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Allele Diversity, Haplotype Frequency and Diversity, and Forensic Genotyping of Fulanis and Yorubas Population in North Central Region of Nigeria

تنوع الأليلات، وتواتر النمط الفردي وتنوعه، والتنميط الجيني الجنائي لكل من قبيلتي الفولاني واليوروبا في شمال وسط نيجيريا

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Abstract

Nigeria is the most populous African nation, comprising over 250 ethnic groups. The Yoruba and Fulani are the second and fourth largest ethnic groups in Nigeria, respectively. Forensic genotyping of short tandem repeats (STRs) is used in computation of Combined DNA Index System databases of individuals and ethnic groups. We examined allele diversity, haplotype frequency, haplotype diversity, and forensic genotyping data of autosomal STRs in Fulani and Yoruba residents in Ilorin, Kwara State, North Central Nigeria, in-order to further provide forensic genotyping data of these ethnic groups.

Samples of 25 Fulani males and 23 Yoruba males whose ethnicity was confirmed by three generations (paternal and maternal) were collected with informed consent using purposive sampling. All individuals in

Keywords: Forensic science, Forensic Anthropology, Autosomal STRs, Forensic Genotyping, Nigeria.

المستخلص

تعتبر نيجيريا أكثر الدول الإفريقية كثافة سكانية، إذ تحتوي على ما يزيد على 250 مجموعة عرقية. وتأتي قبيلة الفولاني في الترتيب الثاني، وقبيلة اليوروبا في الترتيب الرابع كأكبر مجموعات عرقية في البلاد. ويستخدم التنميط الجيني الجنائي لتكرار الترادف القصير (STRs) في حساب الـ DNA المشترك لقواعد البيانات الخاصة بنظام الفهرسة للأفراد والمجموعات العرقية. وقد قمنا بفحص تنوع الأليلات، وتواتر النمط الفردي، وكذلك تنوع النمط الفردي، وبيانات التنميط الجيني الجنائي للتكرار الجسمي للترادف القصير لسكان قبيلتي الفولاني واليوروبا في مدينة هورين بولاية كوارا، في شمال وسط نيجيريا، وذلك لتقديم المزيد من بيانات التنميط الجيني الجنائي لهاتين المجموعتين العرقيتين.

فقد تم أخذ عينات من خمسة وعشرين ذكرًا من قبيلة الفولاني وعينات لثلاث وعشرين أنثى من قبيلة اليوروبا تم تأكيد انتمائهم العرقي لثلاثة أجيال (من ناحية الأب ومن ناحية الأم). وأخذت هذه

الكلمات المفتاحية: علوم الأدلة الجنائية، علم الأجناس الجنائي، التتابعات القصيرة المتكررة الجسدية، التنميط الجيني الجنائي، نيجيريا.

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the samples were unrelated. The samples were amplified and then genotyped using the SureID® 21G PCR Amplification Kit containing Amelogenin and 20 autosomal STR loci.

Statistical analyses of forensic genotyping parameters confirmed no deviation from expectation of Hardy-Weinberg Equilibrium and no dependence of alleles between loci.

All tested loci were polymorphic. Expected Heterozygosity and gene diversity parameters showed lower genetic diversity amongst Fulanis compared to Yorubas. This is possibly due to the prevalent custom of marriage between cousins amongst Fulanis, which is forbidden in Yoruba customs.

العينات بموافقتهم على استخدامها لأغراض معينة. وقد كان جميع أصحاب العينات غير مرتبطين بقرابة النسب.

تم تكثير العينات باستخدام أدوات التضخيم من نوع Surel«R» 21 G PCR الذي يحتوي على الأميلوجينين ، وكذلك أماكن التموضع الـ 20 لتكرار الترادف القصير، وبعد ذلك تم تنميطها بالطريقة الكهربائية الشعرية.

وأكدت التحاليل الإحصائية لمعايير التنميط الجيني الجنائي عدم وجود اختلاف عن المتوقع لتوازن هاردي-وينبيرغ، وعدم الاعتماد للأليلات بين المواقع.

كانت جميع المواقع التي تم اختبارها متعددة الأشكال. وقد أظهرت معايير تغاير الزيكوتات المتوقعة والتنوع الجيني تنوعًا جينيًا أقل بين أفراد قبيلة الفولاني بالمقارنة مع أفراد قبيلة اليوروبا. وقد يعود السبب في ذلك للزواج الشائع بين أبناء وبنات الأعمام والعمات في قبيلة الفولاني، والذي تحرمه العادات في قبيلة اليوروبا.

1. Introduction

Nigeria is located in the Western part of Africa on the Gulf of Guinea, and it has one sixth of Africa's total population [1]. Nigeria, according to the official records of the National Population Commission (NPC), had a population of over 140 million in 2006 [2] and an estimated population of over 174 million in 2013 [1]. Nigeria is the most populous Black nation in the world, and it is composed of over 250 ethnic groups. The Hausas, Yorubas, and Igbos are the first to third largest ethnic groups, respectively [3, 4].

The Fulbe-speaking ethnic Fulanis are known to have extensive intermarriages with the Hausas. They are sometimes grouped with the Hausas as Hausa-Fulani, which together constitute about 30% of the total population of Nigeria, with Hausa as the dominant language of identity. The 1963 census of Nigeria has the Hausa, Yoruba, and Igbo at 20.9%, 20.3%, and 16.6% percent of the total population, respectively. The 1963 census also has the Hausa-Fulani at 29.6% of the national population. Hence, the Fulani as a distinct entity constitute about 9% of Nigeria's total population, and they are arguably the fourth largest ethnic group in Nigeria [3]. The Fulanis are known for travelling over great distances with their herds of cattle, though some Fulanis live in permanent settlements [5].

Recently, there have been reported clashes between herdsmen (mostly of Fulani origin) and farmers across different states and regions of Nigeria. Some of the Fulani herdsmen are also alleged to be foreigners from near and far such as Niger Republic, Chad, Mali, Guinea, and others. Therefore, it is relevant that forensic genotyping of major and minor Nigerian ethnic groups be carried out to provide their Combined DNA Index System (CODIS) database to aid civil and criminal investigations.

