Original Research Article

Single Nucleotide Polymorphism and Allele frequencies of some genetic variants among Type 2 Diabetes Mellitus (T2DM) subjects in Port Harcourt

REVIEW

.

ABSTRACT

Aim: To evaluate Single Nucleotide Polymorphism and Allele frequencies of some genetic variants among T2DM subjects in Port Harcourt.
Study design: Cross-sectional Study.
Place and Duration of Study: Port-Harcourt, Nigeria, Inqaba Biotec (molecular analysis) and Evashalom Medical Diagnostic services, between November 2022 and April 2023.
Methodology: Ninety (90) subjects between aged between 18- 70years among which 54 were females and 26 males were recruited. Eight SNPs, four each from SLC22A1 and CYP2C9 genes respectively were selected and genotyped using Mass Array. Genotypic and allelic distribution employed the Hardy-Weinberg equilibrium test (HWE). The minor allele frequency (MAF) was calculated using Excel, and the Hardy–Weinberg equilibrium (HWE) test was performed using Gene-Calc software. Statistical significance was set at $p < 0.05$. Odd ratios were calculated using the Baptista-Pike test. Statistical significance was set at $p<0.05$.
Results: In this study, the allelic frequency of each SNP was in HWE($p>0.05$). The genotypic frequencies for rs 594709 for homozygote wild type (AA), heterozygote (AG), and homozygote (GG) were 73,33%,16.67% and 0.10% respectively. The MAF observed for rs594709 was 18.33%. The MAF observed was 18.33%. The rs72552763 variant genotype frequencies for the homozygote wild type (ATGATATGAT), heterozygote (ATGATAT) and homozygote (ATAT) were 93.33%, 6.67% and 0.0% respectively. On the other hand, the variant rs9332214 genotype frequencies for homozygote wild type (TT), heterozygote (CT), homozygote (CC) were 93.33%,6.66% and 0.0% respectively. The MAF observed for both SNPs was 0.033%.
Conclusion: This study showed that the rs 594709 for homozygote wild type (AA) had the highest frequency and no polymorphism was observed in four out of the SNPs investigated.

Keywords: Genetic Polymorphism, Metformin, sulphonylureas, Allele frequencies, genetic variants, T2DM subjects, Port Harcourt

1. INTRODUCTION

Diabetes Mellitus is characterized by hyperglycemia which might result in complications and long-term damage to the vasculature, nerves, heart, kidneys and eyes [1]. The basic factors of its epidemic are sedentary lifestyles, global rise in obesity, population aging, and diets high in calories [2]. The pancreas (β and α- cells), skeletal muscle, liver, brain, kidney, adipose

tissue, small intestines are organs involved in the development of T2DM [3]. Shartwz et al. [4] suggests adipokine dysregulation, abnormalities in gut microbiota, inflammation and immune dysregulation as vital physiological factors. Its risk factors involve a combination of metabolic, genetic and environmental factors interacting with one another which is responsible for its prevalence. Prevalence and incidence of T2DM differ according to geographical regions, with over 80% living in low-to middle-income countries [5]. This prevents additional challenges in effective treatment. It is affected by both the environment and genetics. The genetic factors act after an exposure to an environment which is characterized by intake of high caloric diets and sedentary lifestyle. Genome-wide association studies (GWAS) have identified common genetic variants for T2DM which accounts for about 105 of total trait variance [6]. Individuals from different ethnic groups may exhibit different specific phenotypes that elevate predisposition to clusters of cardiovascular disease factors including insulin resistance, hypertension and dyslipidemia [7].

This is the subtle genomic difference between or within populations that makes each or a group of individuals different from others. These variations are observed through the entire genome of an organism however they are unevenly distributed such that some regions are hot spots, and some others stable enough not to show variations among the individuals [8]. The genetic variation of an organism is depicted in its phenotype affecting all traits present in individuals. Variation results in different forms of a gene and makes an organism more adapted to its environment.

