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Original Research Article

Control Region Sequencing of Hyper Variable Segment Ii (HVSII) of Mitochondrial DNA and its Genetic Relationship in Asholio and Atakar Ethnic Group of Nigeria

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Abstract

Africa contains the most genetically divergent group of continental populations and several studies have reported that African populations show a high degree of population stratification. In this regard, it is important to investigate the potential for population genetic structure or stratification of some ethnic groups. Nigeria is one of the West Africa countries that took part in the human history since the dawn of modern man. The population of Nigeria is composed of Asholio and Atakar ethnic groups in the southern part of Kaduna state. DNA genetic marker such as HVII region of the mitochondrial DNA of a sample population of Asholio and Atakar ethnic group was used to get population genetic parameters. Buccal cells of 40 unrelated male individuals, 20 from each ethnic group was extracted using the protocol describe by Bioneer AccuPrep® Genomic DNA extraction kit. DNA samples extracted were analyzed and HVS-II sequences were amplified and purified. Sequencing for the light strand was done followed by sequence alignment, restriction fragment length polymorphism (RFLP) and single nucleotide polymorphism (SNP) analysis. Nucleotide positions 73-340 for HVS-II were compared to the revised Cambridge Reference Sequence (rCRS) and 40 haplotypes were observed with haplotypic diversity of 0.9431 for Asholio and 0.9560 for Atakar. A total of 80 polymorphic sites characterized the haplotypes. All of the haplotypes found have been described in other West African populations of the world. The haplotypes frequencies were used to calculate $F_{ST.}$ Mitochondrial DNA haplogroups present in Africans were represented in the two ethnic groups. The genetic diversity of Asholio was 0.9143 and Atakar, 0.9145. The calculation of F_{ST} (0.003) for the two ethnic groups suggests no difference between them. In order to understand the expansion of the haplotypes of mitochondrial DNA in West Africa, the studied population was compared with neighbor populations. Some African ethnic groups were grouped and the Asholio and Atakar ethnic groups were next to the west African populations. Therefore we can suggest that the populations geographically related and those with the same language (West Africa) are genetically similar, corroborating the fact that these mtDNA marker can be used in the inquiry of the recent history of a population.

Keywords: mtDNA, HVS-II, Control region, Asholio, Atakar and Variation.

INTRODUCTION

Models of Homo sapiens origin and dispersion have been proposed and combated. One of the dispersion models of Man is called multiregional. In this model Homo erectus left Africa scattering to Old World regions. These regional populations then slowly evolved into the modern man [1]. Africa is inhabited by populations that show high levels of genetic diversity compared to most other continental populations today and it is thought to be the ancestral home of modern humans. African populations have the largest number of population specific autosomal, X-chromosomal and mitochondrial DNA haplotypes with non-African populations having only a subset of the genetic diversity present in Africa [2]. Estimates of FST (the classic measure of population subdivision) from mitochondrial DNA are much higher in Africa than other populations, as summarized by Tishkoff *et al*., [3]. In addition, analyses from studies based on autosomal SNPs, STRPs or Alu elements show higher FST values for African populations [2-4]. Recent

studies of world populations based on large genomic data also reported significant population structure among the African groups [5, 6]. However, given the cultural and linguistic diversity of African populations (with over 2000 distinct ethnic groups and languages), these studies have typically included only a handful of African populations indicating that most African populations have not been studied. As previously noted, most existing genetic data on African populations have come from a few countries that are relatively economically developed and/or with key research or medical centers [1]. Availability of more genetic data from sub Saharan Africa will clearly be useful in our understanding of population structure, demographic history and the efforts to map disease-causing genes. Linguistic inclinations, anthropometric records and geographical background of human populations are known to provide a historical basis for human evolution and variation, as well as the reasons underlying such changes [14]. The diversity exhibited by *Homo sapiens* arose during their processes of dispersal into their present regions, thus, the subsets of variation tend to be associated with particular geographic areas and populations [15]. Human variation has been measured using simple visual characters like size, form and skin colour leading to the conclusion that sharing one or more features is an indication of common descent [16], but advances in the science of genetics have revealed greater distinctions [17]. Thus, anthropological genetics, a comparatively new discipline makes attempt at answering questions that concern human origin and variation using methods and theories of genetics [18].

From an evolutionary view point, such relationships spread across the world's genetic map have led to efforts on illuminating the origin and dispersal of anatomically modern man across the world [19] Postulations based on developed models have shown different origins for man, out of which the Recent African origin (RAO) also known as the "out of Africa" model asserts a common descent for all populations from an anatomically modern Homo sapiens ancestor [20, 17]. This makes the African continent, the ancestral home of all humans today. Reconstructing the history of the West African population is considered complex [15], this is due to short and long migration events within the region [14]. One of the earliest indications of West Atlantic occupation by modern humans goes back to about 40 KYA [12], but subsequent changes in the climatic conditions resulted in the significant movement of these occupants [21].