STRs loci are the most descriptive PCR-based genetic markers that can be used for the individualization of biological materials [6]. Therefore, STRs are highly variable diverse genetic markers, and they are widely used as forensic tools in the identification of individuals in civil and criminal cases [7]. For forensic genotyping purposes, 21 Combined DNA Index System (CODIS) STR markers (CSF1PO, D12S391, D13S317, D16S539, D18S51, D19S433, D1S1656, D21S11, D22S1045, D2S1338, D2S441, D3S1358, D5S818, D6S1043, D7S820, D8S1179, FGA, SE33, TH01, TPOX, and vWA) have been previously examined in some Hausa, Igbo, and Yoruba ethnic groups of Nigeria [4].

Gene diversity is described by the proportion of polymorphic loci across the genome. It is an important element of population dynamics, and is directly related to the evolutionary potential of the population, as well as the adverse effects of inbreeding. The number of alleles (allelic richness) is a genetic diversity parameter which can help determine a population's long-term potential for adaptability and persistence [8]. In addition, haplotype refers to alleles of multiple markers inherited or transmitted via a parent. Furthermore, haplotype frequencies are used in the analyses of linkage disequilibrium. Hence, haplotype diversity represents the uniqueness of a specific haplotype in a defined population. Haplotypes are thus used for designating a phenotype to a genetic region, and for detecting associations [9].

Previous studies examined some of the CODIS STR markers in Hausa, Igbo, and Yoruba ethnic groups of Nigeria, but they have not examined them in the Fulani ethnic group of Nigeria [4]. The PENTA loci (pentanucleotide repeat STR D and E) were released by PROMEGA scientists in 2000, as part of the efforts to establish loci with high variability, but low amounts of stutter product formation [10]. PENTA D, aside from being used for forensic identification, paternity, and population testing, is equally being evaluated for its efficiency in genetic testing for Down syndrome, due to its location on chromosome 21 [11]. PENTA D and PENTA E loci have not been examined in previous studies on Fulani, Hausa, Igbo, and Yoruba ethnic groups of Nigeria. Similarly, the allele diversity, haplotype frequency and haplotype diversity have not been reported in previous studies for Yoruba and Fulani ethnic groups of Nigeria. Therefore, this study evaluated allele diversity, haplotype frequency, haplotype diversity, and other population genetics parameters of 20 autosomal CODIS STRs (CSF1PO, D1S1656, D12S11, D12S391, D13S317, D16S539, D18S51, D19S433, D2S1338, D3S1358, D5S818, D6S1043, D7S820, D8S1179, FGA, PENTA D, PENTA E, TH01, TPOX, and vWA) in individuals of Fulani and Yoruba ethnic groups resident in Ilorin, Kwara State, North Central Nigeria, in-order to further provide forensic genotyping data of these ethnic groups. In addition, statistical population genetics of allele frequencies, expected allele frequencies, homozygosity, observed heterozygosity, expected heterozygosity, deviation from hardy Weinberg equilibrium, deviation from expected heterozygosity (inbreeding), polymorphism information content, power of discrimination, power of exclusion, paternity index, random man not excluded, FIS, FST, FIT, and GST were computed for Fulani and Yoruba ethnic groups of Nigeria.

2. Materials and Methods

2.1 Ethical Approval

This research was approved by the University of Ilorin's Ethical Review Committee (approval number UERC/ASN/2018/1261). Experimental procedures were carried out in accordance with the National Ethics and Operational Guidelines for Research on Human Subjects, Number code (1947); the World Medical Association Declaration of Helsinki (1964) and its amendments, and the Council of the International Organization of Medical Sciences (CIOMS) guidelines of 1993 as stated in the research policy of the University of Ilorin, Ilorin, Nigeria. Informed consent was obtained from each voluteer.

2.2 Sample Size and Sample Collection

Peripheral blood samples were collected from a total of forty-eight healthy, unrelated male subjects amongst the Fulani and Yoruba ethnic groups resident in Ilorin, Kwara State, North Central Nigeria **(Figure-1)**. The sample consisted of 25 Fulani males and 23 Yoruba males. The sample size was determined using purposive sampling [12]. The purpo-

sive random sampling technique is a non-probability technique employed in human invasive DNA studies which are dependent solely on the number of individuals who chose to volunteer for the study [12].

The age of the Fulani and Yoruba subjects in the sample ranged from 20-80 years and 20-25 years, respectively. One-mL of whole blood were obtained from each of the 48 subjects, who were either Fulanis or Yorubas since three generations (parents and grandparents).

2.3 Inclusion and Exclusion Criteria for Selection of Subjects

Genealogy of each volunteer was traced back to the third generation, based on self-declaration. Only individuals whose parents, grandparents, and great grandparents belong to the Fulani or Yoruba ethnic group were included in the study.

2.4 Reagents and Equipment

Standard laboratory equipment and high purity grade biochemicals and reagents were used in this study.

2.5 DNA Extraction

Genomic DNA was extracted from collected blood samples according to the manufacturer's instructions, using ZYMO Quick-DNA™ Miniprep Plus Kit (D4068, USA) as previously described [13]. This was done at the laboratory unit of the Department of Haematology and Immunology, Obafemi Awolowo University Teaching Hospital, Ile–Ife, Osun State, Nigeria.

2.6 Gel Electrophoresis and Photography of DNA in Agarose Gels

Agarose gel electrophoresis was performed for the separation, identification, and purification of 0.5- to 25-kb DNA fragments. DNA was photographed in agarose gels and stained with ethidium bromide by illuminating with UV light (>2500 $\mu W/cm^2$), while wearing protective eyeglasses.