Currently, genes discovered to be significantly associated with developing type 2 diabetes mellitus, include transcription factor 7-like 2 (TCF7L2), peroxisome proliferator activated receptor gamma (PPARG), fat mass and obesity associated genes (FTO), potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11), neurogenic locus notch homologous protein 2 (NOTCH2), wolfram syndrome 1 gene (WFS1), cdks regulatory associated protein-1-like 1 (CDKAL1), insulin-like growth factor 2 Mrna 2 (IGF2BP2), solute carrier family 30 member 3(SLC30A8), jazf zinc finger 1(JAZF1), and haematopoetically expressed homeobox protein (HHEX). KCNJ1 encodes the islet ATP-sensitive potassium channel Kir6.2, and TCF7L2 (transcription factor 7-like 2) regulates proglucagon gene expression and thus the production of glucagonlike peptide-1. Moreover, obesity (which is an independent risk factor for type 2 diabetes mellitus) is strongly inherited and Monogenic forms like maturity-onset diabetes of the young (MODY), constitute up to 5% of cases [9].

2. MATERIALS AND METHODS

2.1 Study Area

The study area was in Port Harcourt, capital of Rivers State, Southern Nigeria. It lies along the Bonny River and coordinates 4·49'27"N 7·2'1"E. It has an area of 369km2 and an estimated population of 1,865,000 as at 2016 [10]. It is a major industrial center having large numbers of multinational firms as well as other industrial concerns, particularly business related to the petroleum industry.

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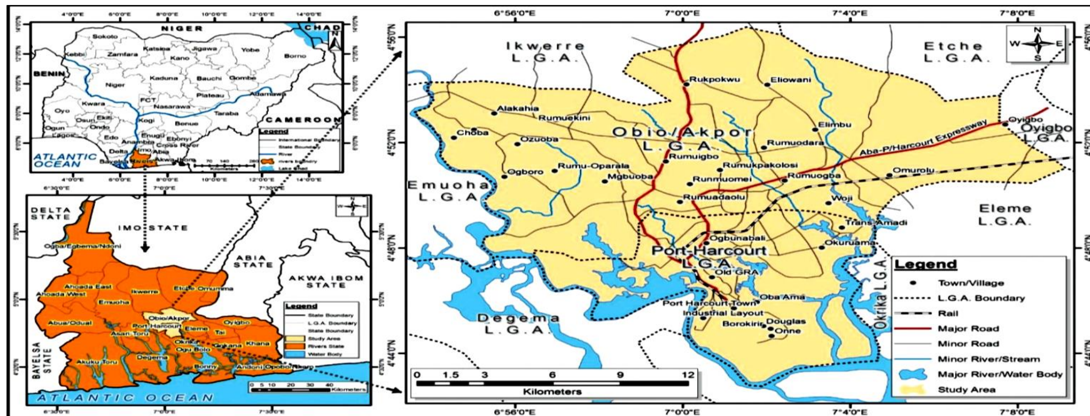

Figure 1: Map of Port Harcourt [10]

2.2 Research Design

This is a cross-sectional study involving the evaluation of some genetic variants.

2.3 Validity of Instruments

Using manufacturer's specifications, all sensitive equipment was calibrated. Sample bottles were pre-treated accordingly before use. Appropriate labelling and all pre-analytical precautions were adhered to, to prevent errors.

2.4 Sample Size Calculation

A total of 90 adults were enrolled for this study. The incident rate of adult with T2DM in Nigeria is 3.84% cases [11]. Making prevalence rate a total of 0.038%. Using the formular,

N=Z2 × p (1-p) / d2

Where N= Minimum sample size

d = Desired level of significance (0.05)

z = Confidence interval (1.96)

p = Prevalence rate or proportion of occurrence (0.038%)

Therefore, N = 3.8416 × 0.038 (1-0.038)/ 0.0025

Minimum participants required =56

2.5 Eligibility Criteria

2.5.1 Inclusion Criteria

Individuals with diagnosed T2DM of at least 1 year, aged between 18–70 years and on continuous metformin/sulphonylureas combination therapy for at least six (6) months prior to the study were included.

