Original Research Article

STUDY OF GENETIC DRIFTS IN SOME FAT MASS AND OBESITY ASSOCIATED GENE VARIATIANTS IN TYPE 2 DIABETICS IN SOME ETHINIC GROUPS RESIDING IN DELTA STATE, NIGERIA.

ABSTRACT

Aim:*Detailing and distribution of some predominant FTO genes among subjects with diabetes mellitus selected from Igbo, Ika, Ijaw and Isoko tribes of Delta state, Nigeria.*
Study Design: *Case-control, observational study.*
Place and Duration of Study: *Federal Medical Center, Asaba, Delta State and Safety Molecular Pathology Laboratory Enugu, Nigeria, between March 2020 and Febuary 2022.*
Methodology*:100 subjects (20 diabetic and 5 non diabetic from each of the four tribes; age range; 28-74 years)Venous blood was collected for the assay of FBS, Hb1ac and molecular assay of four variants of FTO (Fat Mass and Obesity Associated) gene. Results obtained were statistically analysed.*
Result:*The genotypes identified in the subjects were AA, GA, GG, AC, CC, TT, and AT. AA (Ijaw 17, Urhobo 18, Ika 18, Igbo 19) and GG (Ijaw 17, Urhobo 18, Ika 19, Igbo 18) had highest level of occurrence in all tribes. Recombinant genotype and recessive allele had higher number of subjects as compared to the originating genotypes. Hardy- Weinberg statistics demonstrated significant genetic deviations in rs2388405(AA -0.12 x^2 =0.0002, GA 0.12 x^2=0.002) rs201041270(GG-0.12 x^2 =0.0002, GA 2.12 x^2=0.002),and rs8050136 (CC -0.34 x^2 =0.001, AC 2.32, x^2=0.05) proving a chance of finding new SNP in the studied population with a probability of 2.25 as seen in the odd ratio of T allele in Igbo tribe.*
Conclusion: Statistically significant genetic drifts exist in the studied population.

Keywords: Obesity, Diabetes, Ethinic groups, Gene, Genetic drifts, Fat Mass

1.0 INTRODUCTION

Diabetes mellitus, defined as ametabolic conditionthat is characterized by persistent hyperglycemia caused by insulin insufficiency or insulin resistance [1]. Diabetes mellitus (DM) is a major cause of mortality and disability globally, this raises concerns about the financial burden it places on both people and society as a whole [2]. Endocrinology and Metabolism Society of Nigeria (EMSON) reported in 2022 that over 10 million Nigerians are living with diabetes and warned that if preventive measures are not applied, the numbers are expected to double by 2030 [3].An obesity sensitivity gene, whose intron 1 region has multiple SNPs that are strongly associated with Body Mass Index, energy metabolism and anthropometric indices has been identified, the gene is known as the fat mass and obesity-associated (*FTO*) gene [4]. The FTO gene exists in different genetic forms known as variants of FTO polymorphisms. FTO geners9939609 SNP is significantly associated with obesity, With the AA genotype carriers having a 2.02 increased risk of developing obesity[5].interdependeceexistbetween biochemical parameters of T2D with FTO gene,namely insulin, glucose,HOMA, BMI, waist and hips circumference[6].
Results obtained from genetic risk score (GRS) calculated for two FTO single-nucleotide polymorphisms (SNPs) rs8050136 and rs2388405 demonstrated that the two SNPs were significantly related to increased BMI and obesity [7].Understanding the genetic make- up of FTO gene, its various variants, distribution and how it affects and influences manifestation of diabetes can assist with early intervention measures, improvement of diabetes clinical diagnostic processes, and grant insight on developing targeted treatment options for patients with diabetes.Reports of results obtained from various studies for FTO gene variants association with obesity and diabetes varies with population and continents. This research will give insight of FTO gene presence and distribution in various ethnic tribes residing in Delta state region of Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study Area

This research workwas donein Delta State, Nigeria(Figure 1).Samples were collected from subjects attending clinic at Federal Medical Centre, Asaba and General Hospitals in Agbor & Bomadi Delta

State, Nigeria. Participants ware people from Ijaw, Urhobo, Ikah and Igbo ethinic groups residing in Delta State.Nigeria's Delta State capital, Asaba, is located on a terrace of the lower Niger River, with geographical co-ordinates of 6°11′52.23″N, 6°43′42.48″E. Asaba serves as a link between western, eastern, and northern Nigeria through the Asaba Niger Bridge, which connects East and West, and the Niger River in the north.