2.7 PCR Amplification, Genotyping and Bioinformatic Analyses

PCR amplification was performed according to the manufacturer's instructions, using SureID® 21G Kit (Health Gene Technologies Co. Limited, China) as previously described [14], to amplify Amelogenin and the 20 autosomal STRs loci (CSF1PO, D1S1656, D12S11, D12S391, D13S317, D16S539, D18S51, D19S433, D2S1338, D3S1358, D5S818, D6S1043, D7S820, D8S1179, FGA, PENTA D, PENTA E, TH01, TPOX, and vWA) included in the kit at the laboratory unit of Inqaba Biotechnical Industries (Pty) Limited, Pretoria, South Africa. Electrophoresis was performed on the Applied Biosystems® 3500 Genetic Analyzer using POP-4® polymer and Data Collection Software, Version 2.0. GeneMapper® ID 3.2.1 and GeneMapper® ID-X1.2/1.4 software were used for bioinformatics analyses. The SureID® 21G Kit was provided free of charge by Inqaba Biotechnical Industries (Pty) Limited, Pretoria, South Africa. The corresponding dyes repeat numbers, locations of chromosomes, repeat sequences, control DNA for Amelogenin, and each of the 20 CODIS STRs are provided in Table-1.

The forensic genotyping parameters evaluated in this study were calculated as described below.

Allele Frequency: Number of Allele observations/2(n), where n is population size [4, 16].

Expected Allele Frequency: Individuals with Heterozygous + 2 (individual with Homozygous)/total number of individual's allele in the population [16].

Homozygosity (H) = $\Sigma(Pi^2)$, where Pi is frequency of allele.

Observed Heterozygosity (H_O) = $\Sigma Hn/N$, where H_n is the number of heterozygote individuals and N is

the total number of individuals in the population [17].

Expected Heterozygosity (H_E) = 1 – Homozygosity [17]

Deviation from Hardy-Weinberg Equilibrium for each STR locus (X2) = Σ(O-E)2/E, where O and E are the observed and expected number or genotypes.

Observed Genotype Frequency = Number of individuals with each genotype divided by population size.

Expected Hardy Weinberg Genotype Frequency for Homozygous Genotype = P^2.

Expected Hardy Weinberg Genotype Frequency for Heterozygous Genotype = 2_{pipj}.

P_IP_J, where P_I is first allele frequency and p_j is second allele frequency.

The calculated and X^2-Table at $p = 0.05$ and at 1 degree of freedom were compared to confirm deviation from Hardy-Weinberg proportions [16, 18, 19].

Table 1- *SureID® 21G Kit loci, corresponding dyes and STR repeat numbers.*

S/No.	Locus designation	Dye labeled	Repeat number	Chromosome location	Repeat sequence	Control DNA 9947A
1	Amelogenin	TAMRA	X,Y	Xp22.1–22.3 and Y	Not Available	X
2	D8S1179	TAMRA	7-18	8q24.13 (125.976Mb)	TCTA Complex	13
3	FGA	TAMRA	16-46.2	4q28 (155.866Mb)	TTTC Complex	23/24
4	TPOX	TAMRA	6-13	2p25.3 (1.472Mb)	AATG	8
5	vWA	TAMRA	10-24	12p13.31 (5.963Mb)	TCTA Complex	17/18
6	D18S51	6-FAM	8-27	18q21.33 (59.1Mb)	AGAA	15/19
7	D21S11	6-FAM	24-38	21q21.1 (19.476Mb)	TCTA Complex	30
8	D3S1358	6-FAM	12-20	3p21.31 (45.557Mb)	TCTA Complex	14/15
9	PENTA E	6-FAM	5-24	15q26.2 (95.175Mb)	AAAGA	12/13
10	TH01	6-FAM	4-13.3	11p15.5 (2.149Mb)	AATG	8/9.3
11	CSF1PO	JOE	6-15	5q33.1 (149.436Mb)	AGAT	10/12
12	D13S317	JOE	7-15	13q31.1 (81.62Mb)	TATC	11
13	D16S539	JOE	5-15	16q24.1 (84.944Mb)	GATA	11/12
14	D5S818	JOE	7-16	5q23.2 (123.139Mb)	AGAT	11
15	D7S820	JOE	6-15	7q21.11 (83.433Mb)	GATA	10/11
16	PENTA D	JOE	2.2-17	21q22.3 (43.88Mb)	AAAGA	12
17	D1S1656	ROX	11-18.3	1q42 (228.972Mb)	TAGA Complex	18.3
18	D12S391	ROX	14-26	12p12 (12.341Mb)	AGAT/AGAC Complex	18/20
19	D19S433	ROX	9-17.2	19q12 (35.109Mb)	AAGG Complex	14/15
20	D2S1338	ROX	15-28	2q35 (218.705Mb)	TGCC/TTCC	19/23
21	D6S1043	ROX	8-21	6q15 (92.449Mb)	AGAT	12/18

2.8 Deviation from Expected Heterozygosity (Inbreeding = F)

$F = H_E/H_E - H_O/H_E = 1- H_O/H_E$.

2.9 Calculations of Polymorphism Information Content (PIC), Probability of Matching (PM), Power of Discrimination (PD) and Power of Exclusion (PE)

$PIC = 1 - \Sigma (P_I)^2$, where Pi is the allele frequency [17].

$PM = \Sigma_i G_i^2$.[17]

PD = 1 - PM, PM = Probability of Matching [17].

PE = H2 . (1- h.H ^{2}). H = Observed Heterozygosity and h = Homozygosity [17]

2.10 Random Man Not Excluded (RMNE) and Paternity Index (PI)

RMNE = 1 - RMNE, then RMNE = 1 – PE [20]

PI = H/2h+h/2h and H= 1- h, where h is homozygosity [17]

2.11 Calculations of FIS, FST, FIT and GST and Effective Alleles (AE)

F_{IS} = 1 - Mean H_e/H_s

Mean H_e = mean of expected heterozygosity for both Fulani and Yoruba subjects = 2 (multiplication of allele frequencies for Fulani) + 2 (multiplication of allele frequencies for Yoruba subjects)/2

F_{ST} =1-H_S/ H_T,

where HT is the proportion of the heterozygotes in the total population and HS is the average proportion of heterozygotes in subpopulations.

