Original Research Article

Variations in fat-mass and obesity-associated (FTO) genes and Allelic Distribution in some Selected ethnic populations in Niger Delta, Nigeria

ABSTRACT

Aim: To evaluate variations in FTO genes and allelic distribution in some ethnic populations in Niger Delta, Nigeria

Study design: Case-controlled observational study

Place and Duration of Study: Federal Medical Centre, Asaba, Delta State and Safety Molecular Pathology Laboratory, Enugu, Nigeria, between March 2020 and February 2022.

Methodology: The association between sixteen (16) Single Nucleotide Polymorphisms in the FTO gene and some biomarkers of obesity and type 2 diabetes subjects (78 cases and 20 controls) from four different tribes in the Niger Delta region, Nigeria. Multistage sampling method was employed in the subject selection. The subjects were first separated into two groups – new cases (less than a year of diagnosis as Diabetic) and old cases (one year & above). Equal number of samples was then randomly collected from each of the cluster groups. Ten millilitres of blood were collected into EDTA for genotyping using Illumina NextSeq 2000 sequencing platform. Hardy-Weinberg Equilibrium statistical test was used to determine the variation in distribution of the alleles and genotypes within the study population while allelic frequencies were calculated by gene counting. Chi-square (and fisher's test where chi-square was not applicable) and Odd Ratio (OR) were performed to determine the significant differences and associated risks respectively of the allelic and genotypic frequencies of Type 2 diabetic (T2D) and non-diabetic subjects of the FTO gene variants.

Results: The results of Hardy-Weinberg Statistical Test, Genotype and Allelic Distribution of FTO gene Variants in Obese/T2D Subjects in Different Tribes of Niger Delta are presented in Tables(1-4) for rs73609956 (C>T), rs116753298 (T>C), rs201041270 (A>G), rs531215275 (A>C), rs146056278 (C>T), rs1410999299 (G>A), rs79206939 (A>G), rs145884431(G>A), rs61743972 (G>A), rs201496428 (C>T), rs146138389 (T>C), rs886052102 (A>G), rs144743617 (G>A), rs886052103 (T>A), rs9939609 (A>T) and rs8050136 (A>C). However, no significant differences in analyzed genotype frequencies were found between T2D and healthy controls.

Conclusion: Knowledge of the dominant SNPs in some ethnic groups, may provide platform to delay its expression through informed wise choice of lifestyle change and proper dieting.

Keywords: Fat-mass and obesity-associated gene (FTO) *variants, Allelic distribution, ethnic populations, Niger Delta, Nigeria*

1. INTRODUCTION

The FTO gene is of the AlkB related non-haem iron and 2-oxoglutarate-dependent oxygenase superfamily but the exact physiological function of this gene is not known. The Primary (citable) accession number is **Q9C0B1.** Studies in mice and humans indicate a strong association with body mass index, obesity risk, and type 2 diabetes. Disease susceptibility is associated with variations affecting the gene represented in this entry. It is unclear whether variations associated with obesity directly affect FTO function or alter the expression of adjacent genes such as IRX3, rather than FTO itself. A pathogenic intronic FTO variation (rs1421085) disrupts an evolutionarily conserved motif for ARID5B binding. Loss of ARID5B binding results in overexpression of two genes distal to FTO, IRX3 and IRX5. IRX3 and IRX5 overexpression shifts pre-adipocytes differentiation from brown to white fat cells, resulting in increased lipid storage and loss of mitochondrial thermogenesis (Smemo *et al*., 2014; Claussnitzer *et al*., 2015) [2,3]. FTO is a protein-coding gene located at the chromosome region 16q12.2 and associated with the control of food intake and energy balance [4].

Reports of different studies in different populations or geographical locations, appears to suggest different patterns of association of FTO gene variants with obesity and T2D in varying ethnicities, and several researchers have called for more work in other ethnic populations to determine more precisely the extent of the effects in each ethnic group. In addition, some reports have claimed ethnic-specific associations with alternative SNPs, and to that end there has been a degree of inconsistency [5, 6]. There is paucity of data available on the FTO gene allele variants in African and Nigerian Populations. Knowing the FTO gene variants that are common in persons with obesity and T2D in each of the ethnic population to be studied will be adding to the knowledge available in the understanding of the etiology and pathogenesis of these disorders and may be useful in prediction and identifying the possible complications that may arise in patients with these genetic variants. The pathogenesis of obesity and T2D is reportedly related to variations in the fat mass and an obesity-associated gene (FTO); however, as the number of reports increases, particularly with respect to varying ethnicities, there is a need to determine more precisely the nature and extent of the effect of FTO gene polymorphisms in each ethnic group. In addition, some reports have claimed ethnic-specific associations with alternative single nucleotide polymorphisms (SNPs), and to that end there has been a degree of confusion [7].