Figure 1: **Map of Delta state Nigeria, showing Ethnic and Local Government Area** [8]

2.2 Research Design

This is a case-control, observational study involving the identification of FTO gene allele variants, distribution of the gene evaluation ofHbA1c, Fasting blood sugar(FBS), in Type 2 Diabetic (T2D) and non-diabetic control subjects from the selected ethnic groups in Delta State. The bio-data and medical history of the subjects were obtained through questionnaire.

2.3 Sample Size

Sample size for this study was determined using the Cochrane formula:

$$N = \frac{Z^2 pq}{d^2}$$ [9]

N = the desired sample size
Z = The Standard Normal deviate usually set at 1.962 corresponding to the 95% Confidence level
p = The SNPs Prevalence rates. (Minor Alleles Frequency of SNPs set at >0.02) [10]
q = 1- p
d = degree of accuracy desired set at 0.05
Minimum Size – 30
By adding 10% of non-respondent = 33. Therefore, total sample size is 33.
However, this study used 100 subjects, with 20 subjects selected from each of the four ethnic groups in Delta State, and 20 control subjects.

2.4 Sampling Method:

A multistage sampling technique was used to choose the subjects. Participants were grouped into sections based on duration of pathogenesis(1-5years, 6-10years,11-15years,16-20years).

2.5 Selection Criteria

2.5.1 Inclusion Criteria

Individuals who are of the selected tribes in Delta State aged at least 21 years and above, diagnosed with T2D for at least one year.Controls: Five (5) individuals from each of the selected tribes Ika, Urhobo,Ijaw, Igbo with no history of diabetes, and are non-obese and having a fasting blood glucose of less than 6.5mmol/l.

2.5.2 Exclusion Criteria

1. Individuals not from the selected tribes.
2. Critically ill subjects.
3. Pregnant female.

2.6 Sample Collection:

From each subject, venous blood was collected using standard veni-puncture technique, 2ml was dispensed into fluoride-oxalate bottle for the assay of FBS and Hb1ac another 3ml was dispensed into EDTA tubefor molecular assay of FTO genes.
After allowing the sample in the plain tube to retract, it was centrifuged. The analysis was performed within one week of collection at the Federal Medical Centre, Asaba. The first EDTA tube was transported in cold box to Safety Molecular Pathology Laboratory Services located at 44 Rangers Avenue, Enugu for DNA extraction and genotyping (Sequencing). The second EDTA tube for Glycated hemoglobin was for stored at 2-8^{0}C and analysis was done within two days of sample collection.

2.6.1 Fasting Blood Sugar (FBS):

FBS was performed using glucose oxidase method.
Principle: After enzymatic oxidation in the presence of glucose oxidase, glucose is measured. Under the catalysis of peroxidases, the generated hydrogen peroxide combines with phenol and 4-aminophenazone to create a red-violet quinoneimine dye as an indicator.

2.6.2 Glycosylated Haemoglobin (HbA1c)

Quantitative determination of glycosylated Haemoglobin in blood was done using the modified Ion Exchange Resin method with kit from INTECO Diagnostics, UK [11].

2.7 Genetic Analysis

2.7.1 Genomic DNA Extraction

Genomic DNA extractions of the samples was performed using Geneaid DNA Mini Kit (Blood/Cultured Cell).

2.8 Genotyping of SNPs

Genotyping of SNPs of the *FTO* gene was performed with the Illumina next-generation sequencing (NGS) using NextSeq 2000 Sequencing System.Concentration and purity of isolated DNA was determined by UV/VIS spectrophotometer NanoDrop ND-1000(Appendix I).

2.8 Statistical Analysis

Data obtained was categorised based on duration of Type-2 diabetes, ethnicity, age and sex. Utilizing GraphPad Prism, version 8.0.2 (California, USA), **Hardy Weinberg** equilibrium, Student's statistical t-test at *P* =.05, Mean, Standard deviation,Gene counting, Chi-square and Odds ratio (OR)were used to determinegenotypic and allelic frequencies, Variance, SNPs, significant differences in the study population of Type 2 diabetic (T2D) and non-diabetic participants.

