of RE Digested Products

Figure 1 shows the gel bands of the restricted enzyme digested products. Twenty samples are shown in figure 1. L is 100 base pairs (bp) DNA ladder. 178 bp band is KK genotype, 150 bp only is EE genotype while both together is EK genotype. For example, sample 17 is KK genotype, sample 14 is EE genotype and sample 20 is EK genotype.

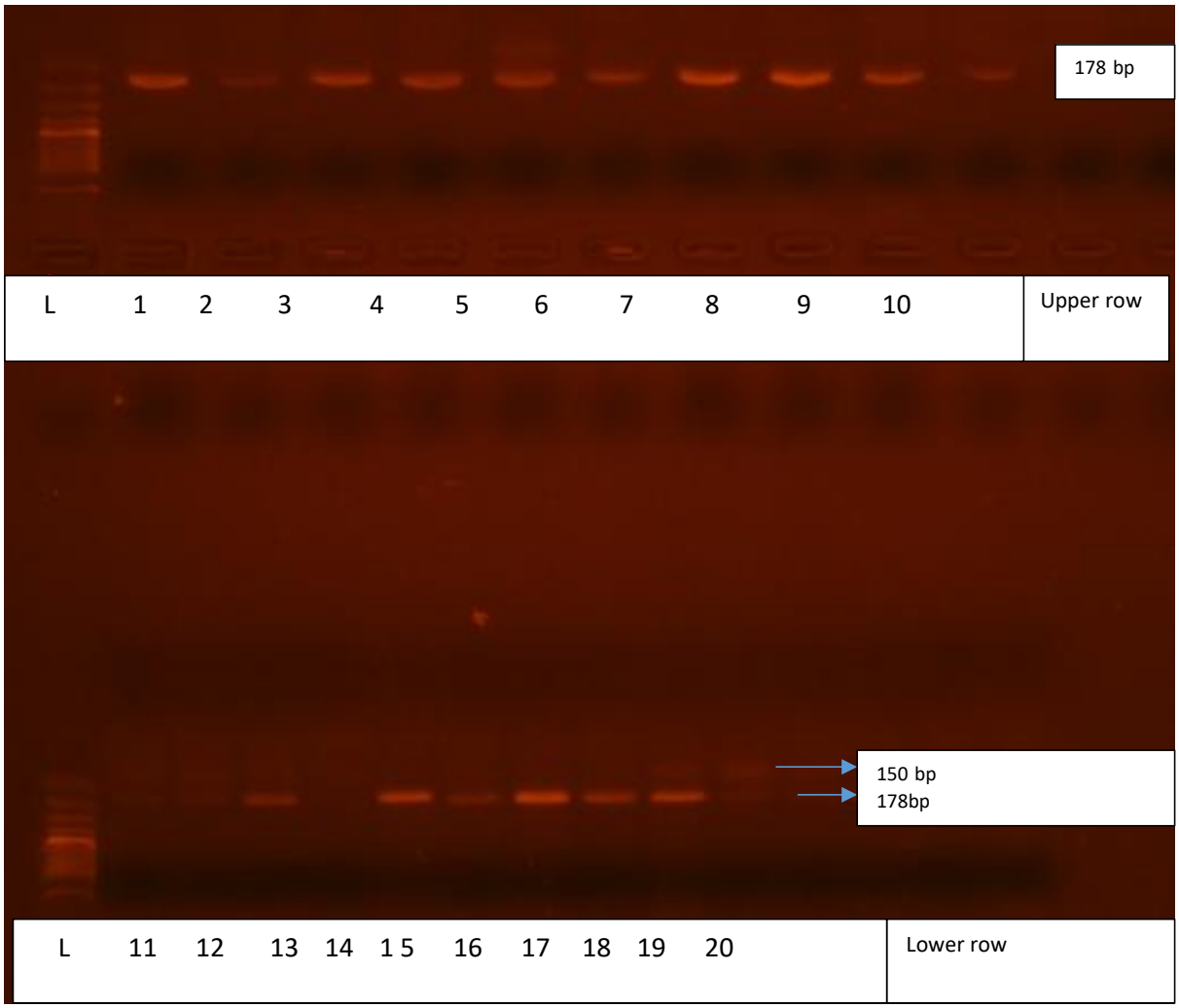

Figure 4.1: Gel bands of RE digested products

4. DISCUSSION AND CONCLUSION

This study examined the association between the E23K polymorphism of the KCNJII gene and type 2 diabetes mellitus in a Nigerian population.

Mann-Whitney U test was performed on the anthropometric variables of the two groups of subjects (diabetic and non-diabetic) enrolled in this study. Only age (p value = 0.0003) and height (p value = 0.0032) showed a statistically significant difference at $p < 0.05$. Other variables including weight (p value = 0.0589), waist circumference (p value = 0.2547), BMI (p value = 0.9212), systolic pressure (p value= 0.7604) and diastolic pressure (p value = 0.3772) did not show any significant difference. Zadhoush *et al*., 2015 found no significant difference between diabetic patients and control with respect to age, weight, hip circumference, waist circumference, BMI and diastolic blood pressure.