2.5.2 Exclusion Criteria

Individuals treated with insulin in the last six months or on any other drug for T2DM prior to the study were excluded. Individuals who were also critically ill or pregnant were also excluded from study.

2.6 Sample Collection

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Ten (10.0) mls of overnight fasting blood sample was obtained from each subject. This was after completing the questionnaire. Their body weight in kilograms, height in meters was also measured and recorded.

2.6.1 Order of Dispensing and Volume of the Blood Sample
About 3.0 ml into vacutainer type plain tubes, 4.0 ml into vacutainer type EDTA K3 (1st Tube) & 3.0ml into vacutainer type EDTA K3 (2nd Tube). All the tubes were appropriately labelled. The sample in the plain tube was allowed to retract, then centrifuged at 3000 rpm. The first EDTA tube was transported in cold box to Molecular Laboratory for DNA extraction and genotyping (Sequencing).

2.7.13 Genetic Analysis

2.7.13.1 Genomic DNA Extraction

Genomic DNA extractions of the samples were performed using Geneaid DNA Mini Kit.

Principle
RBC Lysis Buffer and chaotropic salt are used to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column. Contaminants are removed using a wash buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt elution buffer, TE or water. The entire procedure can be completed within 25 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA, with approximately 20-30 kb, is suitable for use in PCR or other enzymatic reactions.

Stage 1 Guanidinium Thiocyanate or Guanidinium Isothiocyanate (GITC) Procedure

Ten mililitre (10 ml) of cold 1x Red blood cell lysis buffer (RCLB) was added to each sample and the tube properly closed and was mixed by inversion. The tube was then placed on ice for 10 minutes. The tubes were wiped carefully. The tubes were centrifuged at 4000 rpm for 7 minutes. The supernatant was carefully decanted into the waste bucket. Care was taken not to lose the cell pellet. Ten mililitre (10 ml) of cold 1x RCLB was again added to the cell pellet, mixed by vortexing and steps 3-5 above was repeated. Where there were still traces of red cells, 10 ml of cold RCLB was added to the cell pellet again, mixed by vortexing and steps 3-5 repeated. Ten mililitre (10 ml) of sterile Phosphate buffered saline (PBS) was added to the cell pellet, mixed by vortexing and centrifuged at 4000 rpm for 7 min. The supernatant was decanted, 5 ml of sterile PBS was added into each tube and mixed by vortexing and centrifuged at 4000 rpm for 5 min. The supernatant was decanted into the waste bucket carefully not to discard the pellet, and the 15 ml tubes were drained on a clean towel. While draining, the GITC buffer was prepared by adding 10 ul beta-mercaptoethanol (BME) to the 1 ml of GITC.One mililitre (1ml) of activated GITC buffer containing BME was added to the white cell pellets in one tube. A blunt end 18G needle and 2 ml syringe was used to homogenise the GITC lysate 18 times. A sterile Pasteur pipettes was used to transfer the GITC lysate into 2 ml cryovial and labelled accordingly for storage at minus -200C. (The lysate can also be used irnmediately for nucleic acid extraction). For quality control (QC) purpose, also 1ml of the GITC buffer containing BME was transferred into a cryovial, labeled as QC control and treated as a sample during nucleic acid extractions.

Stage 2 DNA Extraction (Geneaid Genomic DNA Mini Kit)

2.7.13.2 Selection of SNPs and Genotyping
Each patient’s genomic DNA was analyzed to determine the genotypes of the following genetic variants in the genes indicated: rs594709 (SLC22A1), rs12208357 (SLC22A1), rs622342

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(SLC22A1), rs72552763 (SLC22A1), rs1057910 (CYP2C9), rs1799853(CYP2C9), rs41291560 (CYP2C9), and rs9332214 (CYP2C9). Two multiplex Mass ARRAY systems (Agena BioscienceTM) were designed and optimized by Inqaba Biotechnical Industries (Pretoria, South Africa) in January 2017. Each multiplex was used to genotype selected SNPs, using an assay that is based on a locus-specific PCR reaction. This reaction is followed by a single base extension using the mass-modified dideoxynucleotide terminators of an oligonucleotide primer, which anneals upstream of the site of mutation. Matrix-assisted laser desorption/ionization—time-of-flight (MALDI-TOF) mass spectrometry was used to identify the SNP of interest.