3.0 RESULTS AND DISCUSION

3.1 Metabolic and Immunologic Parameters

In (**Table. 1**) below presents the Metabolic and Immunologic parameters of subjects with FTO gene variations with varying duration of type 2 diabetes against the control subject. Fasting blood sugar (FBS) values was significantly higher in subjects at various duration of diabetes pathogenesis than control subjects, with a gradual progressive rise over years of disease duration. Thus, confirming disease status of patients. hyperglycaemia is an indicator of diabetes mellitus [1]. the impact of continuous increase in FBS can be seen in progressive glycation of glucose to haemoglobin, as is reflected by values obtained for HbA1c which were significant as compared to control.this changes could be due to the dysregulation of glucose [12; 13]. Increase in HbA1c as seen in (table 1) suggestserves as an indicator of blood glucose control,and a marker of diabetes[14].

Table 1. **Mean±SD of Metabolic and Immunologic Parameters of Subjects with FTO gene variations with Varying Duration of T2D against Control Subjects.**

Parameters	Control	1-5 yrs	6 -10 yrs	11 -15 yrs	16 -20	Fvalue	Pvalue	Remark
FBS (mmol/L)	4.92±0.65^a	8.11±3.41^b	8.27±2.88^b	8.32±3.56^b	7.35±3.37^a	5.168	0.0008	S
HbA1c (%)	6.57±0.99^a	8.84±1.86^b	8.85±1.39^b	9.59±2.08^b	9.46±1.95^b	9.027	<0.0001	S

Keys: S=Significant, NS=Not Significant,, FBS=Fasting Blood Sugar, HbA1c= Glycated Haemoglobin, PostHoc**(Dunnett's Multiple Comparison test):** Values within same row with different superscripts differ significantly compared against the control *P*=.05

3.2 Distribution of FTO Genotypes

Genotypes identified in the studied population are AA, GA, GG, AC, CC, TT, AT. Distribution of these FTO genotypes according to tribes in(Table 2) below showed that, genotype AA and GG had highest level of occurrence in all tribes while genotype GA had the lowest level of occurrence in all tribes. FTO gene prevalence varies by ethnicity and race [15],**AA genotype** was significantly **higher** FTOin studied population [16].

Table 2: Distribution of FTO GenotypesAccording to Tribes

	AA	GA	GG	AC	CC	TT	AT
Ijaw	17	3	17	5	15	14	3
Urhobo	18	2	18	5	15	14	3
Ika	18	2	19	5	15	16	3
Igbo	19	1	18	2	18	17	2
p-value	0.992	0.682	0.990	0.662	0.934	0.931	0.273
□^{2}-value	100.00	1.500	0.111	1.588	0.429	0.443	0.965

3.3 Hardy-Weinberg Statistical Test of FTO gene Variants

In (Tables 3 and 4,)Hardy- Weinberg statistics demonstrated significant negative and positive genetic deviations in rs2388405(AA -0.12 x^2 =0.0002, GA 0.12 x^2=0.002) rs201041270(GG-0.12 x^2 =0.0002, GA 2.12 x^2=0.002),and rs8050136 (CC -0.34 x^2 =0.001, AC 2.32, x^2=0.05) proving that there is a chance of finding new SNP in the studied population, as well as genetic deviations within the population which could be any or a combination of some of the following distribution factors, mutation, natural selection, non-random mating, ***genetic*** drift, or ***gene*** flow. similar finding existsamong Brazilian population [17].changes from expected frequency could be due to cross-breeding that may have occurred in previous generations[18], considering the multi-ethnic nature of the geographical population of the study area this might apply to the genetic drift observed for the studied FTO variants.

Table 3: **Hardy-Weinberg Statistical Test of FTO gene Variants of FTO gene in T2D subjects and control**

FTO gene Variant	**rs2388405**			**rs201041270**		
Genotype	AA	GA	GG	GG	GA	AA
No, Obs	92	8	0	92	8	0
Freq, Allele	0.97	-	0.03	0.97	-	0.03
Freq, Exp	0.94	0.06	0.0009	0.94	0.06	0.0009
No, Exp	92.12	5.88	0.09	92.12	5.88	0.09
Deviation	-0.12	0.12	-0.09	-0.12	2.12	-0.09
X^2	0.0002	0.002	0.99	0.0002	0.002	0.99

Key: A|G=wild (Dominant), G|A=Polymorphic, X^2 =chi-square, Exp=Expected, Obs=Observations

Table 4: **Hardy-Weinberg Statistical Test of some FTO gene Variants in T2D subjects and control**

FTO gene Variant	**rs9939609**			**rs8050136**		
Genotype	TT	AT	AA	CC	AC	AA
No, Obs	77	14	9	81	18	1
Freq, Allele	0.85	-	0.15	0.91	-	0.09
Freq, exp	0.72	0.26	0.02	0.83	0.16	0.008
No, Exp	70.6	25.48	1.96	81.34	15.68	0.8
Deviation	6.4	-11.48	7.04	-0.34	2.32	0.2
X^2	0.5	0.26	0.25	0.001	0.5	0.6