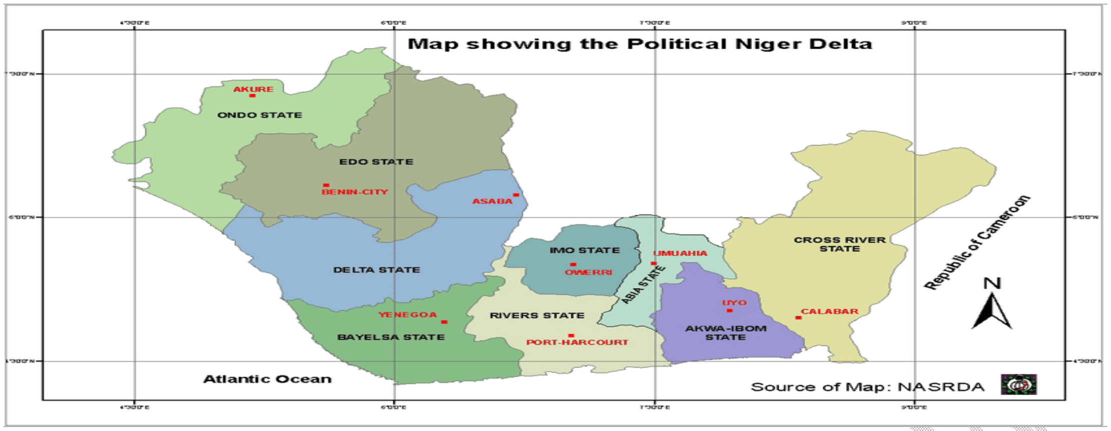
Genome-wide association studies (GWAS) have identified a number of genetic polymorphisms that are associated with an increased risk for obesity and T2D [8, 9]. Also, genome-wide association studies identified a common variant, rs9939609, in the FTO (fat mass and associated obesity) gene that was strongly associated with BMI and obesity in European population [10, 11]. The single nucleotide polymorphism rs9939609 of the gene FTO, which encodes fat mass and obesity–associated protein, is strongly associated with obesity and type 2 diabetes (T2D) in multiple populations; however, the underlying mechanism of this association is unclear [12]. Findings by Liu *et al*. [13] indicate that the two variants (rs9939609 and rs8050136) in the FTO gene contribute to obesity and T2D in the Asian populations. The aim of this study was to evaluate variations in FTO genes and allelic distribution in some ethnic populations in Niger Delta, Nigeria

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Niger Delta region of Nigeria, with Federal Medical Centre, Asaba serving as the major point of the sample collection and some analysis. Some samples were also collected at Agbor & Bomadi. The Igbo participants were drawn from the Igbos of Delta State, Rivers State and Imo State; the Ijaw participants were drawn from the Ijaws of Delta State, Bayelsa State and Rivers State.

The Niger Delta was once known as the Oil Rivers, Nigeria's Niger Delta region is a very densely populated region, a major palm oil producer. After its expansion, it became the Niger Coast Protectorate. Stretching directly on the Gulf of Guinea on the Atlantic Ocean in Nigeria, the Niger Delta used to be historically made up of present-day Bayelsa, Rivers, and Delta states are today, made up of nine coastal states. The federal government of Nigeria's current definition states that the delta extends over about seventy thousand km^2 and makes up almost 7 percent of its landmass. The Niger Delta comprises of level low lying muggy landscape that is befuddled by wandering and anastomosing streams, waterways and brooks [14].



Source of Map: NASRDA

Figure 1: Political Map of the Niger Delta Area.

Asaba, the capital city of Delta State, Nigeria is situated within geographical co-ordinates 6°11′52.23″N6°43′42.48″E. It is situated on a terrace of the lower Niger River, overlooking the point where the Anambra River flows into it. Beyond the river banks, on the high plains which are far more extensive than the river basins, secondary forest vegetation flourishes.

3.2 Research Design

This is a case-controlled observational study involving the evaluation of FTO gene allele variants and allelic distribution in some ethnic groups in Niger Delta, Nigeria. The bio-data and medical history of the subjects were obtained using questionnaire, measuring their weight with a calibrated weighing scale, height and waist circumference.

2.2 Sample Size

A total of 98 subjects enrolled for this study. The sample size was obtained using the formular stated below:

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

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).
q = 1- p
d = degree of accuracy desired set at 0.05
Minimum Size – 30
By adding 10% of non-respondent = 33

2.3 Sampling Method

Multistage sampling method was employed in the subject selection. The subjects were first separated into two groups – new cases (less than a year of diagnosis as Diabetic) and old cases (one year & above). Equal number of samples was then randomly collected from each of the cluster groups.

2.4 Selection Criteria

2.4.1 Inclusion Criteria

Individuals who are purebred of the selected tribes in Niger Delta, aged at least 21 years diagnosed with T2D for at least one year. Controls: Individuals who are from the selected tribes with no history of diabetes, and a fasting blood glucose of less than 6.5mmol/l. The cluster groups were considered also.

2.4.1 Exclusion Criteria

Individuals not of the selected tribes, those who are not purebred from the selected tribes, those who are critically ill subjects and female participants who are pregnancy.

2.5 Sample Collection and Analysis

2.5.1 Sample Collection

Ten millilitres (10ml) of blood were randomly collected from 19-20 subjects from each of the selected tribes following the sampling methodology described earlier and 20 control made of 5 non-diabetic, non-obese subjects from each of the selected tribes. This was after completing the questionnaire and signing the consent form. Their body weight in kilogram, height in meter and waist circumference in centimeter were also measured and recorded. Four ml of blood was collected using a EDTA K_3 tubes and was transported in cold box to Safety Molecular Pathology Laboratory Services located at 44 Rangers Avenue, Enugu for DNA extraction and genotyping (Sequencing).

2.5.2 Sample Analysis

3.11.14 Genetic Analysis

3.11.14.1 Genomic DNA Extraction

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

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

One millitres of whole blood sample was transferred into a 15mL tube and labelled accordingly.

Ten millitres 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 millitres of cold 1x RCLB was again added to the cell pellet, mixed by vortexing as describe above.

Where there were still traces of red cells, 10ml of cold RCLB was added to the cell pellet again, mixed by vortexing as described above.