FIT = 1 - Mean H_E / H_T.

$G_{ST} = (H_T - H_S) / H_T$. [20]

AE is the number of Alleles that are effective [21]

AE = 1/ (1-HE), where HE = expected heterozygosity.

2.12 Calculations of Allele Diversity (AD), Haplotype Frequency (HF) and Haplotype Diversity (HD)

AD = $\frac{(n'-1)}{(n-1)}$ where n' = the number of alleles we would expect following a bottle neck, and n = original number of alleles.

n' = n- $\cdot \sum_{i=1}^{N}(1-p_i)^{2N}$

HF = n/n-1 $[1- \sum p_i^2]$, where n = population size and pi is frequency of allele.

HD = n/n-1l $[1- \sum X_i^2]$ where n = population size and Xi is haplotype frequency.

2.13 Statistical Analyses

Computed data were statistically analysed using SPSS Statistics - Version 23.0. Data were presented as Mean±SD, with determination of level of significance at *p*-value less than or equal to 0.05.

3. Results

3.1 Age, Height, Bodyweight and Body Mass Indices (BMI) of Fulani and Yoruba Subjects

The age, bodyweight, and height for Fulani Subjects ranged from 20-80 years, 51-66kg, and 1.57-1.81m, respectively. The mean values ± standard error of mean of age, height, bodyweight, and BMI for Fulani and Yoruba subjects are as presented in Table-2. The results showed no significant differences ($p \leq 0.05$) between the mean values of height ($p = 0.157$), bodyweight ($p = 0.018$), and BMI ($p = 0.072$) of Fulani and Yoruba subjects, respectively. However, a significant difference ($p < 0.001$) was observed between the mean values of ages of Fulani and Yoruba subjects (Table-2).

3.2 Alleles of PENTA D and PENTA E in Fulani and Yoruba Subjects

PENTA D and PENTA E autosomal STR loci have not been examined in previous studies on Fulani and Yoruba ethnic groups. No partial repeats or

variant alleles of PENTA D and PENTA E were observed for Fulani and Yoruba subjects. The Alleles of PENTA D present in the Fulani subjects are 2.2, 5, 8, 9, 10, 11, 12, and 13. The Alleles of PENTA D present in the Yoruba subjects are 2.2, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14.

The alleles of PENTA E present in Fulani subjects are 5, 7, 8, 9, 10, 11, 12, 13, 14, 16, and 17. The alleles of PENTA E present in Yoruba subjects are 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, and 17.

3.3 Partial Repeats and Variant Alleles of CODIS STRs in Fulani and Yoruba Subjects

No partial repeats were observed for any of the 20 CODIS autosomal STRs in Fulani subjects. In addition, no variant alleles were observed for CSF1PO, D12S11, D12S391, D13S317, D16S539, D18S51, D3S1358, D5S818, D6S1043, D7S820, D8S1179, TPOX, and vWA loci. However, variant alleles were observed for D1S1656 (14.3, 16.3, 17.3 and 18.3), D19S433 (13.2, 14.2 and 15.2), D2S1338 (9.3), FGA (19.2), and TH01 (9.3) loci.

In addition, no partial repeats were observed for any of the 20 CODIS autosomal STRs in Yoruba subjects. And no variant alleles were observed for CSF1PO, D12S11, D12S391, D13S317, D16S539, D18S51, D3S1358, D5S818, D6S1043, D7S820, D8S1179, TPOX, and vWA loci. However, variant alleles were observed for D1S1656 (14.3, 16.3, 17.3, and 18.3), D19S433 (12.2, 13.2, 14.2, 15.2, and 16.2), D2S1338 (9.3), FGA (18.2, 19.2, and 41.2), and TH01 (9.3) loci.

3.4 Population Genetic Parameters

The computed results of AD, HF, HD, H, HO, HE, deviation from hardy Weinberg equilibrium, deviation from expected heterozygosity (inbreeding), PIC, PD, PE, PI, random man not excluded, FIS, FST, FIT and GST for the examined 20 autosomal CODIS STRs in Fulani and Yoruba subjects are presented in Tables-3, 4, 5, and 6, respectively. The computed values of allele frequencies for the examined 20 autosomal CODIS STRs in Fulani and Yoruba subjects have been presented for phylogenetic ancestral relationships between Hausa, Fulani, Igbo, and Yoruba ethnic groups of Nigeria, and other ethnic groups within and outside Africa [22].

Results showed that the highest value of AD in Fulani subjects was 97.1% (D8S1179), while the lowest AD value was 78.1% (D3S1358) (Table-3). For Yoruba subjects, the highest AD value was 99% (D7S820), while the lowest AD value was 76% (D5S818) (Table- 5).

The highest value of haplotype frequency (HF) in Fulani subjects was 88.5% (D6S1043), while the lowest HF value was 60.1% (D3S1358) (Table-3). For Yoruba subjects, the highest HF value was 96.6% (D19S433), while the lowest HF value was 29.6% (D7S820) (Table- 5).

The highest value of haplotype diversity (HD) in

Table 2- *Mean ± Standard Error of Mean of Ages, Height, Bodyweight and Body Mass Indices of Fulanis and Yorubas Subjects.*

Parameters	Fulanis	Yorubas	*p*-value at $p \leq 0.05$
Age (years)	35.20 ± 3.09	21.87 ± 0.37	$p<0.001$
Height (m)	1.71 ± 0.01	1.74 ± 0.01	0.157
Bodyweight (Kg)	59.32 ± 1.17	64.43 ± 1.75	0.018
Body Mass Index (Kg/m^2)	20.41 ± 0.32	21.62 ± 0.59	0.072

Table 3- *Allele Diversity, Haplotype Frequency, Haplotype Diversity and Population Genetics Parameters of the 20 autosomal CODIS STRs in Fulani Subjects.*