T|C=wild (Dominant), A|A=Polymorphic, X^2 =chi-square, Exp=Expected, Obs=Observations

3.4 Genotype and allele distribution of rs2388405 Variant of FTO gene in Obese and T2D subjectsand control

(Table 5 - 8) demonstrates types and numbers of genotypes obtained by gene sequencing and counting, its recombinant genotype and alleles.In this study, rs2388405 Genotype AA had the highest number of occurrences for T2D in all tribes Recombinant genotype of rs2388405 AA+GA and its recessive allele A, had higher number of occurrences as compared to the originating genotype.Similarly,recombinant genotype GG+GA with the recessive allele G of rs201041270 had higher number of subjects as compared to the originating genotypes, having the highest number of subjects. In rs9939609 Genotype TT had the highest number of occurrences for T2D in all tribes with varying number of occurrence, recombinant genotype TT+AT had the highest number of possible occurrence in all tribes. Allele T in rs9939609 had the highest number of subjects in Igbo tribe with an odd ratio value of 2.25, the greatest odds ratio for a T2D risk locus found to be 1.57 suggests that a greater number of as-yet-untested variants may be responsible [19].Genotype CC of rs8050136 was significantly high in Ijaw tribe with an odd ratio of 0.93 as well as it allele C with odd ratio of 0.94 and x^2 of 0.002 in both the genotype and allele. high number of allele occurance and recombinant genotype observed in the studied population suggests that SNPs exist in the studied population allele-specific expression is less often observed in regions of low recombination [20], allele increase may be due to SNP that occurs in the genes of members of a population[21].

Table 5:**Genotype and allele distribution of rs2388405 Variant of FTO gene in Obese and T2D subjectsand control**

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urobo**		**X^2**	**P**	**OR**	**Ikah**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=20	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
AA	17 (89.5%)	5 (100%)	-	-	-	18 (94.7%)	5 (100%)	-	-	-	18 (90%)	5 (100%)	-	-	-	19 (95%)	5 (100%)	-	-	-
GA	3 (10.5%)	0 (0.0%)				2 (5.2%)	0 (0.0%)				2 (10%)	0 (0.0%)				1 (5%)	0 (0.0%)			
GG	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
AA	17 (89.5%)	5 (100%)	0.57	0.45	0.00	18 (94.7%)	5 (100%)	0.27	0.60	0.00	18 (90%)	5 (100%)	0.27	0.60	0.00	19 (95%)	5 (100%)	0.27	0.60	0.00
GA + GG	3 (10.5%)	0 (0.0%)				2(5.2%)	0 (0.0%)				2 (10%)	0 (0.0%)				1 (5%)	0 (0.0%)			
AA+GA	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
GG	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
A	36 (94.7%)	10 (100%)	0.55	0.45	0.00	37 (94.7%)	10 (100%)	0.26	0.60	0.00	38 (95%)	10 (100%)	0.52	0.47	0.00	39 (97.5%)	10 (100%)	0.26	0.61	0.00
G	2 (5.2%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				2 (5%)	0 (0.0%)				1 (2.5%)	0 (0.0%)			

A=wild (Dominant), G=Polymorphic, T2D=Type 2 Diabetes, ND = Non- Diabetes (control), X^2 =chi-square, p= chi-square -pvalue, OR= Odd Ratio

Table 6: **Genotype and allele distribution of rs201041270 Variant of FTO gene in T2D subjects and control**

	Ijaw		**X^2**	**P**	**OR**	**Urobo**		**X^2**	**P**	**OR**	**Ikah**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=20	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
GG	17 (89.5%)	5 (100%)	-	-	-	18 (94.7%)	5 (100%)	-	-	-	19 (95%)	5 (100%)	-	-	-	18 (90%)	5 (100%)	-	-	-
GA	2 (10.5%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (5%)	0 (0.0%)				2 (10%)	0 (0.0%)			
AA	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
GG	17 (89.5%)	5 (100%)	0.57	0.45	0.00	18 (94.7%)	5 (100%)	0.27	0.60	0.00	19 (95%)	5 (100%)	0.27	0.60	0.00	18 (90%)	5 (100%)	0.110	0.73	0.00
GA + AA	3 (10.5%)	0 (0.0%)				2 (5.2%)	0 (0.0%)				1 (5%)	0 (0.0%)				2 (10%)	0 (0.0%)			
GG+GA	20(100%)	5 (100%)	-	>0.99	-	20(100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
AA	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
G	36 (94.7%)	10 (100%)	0.54	0.45	0.00	37 (94.7%)	10 (100%)	0.26	0.60	0.00	39 (97.5%)	10 (100%)	0.25	0.61	0.00	38 (95%)	10 (100%)	0.52	0.47	0.00
A	2 (5.2%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (2.5%)	0 (0.0%)				2 (5%)	0 (0.0%)			