Ten millilitres of sterile Phosphate buffered saline (PBS) was added to the cell pellet, mixed by vortexing and centrifuged at 4000 rpm for 7min.

The supernatant was decanted; 5ml of sterile PBS was added into each tube. Mixed by vortexing and centrifuged at 4000rpm for 5min.

The supernatant was decated into the waste bucket carefully not to discard the pellet and the fifteen millilitres tubes were drained on a clean towel. While draining, the GITC buffer was prepared by adding 10uL beta-mercaptoethanol (BME) to the 1ml of GITC. One millilitre of activated GITC buffer containing BME was added to the cell pellets in one tube.

A blunt end 18G needle and 2ml syringe was used to homogenise the GITC lysate 18 times.

A sterile Pasteur pipettes was used to transfer the GITC lysate into 2mL cryovial and labelled accordingly for storage at minus -20^{0}C. (The lysate can also be used immediately for nucleic acid extraction).

For quality control (QC) purpose, also 1.0mL of the GITC buffer containing BME was transferred into a cryovial, label as QC control and treated as a sample during nucleic acid extractions.

Stage 2: DNA Extraction *[Geneaid Genomic DNA Mini Kit (Blood/Cultured Cell)]*

Protocol for Extraction*:*

Two hundred microlitres of GITC lysate was pipetted into a 1.5mL tube

Two hundred and fifty microliters of Guanidium Chloride buffer (GB buffer), and vortexed for 15 seconds and then incubated at 60^{o}C for 30mins. It was mixed occasionally.

Two hundred and fifty microliters absolute ethanol was added and Vortexed gently for 10 seconds.

The mixture was incubated at room temp for 5 minutes.

The GD column was placed in 2mL collection tube. The mixture was transferred into the GD column.

It was centrifuged at 14000 rpm for 5 minutes.

The 2mL collection tube was discarded and then replaced with a new one.

Four hundred microlitres of W1 buffer was added to the GD column and centrifuged for 1 minute.

The collection tube was discarded and the GD column was placed in a new collection tube.

Six hundred microlitres of wash buffer was added to the GD column and centrifuged for 1 minute.

The GD column was place into another collection tube, centrifuged at 14000rpm for 3 minutes to ensure the column is dry.

The GD column was transferred to 1.5ml tube.

Sixty microlitres of preheated elution buffer was added to the center of the GD column and allowed to stand for 10 minutes at room temperature.

Then centrifuged at 14000 rpm for 30 seconds to elute the purified DNA.

3.11.13.2 Genotyping of SNPs

Genotyping of SNPs of the FTO gene was performed with the Illumina next-generation sequencing (NGS) using NextSeq 2000 Sequencing System. Purity and concentration of isolated DNA was determined by UV/VIS spectrophotometer NanoDrop ND-1000. The primers for the sequence, as designed from Illumina Design Studio (Ampliseq for Illumina Gene DNA for FTO Gene with a 100% coverage):

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Manifest IAA23693_182
Manifest Format Version 1.0
Manifest File Version 20201117
BuildID grch38.p2
source ConvertTsaManifestLite 1.22.0.388

2.6 Statistical Analysis

Hardy-Weinberg Equilibrium statistical test was used to determine the variation in distribution of the alleles and genotypes within the study population while allelic frequencies were calculated by gene counting. Chi-square (and fisher's test where chi-square was not applicable) and Odd Ratio (OR) were performed to determine the significant differences and associated risks respectively of the allelic and genotypic frequencies of Type 2 diabetic (T2D) and non-diabetic subjects of the FTO gene variants. Statistical significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

Table 1: Hardy-Weinberg Statistical Test of FTO gene Variants of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

FTO gene Variant	rs73609956 (C>T)			rs116753298 (T>C)			rs201041270 (A>G)			rs531215275 (A>C)		
Genotype	TT	CT	CC	CC	CT	TT	GG	GA	AA	CC	CA	AA
No, Obs	92	6	0	92	6	0	92	6	0	89	9	0
Freq, Allele	0.97	-	0.03	0.97	-	0.03	0.97	-	0.03	0.95	-	0.05
Freq, Exp	0.94	0.06	0.0009	0.94	0.06	0.0009	0.94	0.06	0.0009	0.90	0.01	0.003
No, Exp	92.12	5.88	0.09	92.12	5.88	0.09	92.12	5.88	0.09	88.2	0.98	0.3
Deviation	-0.12	0.12	-0.09	-0.12	0.12	-0.09	-0.12	0.12	-0.09	0.8	8.02	-0.3
X^2	0.0002	0.002	0.99	0.0002	0.002	0.99	0.0002	0.002	0.99	0.007	0.69	0.3

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

Table 2: Hardy-Weinberg Statistical Test of FTO gene Variants of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

FTO gene Variant	rs146056278 (C>T)			rs1410999299 (G>A)			rs79206939 (A>G)			rs145884431 (G>A)		
Genotype	TT	CT	CC	AA	AG	GG	GG	GA	AA	AA	GA	GG
No, Obs	95	3	0	92	5	1	93	4	1	90	8	0
Freq, Allele	0.98	-	0.02	0.96	-	0.04	0.97	-	0.03	0.96	-	0.04
Freq, exp	0.97	0.04	0.0004	0.93	0.08	0.002	0.94	0.06	0.0009	0.92	0.08	0.002
No, Exp	95	3.84	0.04	91.13	7.53	0.2	92.09	3.94	0.09	90.32	7.84	0.2
Deviation	1	-0.84	-0.04	0.88	-2.53	0.8	0.91	0.06	0.91	-0.32	1.6	-0.2
X^2	0.01	0.18	0.04	0.008	0.85	0.14	0.009	0.0009	0.99	0.001	0.34	0.66