The dearth of archeological evidence (attributable to differences in sea level which may have buried such artifacts) for reconstructing the past has also contributed to this situation, as such language groups were used to genetically classify populations into groups of common descent [22]. This is evident in the fact that cultures may spread without attendant spread of genes, but languages are not easily acquired in later life than other cultural transformations [23]. The recent development of genetic tools has proven that linguistic groups within Africa share common gene pools [24] which have become useful in probing phylogenies. The genetic variation in modern man occurred during the events of early migration into new territories, with concomitant localization of these variations to particular regions [25, 26]. Studies are ongoing to understand the past events involving population expansion, contraction, genetic drift and substructure. Some of these studies employ genetic methods to probe the human genome to investigate and analyse single nucleotide polymorphisms (SNPs) in conjunction with restriction fragment length polymorphism (RFLP) techniques obtained from the hypervariable region of the d-loop [13] [27]. These studies have demonstrated that human mtDNA is geographically structured and may be classified into groups of related haplotypes [13, 28].

SUBJECTS AND METHODS

Subject Sampling

mtDNA yielding specimen (buccal cells) for this study was obtained from the Asholio and Atakar found within the Kaura Local Government Areas of Kaduna State (Figure-1). Collection of specimen was done in randomly selected Secondary Schools from villages where each of the ethnic groups predominates. The villages sampled are: Manchok, where the Asholio subjects will be obtained while Mayit village where the Atakad subjects will be obtained. Twenty unrelated males (n=20 from each ethnic groups) were enrolled for this study. Certification of ethnicities was based on information given by the participants on both parental sides, whose ancestors were known to belong exclusively to a specific ethnic group for at least the last two generations. This information was corroborated by community elders from each of the villages where the participants was drawn.

METHODS

Buccal cells collection was done according to the protocol employed by [29]. For each participant, sterile cotton tipped applicators (swabs) was used to scrape off the cells of the oral mucosa, for 30 seconds and in-between their gum and cheek for 30 seconds. The ends of the swabs was cut, air-dried at room temperature and placed in 15 ml plastic tubes. After the swab, each subject were asked to rinse his mouth with the provided 10 ml of clean water. The water was swished vigorously in the mouth of the subjects for one minute and then poured into 50 ml collection tubes. The tubes was transferred to portable cooling chambers (flasks) containing ice so that the samples was preserved at 4 °C prior to transportation and subsequent extraction.

DNA Extraction, purification and amplification of genomic DNA was carried out at the DNA Laboratory Kaduna. The equipment and laboratory wares (tips and tubes) were autoclaved to prevent contamination.

The amplification of the hypervariable region (HVI) was performed according to the protocol described by Budowle et al. [30] with the following primer pairs:

HVII
C1 (L048): - (5'-CTC ACG GGA GCT CTC CAT GC-3 ') and
D1 (H408): - (5'-CTG TTA AAA GTG CAT ACC GCC A-3 '). Which amplified the region between nucleotides 73 and 340. i.e 260 bp.

Agarose gel electrophoresis was carried out to get the DNA bands of interest. After verifying the presence of DNA through the gel, the amplicons was purified to remove excess dNTPs and unbound primers using ExoSAP DNAse. The PCR amplicons was digested for 10-18 hours at 37 °C with the appropriate restriction enzymes (*Mbo1*and *HpaI*).

Molecular Genetic Analysis

All intra population diversity analyses was done using DNAsp 5.10 [31], XLSTAT 4.06 v13 and Arlequin 3.513 package [32, 24]. The distance matrix was used to measure genetic distances of the study populations. The amount of variation within each study population was determined using the Nei‟s gene/haplotype diversity. Tajima‟s D [33] and Fu‟s F was used to test for any departure from neutrality. mtDNA genealogies were constructed using median-joining network approaches (available in the Network 4.6 program, from the Fluxus Engineering Web site) [34]. AMOVA was used to test the significance of their genetic diversity. Phylogenetic analysis was performed utilising the nucleotide sequence data generated in the study.

RESULT AND DISCUSSION

The extracted DNA were purified and quantified using NanoVue spectrophotometer (GE healthcare) as shown in Table-1. The absorbance ratio (A260/A280) of the DNA was calculated showing high DNA yield from the sample amplified. The HVS-II segments of the control regions of the 40 individuals extracted were amplified. The process utilized primer sets that produced 240 bp fragments for HVS II Figure-1.