STR Locus	AD	HF	HD	PIC	PD	PM	PE	PI	RMNE
CSF1PO	0.8980	0.7904	0.3136	0.8032	0.8544	0.1456	0.2545	2.0886	0.7455
D1S1656	0.8825	0.8250	0.3327	0.8400	0.9280	0.0720	0.6020	2.4038	0.3979
D12S391	0.8750	0.8020	0.6700	0.8400	0.8800	0.1200	0.4600	2.1800	0.5400
D13S317	0.8800	0.6400	0.3700	0.7900	0.7900	0.2100	0.3500	1.3800	0.6500
D16S539	0.9600	0.7450	0.2600	0.4900	0.8900	0.1100	0.7900	4.0000	0.2100
D18S51	0.8823	0.8250	0.3327	0.7900	0.9000	0.1000	0.6000	2.3800	0.4000
D19S433	0.9650	0.7960	0.3800	0.8400	0.9900	0.0100	0.4700	1.9200	0.5300
D2S1338	0.9404	0.8775	0.2396	0.8600	0.9264	0.0736	0.7335	3.1726	0.2665
D21S11	0.9040	0.8080	0.3614	0.8316	0.9310	0.0690	0.5200	2.2300	0.4800
D3S1358	0.7805	0.6092	0.6551	0.5800	0.7600	0.2400	0.3700	1.2000	0.6300
D5S818	0.9700	0.5300	0.5100	0.7600	0.7400	0.2600	0.2700	1.0200	0.7300
D6S1043	0.9430	0.8850	0.8160	0.8600	0.9300	0.0700	0.7900	3.3200	0.8600
D7S820	0.9200	0.8037	0.2040	0.7300	0.8600	0.9400	0.5200	2.2000	0.4800
D8S1179	0.9706	0.8158	0.3484	0.2604	0.9120	0.0880	0.5053	1.5310	0.4900
FGA	0.8470	0.7940	0.3840	0.8100	0.9000	0.1000	0.6500	1.9400	0.3500
PENTA D	0.9200	0.8520	0.1539	0.8200	0.7980	0.2015	0.1829	2.7654	0.8171
PENTA E	0.7900	0.7810	0.2280	0.7136	0.5700	0.4300	0.5300	1.7500	0.4700
THO1	0.9650	0.7000	0.5310	0.8120	0.8510	0.1490	0.3850	1.5240	0.6150
TPOX	0.8707	0.8044	0.3689	0.7372	0.7712	0.2288	0.5935	1.6578	0.4065
vWA	0.9160	0.7442	0.4648	0.3916	0.8770	0.1230	0.5230	1.7507	0.4800

AD; Allele Diversity, HF; Haplotype Frequency, HD; Haplotype Diversity, PIC; Polymorphism Information Content, PD; Power of Discrimination, PM; Probability of Matching, PE; Power of Exclusion, PI; Paternity Index, RMNE; Random man Not Excluded.

Fulani subjects was 81.6% (D6S1043), while the lowest HD value was 15.4% (PENTA D) (Table-3). For Yoruba subjects, the highest HD value was 1.01% (D7S820), while the lowest HD value was 9.5% (PENTA D) (Table- 5).

4. Discussion

The Yoruba subjects in this study were taller and had higher bodyweight (1.60-1.84m and 51-81kg) than their Fulani counterparts (1.57-1.81m and 51-66kg). Notably, Yoruba subjects despite being taller had higher mean values of BMI (21.62 ± 0.59kg/m2) compared with the Fulanis (20.41 ± 0.32kg/m2). This is possibly due to differences in cultural practices, as the Fulanis usually eat more animal protein and dairy foods, being cattle breeders. However, the Yorubas mostly have to purchase meat and dairy foods and therefore have less access to such animal products.

Gene diversity provides descriptive information

Table 4- *Homozygosity, Observed Heterozygosity, Expected Heterozygosity and Population Genetics Parameters of the 20 autosomal CODIS STRs in Fulani Subjects.*

STR Locus	H	H_O	H_E	X^2	F	A_E	F_{IS}	F_{ST}	F_{IT}	G_{ST}
CSF1PO	0.2394	0.8400	0.7606	0.3396	-0.1044	4.1771	0.0000	0.5000	0.5000	0.5000
D1S1656	0.2080	0.8400	0.7920	1.5804	-0.0606	4.7620	0.0000	0.5000	0.5000	0.5000
D12S391	0.2300	0.7200	0.7700	0.4500	0.0700	4.3600	0.0000	0.5000	0.5000	0.5000
D13S317	0.3600	0.6400	0.6400	0.3700	-0.0972	2.7600	0.0000	0.5000	0.5000	0.5000
D16S539	0.1300	0.8400	0.8700	0.5900	-0.0200	7.6900	0.0000	0.5000	0.5000	0.5000
D18S51	0.2100	0.8400	0.7900	0.7400	-0.0600	4.7600	0.0000	0.5000	0.5000	0.5000
D19S433	0.2600	0.8800	0.2600	0.1700	-0.1900	3.8500	0.0000	0.5000	0.5000	0.5000
D2S1338	0.1576	0.9200	0.8424	1.7029	-0.9210	6.3450	0.0000	0.5000	0.5000	0.5000
D21S11	0.2240	0.8800	0.7760	1.0540	-0.1340	4.4640	0.0000	0.5000	0.5000	0.5000
D3S1358	0.4200	0.6800	0.5800	0.6800	-0.1600	2.4000	0.0000	0.5000	0.5000	0.5000
D5S818	0.4900	0.5600	0.5100	0.1600	-0.1000	2.0400	0.0000	0.5000	0.5000	0.5000
D6S1043	0.1500	0.9600	0.8500	0.5900	-0.1300	6.6500	0.0000	0.5000	0.5000	0.5000
D7S820	0.2100	0.9200	0.7900	0.3200	-0.1900	5.8500	0.0000	0.5000	0.5000	0.5000
D8S1179	0.2168	0.7600	0.7832	0.3600	0.02960	4.6100	0.0000	0.5000	0.5000	0.5000
FGA	0.2600	0.8400	0.7400	1.3700	-0.1300	3.8900	0.0000	0.5000	0.5000	0.5000
PENTA D	0.1808	0.8800	0.8200	0.5702	-0.0732	5.5555	0.0000	0.5000	0.5000	0.5000
PENTA E	0.2856	0.8000	0.7144	1.0130	-0.1198	3.5014	0.0000	0.5000	0.5000	0.5000
THO1	0.3280	0.6000	0.6720	0.3790	0.1100	3.0490	0.0000	0.5000	0.5000	0.5000
TPOX	0.3016	0.8800	0.6984	0.2105	-0.2600	3.3157	0.0000	0.5000	0.5000	0.5000
vWA	0.2856	0.8000	0.7144	0.7100	-0.1200	3.5000	0.0000	0.5000	0.5000	0.5000