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

Table 3: Hardy-Weinberg Statistical Test of FTO gene Variants of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

FTO gene Variant	**rs61743972 (G>A)**			**rs201496428 (C>T)**			**rs146138389 (T>C)**			**rs886052102 (A>G)**		
Genotype	AA	GA	GG	TT	CT	CC	CC	TC	TT	GG	GA	AA
No, Obs	92	6	0	92	6	0	91	7	0	92	6	0
Freq, Allele	0.97	-	0.03	0.97	-	0.03	0.96	-	0.04	0.97	-	0.03
Freq, exp	0.94	0.06	0.0009	0.94	0.06	0.0009	0.93	0.08	0.02	0.94	0.06	0.0009
No, Exp	92.12	5.88	0.09	92.12	5.88	0.09	91.14	7.84	1.96	92.12	5.88	0.09
Deviation	-0.12	0.12	-0.09	-0.12	0.12	-0.09	-0.14	-0.84	-1.96	-0.12	0.12	-0.09
X^2	0.0002	0.002	0.99	0.0002	0.002	0.99	0.0002	0.09	0.91	0.0002	0.002	0.99

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

Table 4: Hardy-Weinberg Statistical Test of FTO gene Variants of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

FTO gene Variant	**rs144743617 (G>A)**			**rs886052103 (T>A)**			**rs9939609 (A>T)**			**rs8050136 (A>C)**		
Genotype	AA	GA	GG	AA	TA	TT	TT	AT	AA	CC	AC	AA
No, Obs	93	5	0	92	6	0	77	12	9	81	17	0
Freq, Allele	0.98	-	0.02	0.97	-	0.03	0.85	-	0.15	0.91	-	0.09
Freq, exp	0.96	0.03	0.0004	0.94	0.06	0.0009	0.72	0.26	0.02	0.83	0.16	0.008
No, Exp	94	2.94	0.03	92.12	5.88	0.09	70.6	25.48	1.96	81.34	15.68	0.8
Deviation	1	2.06	-0.03	-0.12	0.12	-0.09	6.4	13.48	7.04	-0.34	2.68	-0.8
X^2	0.01	1.44	0.03	0.0002	0.002	0.99	0.5	0.26	0.25	0.001	0.5	0.6

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

Results of Genotypic and Allelic Distribution of FTO gene Variants in Obese|T2D Subjects in Different Tribes of Niger Delta

Table 5: Genotype and Allele Distribution of rs73609956 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	Ijaw		X^2	P	OR	Urhobo		X^2	P	OR	Ika		X^2	P	OR	Igbo		X^2	P	OR
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
TT	18 (94.7%)	5 (100%)	-	-	-	19 (100%)	5 (100%)	-	-	-	20 (100%)	5 (100%)	-	>0.99	-	18 (90%)	5 (100%)	-	>0.99	-
CT	1 (5.3%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				2 (10%)	0 (0.0%)			
CC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
TT	18 (94.7%)	5 (100%)	0.27	0.60	0.00	19 (100%)	5 (100%)	-	-	-	20 (100%)	5 (100%)	-	>0.99	-	18 (90%)	5 (100%)	0.54	0.46	0.00
CT + CC	1 (5.3%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				2 (10%)	0 (0.0%)			
TT+CT	19	5	-	>0.99	-	19 (100%)	5 (100%)	-	-	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
CC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
T	37 (97.4%)	10 (100%)	0.26	0.60	0.00	38 (100%)	10 (100%)	-	>0.9	-	40 (100%)	10 (100%)	-	>0.99	-	38 (95%)	10 (100%)	0.52	0.47	0.00
C	1 (2.6%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				2 (5%)	0 (0.0%)			

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

Table 6: Genotype and Allele Distribution of rs116753298 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	Ijaw		X^2	P	OR	Urhobo		X^2	P	OR	Ika		X^2	P	OR	Igbo		X^2	P	OR
Genotype	T2D n=19	ND n=5	-	-	-	T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
CC	17 (89.5%)	5 (100%)				18 (94.7%)	5 (100%)	-	-	-	19 (95%)	4 (80%)	-	-	-	19 (95%)	5 (100%)	-	-	-
CT	2 (10.5%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (5%)	1 (20%)				1 (5%)	0 (0.0%)			
TT	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	17 (89.5%)	5 (100%)	0.57	0.45	0.0	18 (94.7%)	5 (100%)	0.27	0.60	0.0	19 (95%)	4 (80%)	1.22	0.27	4.8	19 (95%)	5 (100%)	0.27	0.60	0.0
CT + TT	2 (10.5%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (5%)	1 (20%)				1 (5%)	0 (0.0%)			
CC+CT	19 (100%)	5 (100%)		>0.99		19 100%)	5 (100%)		>0.99		20 (100%)	5 (100%)		>0.99		20 100%)	5 (100%)		>0.99	
TT	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	36 (94.7%)	10 (100%)	0.55	0.47	0.0	37 (94.7%)	10 (100%)	0.26	0.60	0.0	39 (97.5%)	9 (90%)	1.17	0.28	4.3	39 (97.5%)	10 (100%)	0.26	0.61	0.0
T	2 (5.3%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (2.5%)	1 (10%)				1 (2.5%)	0 (0.0%)			

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

Table 7: Genotype and Allele Distribution of rs201041270 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