G=wild (Dominant), A=Polymorphic, T2D=Type 2 Diabetes, ND = Non- Diabetes (control), X^2 =chi-square, p= chi-square -pvalue, OR= Odd Ratio

Table 7: **Genotype and allele distribution of rs9939609 Variant of FTO gene in T2D subjects and control**

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urobo**		**X^2**	**P**	**OR**	**Ikah**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=20	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
TT	14 (73.7%)	4 (80%)	0.58	0.74	-	14 (73.7%)	4 (80%)	1.09	0.57		16 (80%)	4 (80%)	1.87	0.39	-	17 (85%)	4 (80%)	1.63	0.44	-
AT	3 (15.8%)	1 (20%)				3 (10.5%)	1 (20%)				3 (15%)	0 (0.0%)				2 (10%)	0 (0.0%)			
AA	3 (10.5%)	0 (0.0%)				3 (15.8%)	0 (0.0%)				1 (5%)	1 (20%)				1 (5%)	1 (20%)			
TT	14 (73.7%)	4 (80%)	0.08	0.77	0.70	14 (73.7%)	4 (80%)	0.08	0.77	0.70	16 (80%)	4 (80%)	0.00	>0.99	1.0	17 (85%)	4 (80%)	0.07	0.78	1.41
AT + AA	6 (26.3%)	1 (20%)				6 (26.3%)	1 (20%)				4 (20%)	1 (20%)				3 (15%)	1 (20%)			
TT+AT	17 (94.7%)	5 (100%)	0.57	0.45	0.00	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
AA	3 (10.5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
T	31 (81.6%)	9 (90%)	0.40	0.52	0.49	30 (78.9%)	9 (90%)	0.63	0.42	0.41	35 (87.5%)	8 (80%)	0.37	0.54	1.75	36 (90%)	8 (80%)	0.75	0.38	2.25
A	7 (18.4%)	1 (10%)				8 (21.1%)	1 (10%)				5 (12.5%)	2 (20%)				4 (10%)	2 (20%)			

A=wild (Dominant), T=Polymorphic, T2D=Type 2 Diabetes, ND = Non- Diabetes (control), X^2 =chi-square, p= chi-square -pvalue, OR= Odd Ratio

Table 8: **Genotype and allele distribution of rs8050136 Variant of FTO gene in T2D subjects and control**

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urobo**		**X^2**	**P**	**OR**	**Ikah**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=20	ND n=5				T2D n=20	ND n=5					T2D n=20	ND n=5				T2D n=20	ND n=5		
CC	15 (78.9%)	4 (80%)	-	-	-	15 (78.9%)	5 (100%)	-	-	-	15 (75%)	5 (100%)	-	-	-	18 (90%)	4 (80%)	-	-	-
AC	5 (21.1%	1 (20%)				5 (21.1%	0 (0.0%)				5 (25%)	0 (0.0%)				2 (10%)	1 (20%)			
AA	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
CC	15 (78.9%)	4 (80%)	**0.002**	0.95	0.93	15 (78.9%)	5 (100%)	1.26	0.26	0.00	15 (75%)	5 (100%)	1.56	0.21	0.00	18 (90%)	4 (80%)	0.37	0.53	2.25
AC + AA	5 (21.1%	1 (20%)				5 (21.1%	0 (0.0%)				5 (25%)	0 (0.0%)				2 (10%)	1 (20%)			
CC+AC	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
AA	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
C	34 (89.5%)	9(90%)	**0.002**	0.96	0.94	34 (89.5%)	10 (100%)	1.14	0.28	0.00	35 (87.5%)	10 (100%)	1.38	0.23	0.00	38 (95%)	9 (90%)	0.35	0.55	2.11
A	4 (10.5%)	1 (10%)				4 (10.5%)	0 (0.0%)				5 (12.5%)	0 (0.0%)				2 (5%)	1 (10%)			