2.8 Statistical Analysis

The general characteristics of the participants were expressed as frequency (percentages), mean (X) and standard deviation (SD). Statistical Analysis was carried out using GraphPad Prism 9.03. Statistical comparisons of the means between groups were made using t-test and oneway analysis of variance (ANOVA). Genotypic and allelic distribution employed the Hardy-Weinberg equilibrium test (HWE). The minor allele frequency (MAF) was calculated using Excel. Statistical significance was set at $p < 0.05$. Odd ratios were calculated using the Baptista-Pike test. Statistical significance was set at $p<0.05$

3. RESULTS AND DISCUSSION

Table 1: SNP Information In The Study Population

SNP	Gene	Chromosomal Location	Location	Amino Acid Change	Drug
rs1057910	CYP2C9	10:94981296	Exon	A >C	Glimepiride
rs12208357	SLC22A1	6:160122116	Exon	C >T	Metformin
rs1799853	CYP2C9	10:94942290	Exon	C> A	Sulphonylureas
rs41291560	CYP2C9	10:94942999	Exon	C>T	Sulphonylureas
rs594709	SLC22A1	6:160134722	Intron	A>G	Metformin
rs622342	SLC22A1	6:160151834	Intron	A>C	Metformin
rs72552763	SLC22A1	6:160139851	Inframe Deletion	GAT>Del	Metformin
rs9332214	CYP2C9	10:94983351	Intron	T>A	Sulphonylureas

Key: SNP Single Nucleotide Polymorphisms, A-Adenosine, C-Cyosine, T-Thymine, G-Guanine

Table 2: Genotype and allelic distribution of Variants of SLC22Al and CYP2C9 Genes in Type 2 Diabetic Subjects.

SNP	Genotype	%	95%CI	Allele	%	95%CI	HWE (p)
rs1057910	AA	100	95.1-100.0	A	100	97.5-100.0	
	AC	0.0	0.0 - 4.9	C	0.0	0.0-2.5	
	CC	0.0	0.0 – 4.9				
rs12208357	CC	100	95.1-100.0	C	100	97.5-100.0	
	CT	0.0	0.0 - 4.9	T	0.0	0.0-2.5	
	TT	0.0	0.0 – 4.9				
rs1799853	CC	100	95.1- 100.0	C	100	97.5-100.0	

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	CT	0.0	0.0 - 4.9	T	0.0	0.0-2.5	
	TT	0.0	0.0 - 4.9				
rs41291560	TT	100	95.1- 100.0	T	100	97.5-100.0	
	TC	0.0	0.0 - 4.9	C	0.0	0.0-2.5	
	CC	0.0	0.0 – 4.9				
rs594709	AA	73.3	64.2 - 82.5	A	81.7	76.1- 87.2	0.8167
	AG	16.7	9.1- 24.3	G	18.3	12.7 -24.0	
	GG	0.1	3.8 -16.2				
rs622342	AA	63.3	53.4 - 68.4	A	81.7	76.1- 87.2	0.8167
	CA	36.7	26.7 - 46.7	C	18.3	12.7 -24.0	
	CC	0.0	0.0 – 4.9				
rs72552763	ATGATATGAT	93.3	88.1 -98.5	ATGAT	96.7	93.9 -99.4	0.9667
	ATGAT AT	6.7	5.3 - 8.0	AT	0.03	0.7 -6.0	
	ATAT	0.0	0.0 -4.9				
rs9332214	TT	93.3	88.1 -98.5	T	96.7	93.9 -99.4	0.9667
	CT	6.7	5.3 - 8.0	C	0.03	0.7 -6.0	
	CC	0.0	0.0 - 4.9				