	Ijaw		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	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	0.0	18 (94.7%)	5 (100%)	0.27	0.60	0.0	19 (95%)	5 (100%)	0.27	0.60	0.0	18 (90%)	5 (100%)	0.11	0.73	0.0
GA + AA	2 (10.5%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (5%)	0 (0.0%)				2 (10%)	0 (0.0%)			
GG+GA	19 (100%)	5 (100%)	-	>0.99	-	19 (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	0.0	37 94.7%)	10 (100%)	0.26	0.60	0.0	39 (97.5%)	10 (100%)	0.25	0.61	0.0	38 (95%)	10 (100%)	0.52	0.47	0.0
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= p value, OR= Odd Ratio

Table 8: Genotype and Allele Distribution of rs531215275 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
CC	17 (89.5%)	5 (100%)	-	-	-	17 (89.5%)	4 (80%)	-	-	-	19 (95%)	5 (100%)	-	-	-	18 (90%)	4 (80%)	-	-	-
CA	2 (10.5%)	0 (0.0%)				2 (10.5%)	1 (20%)				1 (5%)	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	17 (89.5%)	5 (100%)	0.57	0.45	0.00	17 (89.5%)	4 (80%)	0.32	0.57	2.13	19 (95%)	5 (100%)	0.27	0.60	0.00	18 (90%)	4 (80%)	0.37	0.53	2.25
CA + AA	2 (10.5%)	0 (0.0%)				2 (10.5%)	1 (20%)				1 (5%)	0 (0.0%)				2 (10%)	1 (20%)			
CC+CA	19 (100%)	5 (100%)	-	>0.99	-	19 (100%)	5 (100%)	-	>0.99	-	20	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	36 (94.7%)	10 (100%)	0.55	0.45	0.00	36 (94.7%)	10 (100%)	0.55	0.45	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%)				2 (5.2%)	0 (0.0%)				1 (2.5%)	0 (0.0%)				2 (5%)	0 (0.0%)			

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

Table 9: Genotype and Allele Distribution of rs146056278 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
TT	19 (100%)	5 (100%)	-	-	-	19 (100%)	5 (100%)	-	-	-	20 (100%)	4 (80%)	-	-	-	18 (90%)	5 (100%)	-	-	-
TC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	1 (20%)				2 (10%)	0 (0.0%)			
CC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
TT	19 (100%)	5 (100%)	-	>0.99	-	19 (100%)	5 (100%)	-	>0.99	-	20 (100%)	4 (80%)	4.17	**0.04**	<0.0	18 (90%)	5 (100%)	0.54	0.46	0.000
TC + CC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	1 (20%)				2 (10%)	0 (0.0%)			

TT+TC	19 (100%)	5 (100%)	-	>0.99	-	19 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
CC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
T	38 (100%)	10 (100%)	-	>0.99	-	38 (100%)	10 (100%)	-	>0.99	-	40 (100%)	9 (90%)	4.17	**0.04**	<0.0	38 (95%)	10 (100%)	0.52	0.47	0.00
C	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	1 (10%)				2 (5%)	0 (0.0%)			

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

Table 10: Genotype and Allele Distribution of rs1410999299 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	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%)	-	-	-	19 (95%)	4 (80%)	-	-	-	19 (95%)	5 (100%)	-	-	-
AG	2 (10.5%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (5%)	0 (0.0%)				1 (5%)	0 (0.0%)			
GG	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	1 (20%)				0 (0.0%)	0 (0.0%)			
AA	17 (89.5%)	5 (100%)	0.57	0.45	0.0	18 (94.7%)	5 (100%)	0.27	0.60	0.0	19 (95%)	4 (80%)	0.28	0.59	2.25	19 (95%)	5 (100%)	0.27	0.60	0.0
AG + GG	2 (10.5%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (5%)	1 (20%)				1 (5%)	0 (0.0%)			
AA+AG	19 (100%)	5 (100%)	-	>0.99	-	19 (100%)	5 (100%)	-	>0.99	-	20 (100%)	4 (80%)	4.17	**0.04**	<0.0	20 (100%)	5 (100%)	-	>0.99	-
GG	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	1 (20%)				0 (0.0%)	0 (0.0%)			
A	36 (94.7%)	10 (100%)	0.55	0.45	0.0	37 (94.7%)	10 (100%)	0.26	0.60	0.0	39 (97.5%)	8 (80%)	4.34	**0.03**	9.75	39 (97.5%)	10 (100%)	0.26	0.61	0.0
G	2 (5.2%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				1 (2.5%)	2 (20%)				1 (2.5%)	0 (0.0%)			

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

Table 11: Genotype and Allele Distribution of rs79206939 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
GG	18 (94.7%)	5 (100%)	-	-	-	19	5 (100%)	-	-	-	18 (90%)	4 (80%)	-	-	-	19 (95%)	5 (100%)	-	-	-
GA	1 (5.2%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				2 (10%)	0 (0.0%)				1 (5%)	0 (0.0%)			
AA	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	1 (20%)				0 (0.0%)	0 (0.0%)			
GG	18 (94.7%)	5 (100%)	0.27	0.60	0.00	19 (100%)	5 (100%)	-	>0.99	-	18 (90%)	4 (80%)	0.38	0.53	2.25	19 (95%)	5 (100%)	0.27	0.60	0.00
GA + AA	1 (5.2%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				2 (10%)	1 (20%)				1 (5%)	0 (0.0%)			
GG+GA	19 (100%)	5 (100%)	-	>0.99	-	19 (100%)	5 (100%)	-	>0.99	-	20 (100%)	4 (80%)	4.17	**0.04**	<0.0	20 (100%)	5 (100%)	-	>0.99	-
AA	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	1 (20%)				0 (0.0%)	0 (0.0%)			
G	37 (94.7%)	10 (100%)	0.26	0.60	0.00	38 (100%)	10 (100%)	-	>0.99	-	38 (95%)	8 (80%)	2.44	0.11	4.75	39 (97.5%)	10 (100%)	0.26	0.61	0.00
A	1 (5.2%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				2 (5%)	2 (20%)				1 (2.5%)	0 (0.0%)			