C=wild (Dominant), A=Polymorphic, T2D=Type 2 Diabetes, ND = Non- Diabetes (control), X^2 =chi-square, p= chi-square -pvalue, OR= Odd Ratio

4.0 CONCLUSIONS

The findings from this investigation showed that genotype AA and GG has the highest level of occurrence in all tribes ofthe studied population. Analysis and comparism ofgenotype and alleleobtained within the four variants of FTO gene analysedfor sample size showed that Genotype AA in rs2388405, Genotype GG in rs201041270, Genotype TT Inrs9939609, and Genotype CC in rs8050136 had the highest number of occurrences in all tribes.Statistically significant genetic drifts exist in the studied population, which has caused a deviation from expected frequencies in Hardy-Weinberg statistics. This correlates with higher numbers observed in recombinant genotypes rs2388405, rs201041270, rs9939609 and rs8050136, than the originating genotypes as well as notable odd ratios of 2.25in Igbo tribe and a significant allele with x^2 of 0.002 and odd ratio of C in rs8050136.

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.

ETHICAL APPROVAL

Ethical approval was sought and obtained from the Ethical Committee of Federal Medical Centre, Asaba, Delta State, Nigeria. Informed consent of the participants involved was also obtained using the consent form.

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APPENDIX I

Materials

Statfax–2200 ELISA Machine (Reader, Washer & Incubator) by Awareness Technology, USA; MB100-4A Thermo-shaker (USA); ErbaChem 5 V3 by ERBA Diagnostics Mannheim GmbH, Germany; 721-Vis Spectrophotometer (Scottfield, England); HettichRotofix 32A Centrifuge by Hettich Germany; ELMI Centrifuge & Vortex, USA; Minispin Centrifuge, Eppendorf (Hamburg), Thermostat; Kiptrack blood glucose machine (Taiwan); Grant T100 water bath (UK); Illumin NextSeq 2000 platform; 10ml Syringe &Needle; Vacutainer-Type EDTA & Plain Tubes, Hand Gloves, Alcohol Swabs, Tourniquet; Sharp Container; Eppendorf Tube; Cryo Tube; Pen; Marker; Semi-Auto pipette (5-20ul, 10-50, 10-100, 20-200, 100-1000uL, 10ul. 20uL. 50uL, 200uL, 1000uL); Weighing scale; Measuring tape; Refrigerator with freezer; Test tubes; Test tube rack; Timer; 1.5-ml micro centrifuge tubes *or* 15- or 50-ml conical polypropylene centrifuge tubes (e.g., Falcon), DNA source plate: 384-well deep-well PCR plate containing 2.5ng/µl DNA of interest, 100mM dNTPs (Applied Biosystems; store at −20°C), 25mM MgCl2 (QIAGEN; store at −20◦C), Ultrapure PCR-grade H_2O (Invitrogen), 5U/µlHotStarTaq Plus DNA polymerase with 10× PCR buffer (QIAGEN; store at −20°C), Forward and reverse primers: 1µM each in multiplex pool, 384-well PCR reaction plate (Eppendorf twin.tec), 1× SAP buffer (Sequenom), 1.7U/µl shrimp alkaline phosphatase (SAP; Sequenom; store at 0°C), PCR products in 384-well PCR plates, Ultrapure PCR-grade H_2O (Invitrogen), Extend primers: from 5 to 10µM each in multiplex pool, PCR products in 384-well PCR plates, cleaned up by SAP reaction, Ultrapure PCR-grade H_2O (Invitrogen), SpectroCLEAN resin (Sequenom; store at room temperature), Post–primer extension products in 384-well plates kept at 4°C, 100% and 50% ethanol, Plates from primer extension, cleaned up with resin and centrifuged, 3-point calibrant (Sequenom),

Sample Purification Beads (SPB), Resuspension Buffer (RSB), Freshly prepared 80% ethanol (EtOH), 96-well 0.8 ml Polypropylene Deepwell Storage Plate (midi plate) (2), 96-well PCR plate, Microseal 'B' adhesive seal, Microseal 'F' foil seal, 1.7 ml microcentrifuge tubes, Nuclease-free water, Enhanced PCR Mix (EPM), Index adapters (tubes or plate), 20 µl multichannel pipettes, 200 µl multichannel pipettes, Tagment Stop Buffer (TSB), Tagment Wash Buffer (TWB), 96-well plate magnet, Microseal 'B' adhesive seal, Bead-Linked Transposomes (BLT), Tagmentation Buffer 1 (TB1), 96-well PCR plate, 8-tube strip.