H; Homozygosity, HO; Observed Heterozygosity, HE; Expected Heterozygosity, X2; Deviation from Hardy-WeinbergEquilibrium, F; Inbreeding, AE; Effective Allele, FIS; Variance of an allele frequencies within populations, FST; Variance of allele frequencies between populations, FIT; Inbreeding coefficient of an individual relative to the total population, GST; coefficient of gene differentiation.

on polymorphic loci proportion across the genome, and is directly related to the evolutionary potential of the population and inbreeding. Similarly, the number of alleles (allelic richness) can help determine a population's long-term potential for adaptability and persistence [23]. Furthermore, haplotype frequencies (HF) are used in the analyses of linkage disequilibrium, while haplotype diversity (HD) represents the uniqueness of a specific haplotype in a defined population [24]. Haplotypes are, therefore, used for designating a phenotype to a genetic region and for detecting associations [24]. The computed AD values for Fulanis and Yorubas were either approximately 0.5 or higher than 0.5 [25]. Therefore, computed AD, HF, and HD values for Fulani and Yoruba subjects indicate that the tested 20 autosomal CODIS STRs are highly informative and effective polymorphic loci for the evaluations of

Table 5- *Allele Diversity, Haplotype Frequency, Haplotype Diversity, Variant Alleles and Population Genetics Parameters of the 20 autosomal CODIS STRs in Yoruba Subjects.*

STR Locus	AD	HF	HD	PIC	PD	PM	PE	PI	RMNE
CSF1PO	0.9658	0.7990	0.6496	0.7891	0.8128	0.1872	0.2684	2.1616	0.7316
D1S1656	0.8673	0.8992	0.2001	0.8850	0.9366	0.0630	0.5599	3.5714	0.4401
D12S391	0.8520	0.8890	0.8250	0.8700	0.9100	0.0900	0.6700	3.2400	0.3300
D13S317	0.9200	0.7090	0.3000	0.7800	0.9400	0.0600	0.5000	1.6400	0.5000
D16S539	0.9200	0.8030	0.2040	0.8200	0.8900	0.1100	0.5000	2.1100	0.5000
D18S51	0.8760	0.8903	0.2168	0.8500	0.9400	0.0600	0.6200	3.3700	0.3800
D19S433	0.8830	0.9660	0.0690	0.7800	0.9200	0.0800	0.8100	3.0300	0.1900
D2S1338	0.8981	0.8759	0.2434	0.8736	0.8428	0.1572	0.6635	3.0845	0.3365
D21S11	0.9130	0.8600	0.2710	0.8590	0.9150	0.0853	0.5990	2.8600	0.4010
D3S1358	0.9701	0.8191	0.3441	0.7800	0.8900	0.1100	0.3800	2.3000	0.6200
D5S818	0.7600	0.6740	0.3300	0.7900	0.8300	0.1700	0.3600	1.4700	0.6400
D6S1043	0.8540	0.9200	0.8830	0.8900	0.9400	0.0600	0.7500	4.1700	0.2500
D7S820	0.9900	0.2964	1.0070	0.6500	0.8900	0.1100	0.5000	2.1000	0.5000
D8S1179	0.8961	0.8201	0.3423	0.1661	0.8700	0.1300	0.5998	2.8200	0.4002
FGA	0.8350	0.8790	0.2370	0.8600	0.9300	0.0700	0.7200	3.1500	0.2800
PENTA D	0.8500	0.9070	0.0950	0.8530	0.9498	0.0502	0.5600	3.4530	0.2174
PENTA E	0.8800	0.8940	0.1080	0.8340	0.9660	0.0340	0.1600	3.5200	0.8400
THO1	0.9125	0.7240	0.4967	0.7660	0.8250	0.1790	0.4110	1.6340	0.5890
TPOX	0.8707	0.8044	0.3689	0.8167	0.8696	0.1266	0.6243	2.1683	0.3757
vWA	0.8758	0.8420	0.3043	0.1664	0.8800	0.1200	0.5918	2.5689	0.4082

AD; Allele Diversity, HF; Haplotype Frequency, HD; Haplotype Diversity, PIC; Polymorphism Information Content, PD; Power of Discrimination, PM; Probability of Matching, PE; Power of Exclusion, PI; Paternity Index, RMNE; Random man Not Excluded.

gene diversity and evolutionary potentials of populations.

Homozygosity values were higher in Fulani subjects than in Yoruba subjects in 17 out of the evaluated 20 autosomal CODIS STRs, except D16S539, D2S1338, and D7S820 (Tables 4 and 6), indicating lower genetic diversity amongst the Fulani subjects compared with Yoruba subjects. This is equally due to differences in customs and norms between Fulanis and Yorubas, which permit Fulanis to marry their cousins, who are of closer genetic matches. The customs and norms of Yorubas do not permit them to marry their cousins or children of close relatives.

The highest percentage of expected heterozygosity (HE) was 87% (D16S539), while the lowest HE percentage was 51% (D5S818) in Fulani subjects (Table-4). For the Yoruba subjects, the highest percentage of HE was 88% (D6S1043), while the lowest HE percentage was 66% (D5S818) (Table-6). These observations equally indicate a lower

degree of genetic diversity amongst Fulani subjects compared with Yoruba subjects.