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

Table 12: Genotype and Allele Distribution of rs145884431 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
AA	17 (89.5%)	5 (100%)	-	-	-	17 (89.5%)	4 (80%)	-	-	-	18 (90%)	5 (100%)	-	-	-	19 (95%)	5 (100%)	-	-	-
GA	2 (10.5%)	0 (0.0%)				2 (10.5%)	1 (20%)				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	0.0	17 (89.5%)	4 (80%)	0.32	0.56	2.13	18 (90%)	5 (100%)	0.27	0.60	0.0	19 (95%)	5 (100%)	0.27	0.60	0.0
GA + GG	2	0				2	1				2	0				1	0			

	(10.5%)	(0.0%)				(10.5%)	(20%)				(10%)	(0.0%)				(5%)	(0.0%)			
AA+GA	19 (100%)	5 (100%)	-	>0.99	-	19 (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	0.0	36 (94.7%)	9 (90%)	0.30	0.58	2.0	38 (95%)	10 (100%)	0.52	0.47	0.0	39 (97.5%)	10 (100%)	0.26	0.61	0.0
G	2 (5.2%)	0 (0.0%)				2 (5.2%)	1 (10%)				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= p value, OR= Odd Ratio

Table 13: Genotype and Allele Distribution of rs61743972 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	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	2 (10.5%)	0 (0.0%)				1 (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	2 (10.5%)	0 (0.0%)				1 (5.2%)	0 (0.0%)				2 (10%)	0 (0.0%)				1 (5%)	0 (0.0%)			
AA+GA	19 (100%)	5 (100%)	-	>0.99	-	19 (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= p value, OR= Odd Ratio

Table 14: Genotype and Allele Distribution of rs201496428 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	Ijaw		X^2	P	OR	Uhrobo		X^2	P	OR	Ika		X^2	P	OR	Igbo		X^2	P	OR
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
TT	17 (89.5%)	5 (100%)	-	-	-	16 (89%)	5 (100%)	-	-	-	19 (95%)	5 (100%)	-	-	-	20 (100%)	5 (100%)	-	-	-
CT	2 (10.5%)	0 (0.0%)				3 (11%)	0 (0.0%)				1 (5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
CC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
TT	17 (89.5%)	5 (100%)	0.57	0.45	0.00	16 (89%)	5 (100%)	0.90	0.34	0.00	19 (95%)	5 (100%)	0.27	0.60	0.00	20 (100%)	5 (100%)	-	>0.99	-
CT + CC	2 (10.5%)	0 (0.0%)				3 (11%)	0 (0.0%)				1 (5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
TT+CT	19 (100%)	5 (100%)	-	>0.99	-	19 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
CC	0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
T	36 (94.7%)	10 (100%)	0.55	0.45	0.00		10 (100%)	0.84	0.36	0.00	39 (97.5%)	10 (100%)	0.26	0.61	0.00	40 (100%)	10 (100%)	-	>0.99	-
C	2 (5.2%)	0 (0.0%)				3 (7.9%)	0 (0.0%)				1 (2.5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			

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

Table 15: Genotype and Allele Distribution of rs146138389 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	Ijaw		X^2	P	OR	Urhobo		X^2	P	OR	Ika		X^2	P	OR	Igbo		X^2	P	OR
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
CC	19 (100%)	4 (80%)	-	-	-	17 (89.5%)	5 (100%)	-	-	-	18 (90%)	5 (100%)	-	-	-	19 (95%)	4 (80%)	-	-	-
TC	0 (0.0%)	1 (20%)				2 (10.5%)	0 (0.0%)				2 (10%)	0 (0.0%)				1 (5%)	1 (20%)			
TT	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	19 (100%)	4 (80%)	3.96	**0.04**	0.0	17 (89.5%)	5 (100%)	0.57	0.45	0.0	18 (90%)	5 (100%)	0.27	0.60	0.00	19 (95%)	4 (80%)	1.22	0.26	4.75
TC + TT	0 (0.0%)	1 (20%)				2 (10.5%)	0 (0.0%)				2 (10%)	0 (0.0%)				1 (5%)	1 (20%)			
CC+TC	19	5	-	>0.9	-	19	5	-	>0.99	-	20	5	-	>0.99	-	20	5	-	>0.99	-

	(100%)	(100%)		9		(100%)	(100%)				(100%)	(100%)				(100%)	(100%)			
TT	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	38 (100%)	9 (90%)	3.88	**0.04**	0.0	36 (94.7%)	10 (100%)	0.55	0.45	0.0	38 (95%)	10 (100%)	0.52	0.47	0.00	39 (97.5%)	9 (90%)	1.17	0.27	4.33
T	0 (0.0%)	1 (10%)				2 (5.2%)	0 (0.0%)				2 (5%)	0 (0.0%)				1 (2.5%)	1 (10%)			