A comparison between the levels of selected biochemical variables present in the diabetic and non-diabetic subjects (Table 4.5) showed that only HbAlc had a significant difference (p value =0.01). Leptin (p value = 0.95), C-peptide (p value = 0.47), cystatin C (p value = 0.86), insulin (p value = 0.65) and HOMA (p value = 0.65) were not statistically significant. Souza *et al*., (2017) found that HbAlc medians were significantly higher in T2DM patients than the control group (p value = 0.001). The reason for this may be due to the fact that while other variables show the levels present in the participants at the time of sampling, HbAlc measures glycaemic control over the past 3 months. Also, the diabetic participants in the study were mostly people who were managing the disease with drugs and lifestyle interventions. In a study by Zadhoush *et al*., 2015 biochemical changes in the diabetic group without metabolic syndrome (group 1) were found to be statistically insignificant when compared with the control group but were significant when the diabetic group with metabolic syndrome (group 2) was compared with the control group. This suggests that poor disease management is associated with abnormalities in plasma parameters.

The KK genotypes were preponderant in the diabetic participants (52%) compared the non-diabetic participants (7.4%). From our study, having a KK genotype increases risk of T2DM by seven fold (OR =7.03, 95% Cl: 1.59 -31.16). Similarly, Chistiakov *et al*., (2008) and Rastegari *et al*., (2015) found that people with this homozygous genotype had more risk of T2DM with P values of 0.004 and 0.016 respectively. The K allele was more predominant in the diabetic subjects (86.3%) than in the non-diabetic subjects (33.3%). An increased risk of nearly 3 fold (OR=2.59, 95% CI: 1.13 – 5.92) was found in heterozygous carriers of the K allele. In 2013, Asaf *et al*., reported that carriers of the K allele were more predisposed to T2DM provided other factors such as physical and environmental factors were present. This agrees with the findings of Rastegari *et al*., (2015) p value = 0.048 and Christiakov *et al*., (2008) p value = 0.023. Of the three genotypes the KK genotype from this study has the strongest association to risk of developing diabetes complications (p value = 0.002; OR = 12.67; 95% CI: 1.618 -99.150). This may be because substitution of the wild type E (glutamic acid) with an oppositely charged K (lysine) at position 23 in the translated protein would result in a potentially significant restructuring of the pore structure and disruption of interactions with other Kir 6.2 subunits; thus providing a basis for altered high-fidelity of the K_{ATP} channel, especially in the homozygous state (Yang *et al.,* 2007). Functional studies have revealed that the KK genotype markedly reduced glucose-induced β-cell insulin release by

inducing spontaneous over activity of the pancreatic cells leading to an increase in the ATP concentration for insulin release (Riedel *et al*., 2003).

The frequency of the EK genotype in this study was more in the diabetic participants (34.2%) than in the non-diabetic participants (25.9%) but this is not statistically significant (p value = 0.5639). This is different from a study carried out in an Iranian population where it was observed that the frequency of the EK genotype was more in the non-diabetic subjects (p value = 0.049) (Rastegari *et al*., 2015). However, in both studies the E allele had a higher frequency in the non-diabetic patients: 92.6% for the non-diabetic participants and 47.9% for the diabetic participants (p value = 0.0552) in this study and a p value of 0.048 for the Iranian study (Rastegari *et al*., 2015).

The EE genotype was found to be more in non-diabetic subjects (66.7%) than in diabetic subjects (13.7%) (p value = 0.0003). Thus the E allele carriers (OR = 0.52; 95% CI: 0.26 – 1.02) probably have a lower risk of T2DM compared with the carriers of K allele (OR = 2.59; 95% CI: 1.13 – 5.92). This is in agreement with the work of Rastegari *et al*., 2015 who that state that the higher prevalence of the E allele in non-diabetic subjects suggests that the E allele confers on carriers a lower risk ofT2DM when compared with carriers of the K allele.

HbAlc, which is the only glycaemic variable that was statistically significant, shows no significant differences in its levels in the different genotypes (p value =0.64, Kruskal Wallis Test).

Although the data on the association of this polymorphism to the risk of developing T2DM is inconsistent, (Gloyn *et al.,* 2003; Souza *et al.,* 2017; Nielson *et al.,* 2003; Koo *et al.,* 2007; Alsmadi *et al.,* 2008), this study found an association between the E23K polymorphism and the development of T2DM and this is in agreement with other studies (Zhou *et al.,* 2009; Alsmadi *et al.,* 2008; Abdelhamid *et al.,* 2014; Nielson *et al.,* 2003). The inconsistency in data may be due to the failure of some studies to detect the modest impact of individual loci, aetiological heterogeneity across populations and small sample sizes (Hirschhorn *et al*., 2002; Souza *et al*., 2017).

The susceptibility of E23K allele has a modest effect (OR 1.15) on T2DM but because it is a high frequency allele it may likely contribute more to population attributable risk (Gloyn *et al.,* 2003; Souza *et al.,* 2009). In addition to the risk conferred on the population by the high frequency allele of E23K is the risk conferred by environmental and physical factors like BMI which have a higher predictive value (Souza *et al.,* 2017). A 2003 study by Nielson *et*

al., reveals an association between E23K and a higher BMI values. Another study suggests that mutation in both the KCNJ11 and ABCC8 genes caused the development of T2DM in obese subjects (Souza *et al.,* 2017). Therefore higher BMI values/obesity may account for the variations seen in the contribution of E23K to the development of T2DM in different populations.

Conclusion

There is significant association between the E23K polymorphism and T2DM was found in the Nigerian population that was studied. The KK genotype of the E23K polymorphism of the KCNJ11 gene could be an independent predictor of Type 2 diabetes mellitus.

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