In comparison with previous studies, the highest HE percentages were 87.5% (D18S51), 88% (vWA), and 88% (D2S1338) for Hausas, Igbos, and Yorubas, respectively [4]. The lowest HE percentages were 69% (CSF1PO), 65.5% (D13S317), and 66% (D13S317) for Hausas, Igbos, and Yorubas, respectively [4].

The highest value of power of discrimination (PD) in Fulani subjects was 99% (D19S433), while the lowest PD value was 57% (PENTA E) (Tables-3). For Yoruba subjects, the highest PD value was 96.6% (PENTA E), while the lowest PD value was 81.3% (CSF1PO) (Table- 5). The PD values indicate that all evaluated autosomal CODIS STRs are highly informative in discriminating between unrelated individuals within each ethnic group.

In comparison with previous studies, the highest PD values were 97% (D18S51), 99% (vWA), and 97% (D18S51) for Hausas, Igbos, and Yorubas, respectively [4], while the lowest PD values were 32% (D2S1338), 31% (D2S1338), and 26% (D2S1338) for Hausas, Igbos, and Yorubas, respectively [4].

The highest values of polymorphism information content (PIC) were 86% (D2S1338 and D6S1043), while the lowest PIC values were 26% (D8S1179) and 39.2% (vWA) in Fulani subjects (Table-3). For Yoruba subjects, the highest PIC value was 89% (D6S1043), while the lowest PIC values were 16.6% (D8S1179 and vWA) (Table-5). The PIC values indicate that 18 autosomal CODIS STRs were highly polymorphic in Fulani and Yoruba subjects, except D8S1179 and vWA loci, which were less polymorphic compared to the other 18 loci in the two ethnic groups.

In comparison with previous studies, the highest PIC values were 97% (FGA), 97% (FGA), and 97% (FGA) for Hausas, Igbos, and Yorubas, respectively [4], while the lowest PIC values were 63.8% (D13S317), 60.3% (D13S317), and 60.1% (D13S317) for Hausas, Igbos, and Yorubas, respectively [4].

The FIS, FST, FIT and GST of 0.0000 and 0.5000 for all evaluated 20 autosomal CODIS STRs (Tables-4 and 6) implied little genetic differentiation and total lack of sub-structuring in the Fulani and Yoruba populations. Similarly, the X2 values were lesser than the X2- table at 1 degree of freedom, indicating that the population genetics data for all evaluated 20 autosomal CODIS STRs (Tables- 4 and 6) did not deviate from Hardy-Weinberg proportions.

5. Conclusion

This study provides further forensic genotyping data for the Fulani and Yoruba ethnic groups of Nigeria, which can be included in forensic CODIS databases and for forensic analyses by relevant civil and security agencies. The findings of this study are quite relevant as they provide the CODIS database for settler Fulanis, as against nomadic Fulanis who have been alleged to be foreigners from near and far countries, and who have been alleged to constantly clash with farmers across Nigeria partly because they are quite not familiar with the customs and agricultural practices of Nigerian farmers. We, therefore, recommend that open grazing be banned throughout Nigeria, while interested livestock farmers should be required to establish ranches for cattle and livestock breeding. This will make it easier to evaluate the forensic genotyping of 'settler' individuals and ethnic groups working in such ranches for civil and criminal investigations.

In addition, the results of the population genetic parameters in Fulani and Yoruba subjects in this study confirmed that the incorporation of PENTA D and PENTA E loci provided additional power of discrimination to previously tested 18 autosomal CODIS STRs (CSF1PO, D1S1656, D12S11, D12S391, D13S317, D16S539, D18S51, D19S433, D2S1338,

Table 6- *Homozygosity, Observed Heterozygosity, Expected Heterozygosity and Population Genetics Parameters of the 20 autosomal CODIS STRs in Yoruba Subjects.*

STR Locus	H	H_O	H_E	X^2	F	A_E	F_{IS}	F_{ST}	F_{IT}	G_{ST}
CSF1PO	0.2313	0.9130	0.7687	0.6423	-0.1877	4.3233	0.0000	0.5000	0.5000	0.5000
D1S1656	0.1400	0.7826	0.8600	2.9880	0.0900	1.1630	0.0000	0.5000	0.5000	0.5000
D12S391	0.1500	0.8700	0.8500	3.1700	-0.0300	6.4900	0.0000	0.5000	0.5000	0.5000
D13S317	0.3000	0.7800	0.7000	0.1500	-0.1200	3.2900	0.0000	0.5000	0.5000	0.5000
D16S539	0.2200	0.8300	0.7800	1.1500	0.0600	4.5500	0.0000	0.5000	0.5000	0.5000
D18S51	0.1500	0.8300	0.8500	0.9900	0.0200	6.6700	0.0000	0.5000	0.5000	0.5000
D19S433	0.1700	0.9600	0.8400	1.0800	-0.1500	6.0600	0.0000	0.5000	0.5000	0.5000
D2S1338	0.1621	0.8696	0.8376	2.3630	-0.0378	6.1690	0.0000	0.5000	0.5000	0.5000
D21S11	0.1750	0.7400	0.8250	0.4780	0.3900	5.7140	0.0000	0.5000	0.5000	0.5000
D3S1358	0.2200	0.6500	0.7800	0.3700	0.1700	4.6000	0.0000	0.5000	0.5000	0.5000
D5S818	0.3400	0.6500	0.6600	0.2200	0.0100	2.9500	0.0000	0.5000	0.5000	0.5000
D6S1043	0.1200	0.9100	0.8800	2.5700	-0.0300	8.3300	0.0000	0.5000	0.5000	0.5000
D7S820	0.2400	0.7800	0.7600	0.0800	-0.0300	5.2200	0.0000	0.5000	0.5000	0.5000
D8S1179	0.1773	0.8260	0.8227	0.6000	-0.0040	5.6400	0.0000	0.5000	0.5000	0.5000
FGA	0.1600	0.9100	0.8400	1.7100	-0.0700	6.2900	0.0000	0.5000	0.5000	0.5000
PENTA D	0.1448	0.7830	0.8552	0.4175	0.0844	6.9061	0.0000	0.5000	0.5000	0.5000
PENTA E	0.1420	0.8260	0.8580	0.5370	0.0348	7.0423	0.0000	0.5000	0.5000	0.5000
THO1	0.3060	0.6960	0.6940	0.9350	-0.0028	3.2680	0.0000	0.5000	0.5000	0.5000
TPOX	0.2306	0.8696	0.7694	0.5882	-0.1302	4.3365	0.0000	0.5000	0.5000	0.5000
vWA	0.1946	0.8261	0.8054	0.9200	-0.0260	5.1400	0.0000	0.5000	0.5000	0.5000