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

Table 16: Genotype and Allele Distribution of rs886052102 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
GG	17 (89.5%)	5 (100%)	-	-	-	17 (89.5%)	5 (100%)	-	-	-	20 (100%)	4 (80%)	-	-	-	20 (100%)	4 (80%)	-	-	-
GA	2 (10.5%)	0 (0.0%)				2 (10.5%)	0 (0.0%)				0 (0.0%)	1 (20%)				0 (0.0%)	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%)			
GG	17 (89.5%)	5 (100%)	0.57	0.45	0.0	17 (89.5%)	5 (100%)	0.57	0.45	0.0	20 (100%)	4 (80%)	4.17	**0.04**	0.0	20 (100%)	4 (80%)	4.17	**0.04**	0.0
GA + AA	2 (10.5%)	0 (0.0%)				2 (10.5%)	0 (0.0%)				0 (0.0%)	1 (20%)				0 (0.0%)	1 (20%)			
GG+GA	19 (100%)	5 (100%)	-	>0.99	-	19 (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%)	0.55	0.45	0.0	36 (94.7%)	10 (100%)	0.55	0.45	0.0	40 (100%)	9 (90%)	4.17	**0.04**	0.0	40 (100%)	9 (90%)	4.17	**0.04**	0.0
A	2 (5.2%)	0 (0.0%)				2 (5.2%)	0 (0.0%)				0 (0.0%)	1 (10%)				0 (0.0%)	1 (10%)			

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

Table 17: Genotype and Allele Distribution of rs144743617 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	Ijaw		X^2	P	OR	Urhobo		X^2	P	OR	Ika		X^2	P	OR	Igbo		X^2	P	OR
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
AA	17 (89.5%)	4 (80%)	-	-	-	18 (94.7%)	5 (100%)	-	-	-	19 (95%)	5 (100%)	-	-	-	20 (100%)	5 (100%)	-	-	-
GA	2 (10.5%)	1 (20%)				1 (5.2%)	0 (0.0%)				1 (5%)	0 (0.0%)				0 (0.0%)	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%)	4 (80%)	0.32	0.56	2.12	18 (94.7%)	5 (100%)	0.27	0.60	0.0	19 (95%)	5 (100%)	0.27	0.60	0.0	20 (100%)	5 (100%)	-	>0.99	-
GA + GG	2 (10.5%)	1 (20%)				1 (5.2%)	0 (0.0%)				1 (5%)	0 (0.0%)				0 (0.0%)	0 0.0%)			
AA+GA	19 (100%)	5 (100%)	-	>0.99	-	19 (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%)	9 (90%)	0.30	0.58	2.00	37 (94.7%)	10 (100%)	0.26	0.60	0.0	39 (97.5%)	10 (100%)	0.26	0.61	0.0	40 (100%)	10 (100%)	-	>0.99	-
G	2 (5.2%)	1 (10%)				1 (5.2%)	0 (0.0%)				1 (2.5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			

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

Table 18: Genotype and Allele Distribution of rs886052103 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	Ijaw		X^2	P	OR	Urhobo		X^2	P	OR	Ika		X^2	P	OR	Igbo		X^2	P	OR
Genotype	T2D n=19	ND n=5				T2D n=19	ND n=5				T2D n=20	ND n=5				T2D n=20	ND n=5			
AA	17 (89.5%)	5 (100%)	-	-	-	17 (89.5%)	4 (80%)	-	-	-	19 (95%)	5 (100%)	-	-	-	20 (100%)	5 (100%)	-	-	-
TA	2 (10.5%)	0 (0.0%)				2 (10.5%)	1 (20%)				1 (5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
TT	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	0.0	17 (89.5%)	4 (80%)	0.32	0.56	2.12	19 (95%)	5 (100%)	0.27	0.60	0.0	20 (100%)	5 (100%)			
TA + TT	2 (10.5%)	0 (0.0%)				2 (10.5%)	1 (20%)				1 (5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			
AA+TA	19 (100%)	5 (100%)	-	>0.99	-	19 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
TT	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	0.0	36 (94.7%)	9 (90%)	0.30	0.58	2.00	39 (97.5%)	10 (100%)	0.26	0.61	0.0	40 (100%)	10 (100%)	-	>0.99	-
T	2 (5.2%)	0 (0.0%)				2 (5.2%)	1 (10%)				1 (2.5%)	0 (0.0%)				0 (0.0%)	0 (0.0%)			

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

Table 19: Genotype and Allele Distribution of rs9939609 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	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%)				2 (10.5%)	1 (20%)				3 (15%)	0 (0.0%)				2 (10%)	0 (0.0%)			
AA	2 (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	5 (26.3%)	1 (20%)				5 (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	19 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-	20 (100%)	5 (100%)	-	>0.99	-
AA	2 (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%)			

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

Table 20: Genotype and Allele Distribution of rs8050136 Variant of FTO gene in Obese|T2D subjects in Different Tribes of Niger Delta

Tribes	**Ijaw**		**X^2**	**P**	**OR**	**Urhobo**		**X^2**	**P**	**OR**	**Ika**		**X^2**	**P**	**OR**	**Igbo**		**X^2**	**P**	**OR**
Genotype	T2D n=19	ND n=5				T2D n=19	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	4 (21.1%	1 (20%)				4 (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	0.9	15 (78.9%)	5 (100%)	1.26	0.26	0.0	15 (75%)	5 (100%)	1.56	0.21	0.0	18 (90%)	4 (80%)	0.37	0.53	2.2
AC + AA	4 (21.1%	1 (20%)				4 (21.1%	0 (0.0%)				5 (25%)	0 (0.0%)				2 (10%)	1 (20%)			
CC+AC	19 (100%)	5 (100%)	-	>0.99	-	19 (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	0.9	34 (89.5%)	10 (100%)	1.14	0.28	0.0	35 (87.5%)	10 (100%)	1.38	0.23	0.0	38 (95%)	9 (90%)	0.35	0.55	2.1
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= p value, OR= Odd Ratio k