From the results in Table 1, the allelic frequency of each SNP was in HWE(p>0.05). The genotypic frequencies for rs 594709 for homozygote wild type (AA), heterozygote (AG), and homozygote (GG) were 73,33%,16.67% and 0.10% respectively. The MAF observed for rs594709 was 18.33%. No polymorphisms were observed in four (rs1057910, rs1220835, rs1799853, rs4129156) out of the eight SNPs investigated, they were basically monomorphic. Furthermore, none of the participants were homozygous for the variant allele of rs622342, rs1799853 and rs4129156. the variant rs622342 genotype frequencies for homozygote wild type (AA), heterozygote (CA), homozygote (CC) was 63.3%, 36.7% and 0.0% respectively. The MAF observed was 18.33%. The rs72552763 variant genotype frequencies for the homozygote wild type (ATGATATGAT), heterozygote (ATGATAT) and homozygote (ATAT) were 93.33%, 6.67% and 0.0% respectively. On the other hand, the variant rs9332214 genotype frequencies for homozygote wild type (TT), heterozygote (CT), homozygote (CC) were 93.33%,6.66% and 0.0% respectively. The MAF observed for both SNPs was 0.033%.
There is a need for effective management of diabetes, which is multifactorial in nature and of a global health concern. Oral anti-diabetes drugs are however designed to help patients to keep their blood sugar level under control and achieving a HbAlc target of less than 6.5, reduce many cardiovascular risk factors and composite cardiovascular outcome [12]. Metformin and Sulfonylureas is the most prescribed combination therapy, but the outcome of treatment varies in individuals probably due to genetic factors therefore this study investigated the association of eight SNPs belonging to SLC22A1 and CYP2C9 genes with glycemic response to metformin and sulfonylureas combination therapy in type 2 Diabetes Mellitus (T2DM).
The allelic frequencies of each SNP in the population studied was in Hardy Weinberg Equilibrum (HWE) p>0.05. In this study, three variants (rs1057910, rs1779853, rs4129156) of CYP2C9 and one variant (rs12208357) of SLC22A1 genes were basically monomorphic that is no polymorphisms were observed, this is like the study of Jacobs et al. [13]. for rs12208357, it was observed that none of the participant carried the variant allele in a Xhosa (South African), Luhya (Kenya), African-Americans, Asian or any other Sub-Saharan African population but Mahldi et al. [14] observed that the MAF for rs1057910 and rs1779853 were remarkably lower in African and Asian populations when compared with Caucasian population, they reported a MAF of 96.39% for rs1057910 in a diabetic North Indian population and 54.74% for rs1779853 which is also higher than the MAF reported for populations of Egyptians (12%), Lebanese (11.31%), British (12.5%), American (8%), Turkish (10.6%), Swedish (10.7%), Korean(0%), African-American (0%), Chinese-Taiwanese (0%) and Japan (0%). The

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MAF observed for rs594709 was 18.33%. Furthermore, none of the participants were homozygous for the variant allele for rs622342, rs72552763 and rs9332214.The MAF observed for rs622342 is 18.33% which is like that of Cape admixed (18%) and Lebanese (17.5%) populations [15], but lower than what was observed in Caucasian (37%) and Xhosa (22%) populations [13]. The studied variants rs72552763 and rs9332214 are expressed in less than 0.5 % of the population, the MAF observed for rs72552763 is 0.033% which appears to be different from Caucasians (18.5%), Scotland (0.198%), South African (4%), and Ethiopia (9.3%). The difference in MAF could be because of Sub-Saharan African been the most genetically diverse in the world compared with people of non-African ancestry.

4. CONCLUSION

This study showed that the rs 594709 for homozygote wild type (AA) had the highest frequency and out of the eight SNPs investigated, no polymorphism was observed in four.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

Ethical approval and permission were sought and obtained from the Rivers State Health Research Ethics Committee of Rivers State Health Management Board. Informed consent of the participants involved was obtained and anthropometric data was also obtained via a questionnaire.

Disclaimer (Artificial intelligence)

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