H; Homozygosity, H_O; Observed Heterozygosity, H_E; Expected Heterozygosity, X^2; Deviation from Hardy-Weinberg Equilibrium, F; Inbreeding, A_E; Effective Allele, F_{IS}; Variance of ~~an~~ allele frequencies within populations, F_{ST}; Variance of allele frequencies between populations, F_{IT}; Inbreeding coefficient of an individual relative to the total population, G_{ST}; coefficient of gene differentiation.

D3S1358, D5S818, D6S1043, D7S820, D8S1179, FGA, TH01, TPOX, and vWA). Furthermore, our findings implied that the tested 20 autosomal CODIS STR loci are highly polymorphic and informative, and that these loci can be used for the individualization of biological materials, paternity testing, estimation of strength of relationships between populations, forensic genotyping of individuals and ethnic groups, genetic mapping, and for forensic identification of individuals in civil and criminal investigations.

6. Limitations of the Study

This study is limited to forensic genotyping data of autosomal short tandem repeat (STR) loci and does not include genetic analyses of the Y-Chromosomes and Mitochondrial DNA. Y-Chromosome and Mitochondrial DNA analyses and other Genome-wide association studies shall be carried out in future research studies, in-order to provide further data on the genetic structures of Fulanis and Yorubas of Nigeria.

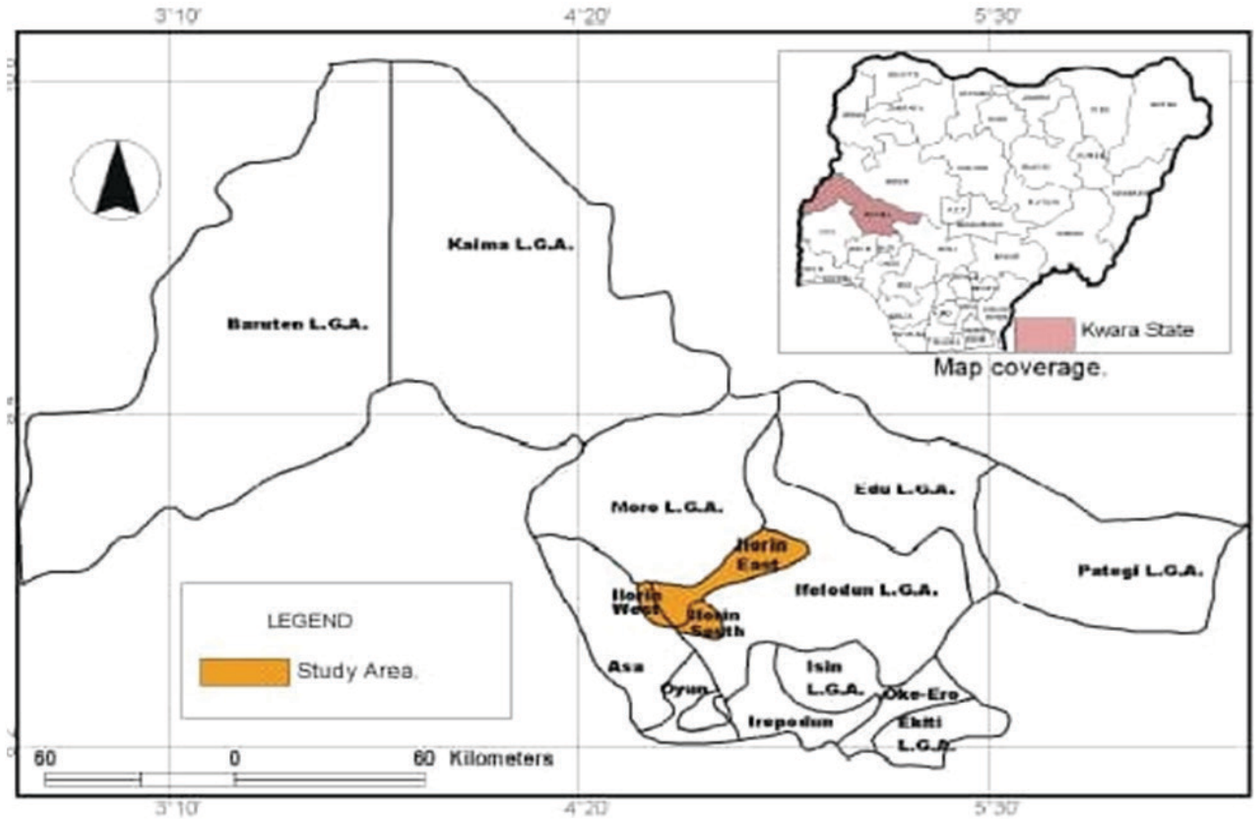

Figure 1- *Location map of iiorin, Kwara state, North Central of Nigeria [15]*

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Conflicts of interest

There are no conflicts of interest.

Authors' Contributions

AA conceptualized and designed the study methodology and was the main contributor in writing the manuscript. AA, NS, MM, DO, MO, MA, YA, MY, PO, TB, TO, SS, and RB performed the project procedures, analyzed, interpreted the data and contributed to writing of the manuscript. All authors read and approved the final manuscript.

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