This study investigated the association of FTO gene variants in the Niger Delta region in Southern Nigeria, involving several FTO gene variants. Sixteen (16) variants common to all the Niger Delta tribes was identified after sequencing and analysed after excluding some that significantly deviated from Hardy-Weinberg equilibrium, in this novel study of the FTO gene in that region. They are rs73609956, rs116753298, rs201041270, rs531215275, rs146056278, rs1410999299, rs79206939, rs145884431, rs61743972, rs201496428, rs146138389, rs886052102, rs144743617, rs886052103, rs9939609 and rs8050136. These are SNPs have been found in previous studies involving African population, European and Asian populations [10, 16-19].

Most FTO SNPs in all the tribes (Tables 1-4) by the deviation values showed weak linkage disequilibrium (LD) except for the FTO SNPs in the first intron of the gene such as rs9939609 which showed a high LD as against findings in populations of European ancestry and African American. This is consistent with the findings by other studies in Sub-Sahara Africa and amongst the Malaysian Malays [17-18]. The potential explanation to this may be in the homogeneity of passage along ancestral line through intra tribe marriages and low migration as against what obtains in Europe and our study subjects are also purebreds.

The analysis observed minor allele frequency (MAF) consistent with other findings in African populations much lower than those reported for European populations [16-17] and a similar allelic or genotype frequencies with ten FTO SNPs between the case and control in all the tribes (Tables 5-20). The MAF for the rs9939609 FTO gene variants in the Niger Delta population from this study is approximately 21% (Tables 4 and 18) with about 15% carrying the risk allele (A). The FTO variant MAF in white Europeans is put at approximately 45%, such that 66% of Europeans carry at least one risk allele and 18% carry two risk alleles [20]. The prevalence of the risk alleles of rs9939609 FTO SNP in East Asians (approx. 20%) and South Asians (approx. 30%) is substantially lower than in Europeans [21]. Peng et al. [22] in a meta-analysis of several studies for five FTO polymorphs, estimated the MAF for control subjects across all studies to be within the range of 11-45%, 40-46%, 11-44%, 36-60% and 21-44% for rs9939606, rs1421085, rs8050136, rs17817449 and rs1121980 respectively. A meta-analysis reported that the minor allele frequency (MAF) for rs9939609 varies across the global population. Apalasamy et al. [18] found the MAF of the FTO rs9939609 polymorphism was lower (0.31) in the Malaysian Malay population compared to the previously reported range of 0.38 to 0.46 in European population. Grant *et al.* in a study in 2008 found a varied frequencies among African-American unlike what was observed by this study, while Henning *et al*. 2009 in a study in an African population found otherwise. The discrepancies in the African-American was attributed to the genetically heterogeneous nature of the population as against the study nature of the study by Henning and colleagues which was in West Kiage region of Gambia which is ethnically homogenous.

The findings in this study however, observed a significant distribution difference mostly with the dominant allele with rs146056278 (TT,T; p<0.04) in Ika, rs1410999299 [(AA+AG, p<0.04), (A, p<0.03)] in Ika, rs79206939 (GG+GA, p<0.04), rs146138389 (CC, C, p<0.04), rs886052102 (GG, G, p<0.04) in Ika and Igbo tribes and rs8050136 (CC, C, p<0.002) in the Ijaw tribes. Bressler et al. [23] observed there was significant difference in four FTO genotype (rs9939609, rs17817449, rs805136, rs1421085) frequencies in the whites, but a difference was only revealed for one (rs1421085) in African-American. Apalasamy et al. [18] in a study among the Malays of Malaysia observed no significant differences in allelic or genotype between the obese and non-obese groups.

4. CONCLUSION

The study found a strong association between the variant genotype rs9939609 with obesity in Niger Delta tribes, but no significant independent association with T2D. The type 2 diabetes risk resulting from rs9939609 variant of the FTO gene in this region may be obesity mediated as evidenced by the increases observed in the BMI of the carriers of the risk allele (A). However, findings showed a significant association between some other variants with type 2 diabetes independent of body mass index, especially the rs886052102 and rs201041270 genotype variants.

Findings in this study showed no obvious disparity in the associations of FTO gene variants in the lineage groupings, disease duration, or sex, neither were there any major significant difference in pattern in the FTO genetic allelic distributions within the different tribes in the region. Findings suggest a lower risk of insulin resistance with the Ijaw diabetics than the other tribes and the Urhobo/Isoko showed higher levels of insulin resistance. The study also observed a higher prevalence of obesity in the Urhobo/Isoko & Ika tribes and that there were a greater number of obese subjects among the female T2D than their male counterpart, but there were more males with issues of insulin resistance/insulin insufficiency among T2D subject in the Niger Delta region of Nigeria. Knowledge of the dominant SNPs that are consistent with some specific biomarkers in some ethnic groups, may provide platform to delay its expression through informed wise choice of lifestyle change and proper dieting.

ETHICAL APPROVAL AND CONSENT

Ethical approval and permission were sought and obtained from the ethical committee of Federal Medical Centre, Asaba. Informed consent of the participants involved was also obtained using the consent form and anthropometric data was obtained via a questionnaire.

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