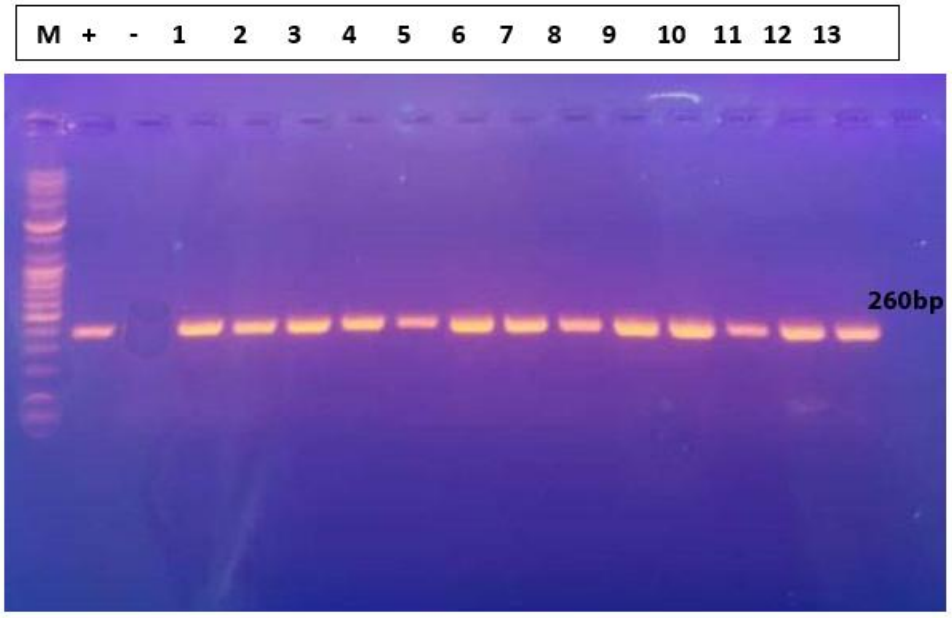

Fig-1: PCR amplicons of HVS II visualized on a 2% agarose gel. First lane M: Molecular DNA genetic marker, Lane +: POSITIVE control, Lane - : NEGATIVE control, Lane 1-13 are the PCR products

Table-1: Yields and quality of DNA from buccal swabs

	HVS II
Average DNA yield per swab and range (in parenthesis) in µg	3.82 (1.35-7.1)
Mean OD260/OD280 ratio ± SD and range (in parenthesis)	1.85±0.17* (1.27-1.82)

*P< 0.001

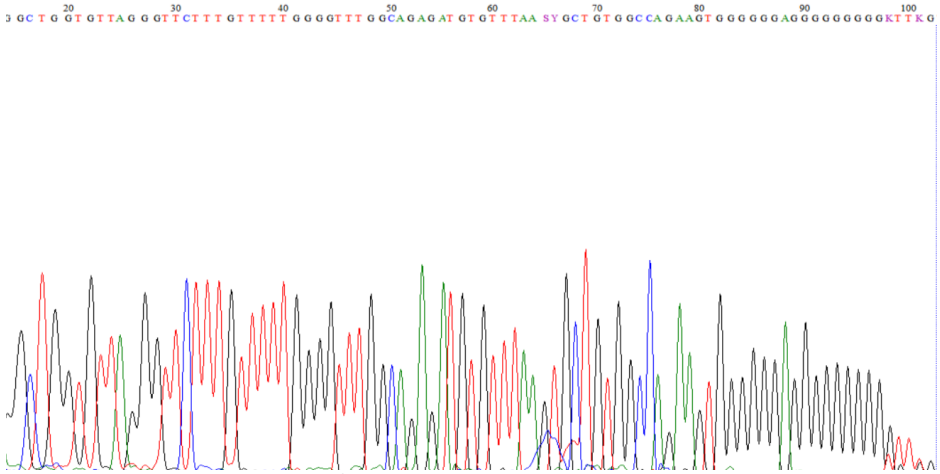

Fig-2: Representative mtDNA sequence chromatogram for HVS II. Nucleotide peaks intensities and reading positions are colour coded to aid identification of sequenced nucleotide bases

Table-2: Mutation sites observed in mtDNA control region sequences with respect to rCRS

SAMPLE	HAPLOGROUP	HVII (nt 73 – nt 340)	ASHOLIO	ATAKAR
V1	L	73 150 228 263 315	1	
V2	L	73 150 195 263 315.1		1
V3	L0	204 263 309.1 315	1	
V4	L0a	73 146 152 195 309.1 315.1	1	
V5	L0a	73 151 152 263 315.1	1	
V6	L1	73 152 182 186A 189C 195 247 263 297 316		1
V7	L1	73 150 263 315.1	1	
V8	L1	73 143 150 263 309.1 315.1		1
V9	L1	263 309.1 315.1		1
V10	L1	118 146 152 263 298 315.1	1	
V11	L1	73 150 195 263 295 315.1 316	1	
V12	L1b	73 146 152 263 315.1		1
V13	L1b	263 309.1 315.1		1
V14	L1b1	73 152 263 295 309.1 315.1	1	
V15	L1b1	73 263 309.1 309.2 315.1		1
V16	L1c	73 152 263 315.1		1
V17	L2a	73 152 263 315.1	1	
V18	L2a1	73 143 146 152 182 189G 195 263 309.1 315.1	1	
V19	L2a	73 263 315.1	1	
V20	L2a1	73 150 263 315.1		1
V21	L3a	73 143 150 152 153 189 263 309.1 315.1		1
V22	L3b	73 143 146 152 182 189 195 263 315.1	1	
V23	L3b	73 152 263 309.1 309.2 31.1		1
V24	L3c	73 152 182T 185T 195 247 263 309.1 315.1	1	
V25	L3e	73 263 315.1		1

Table-3: Haplotypes and segregating sites compared to the Cambridge reference sequence

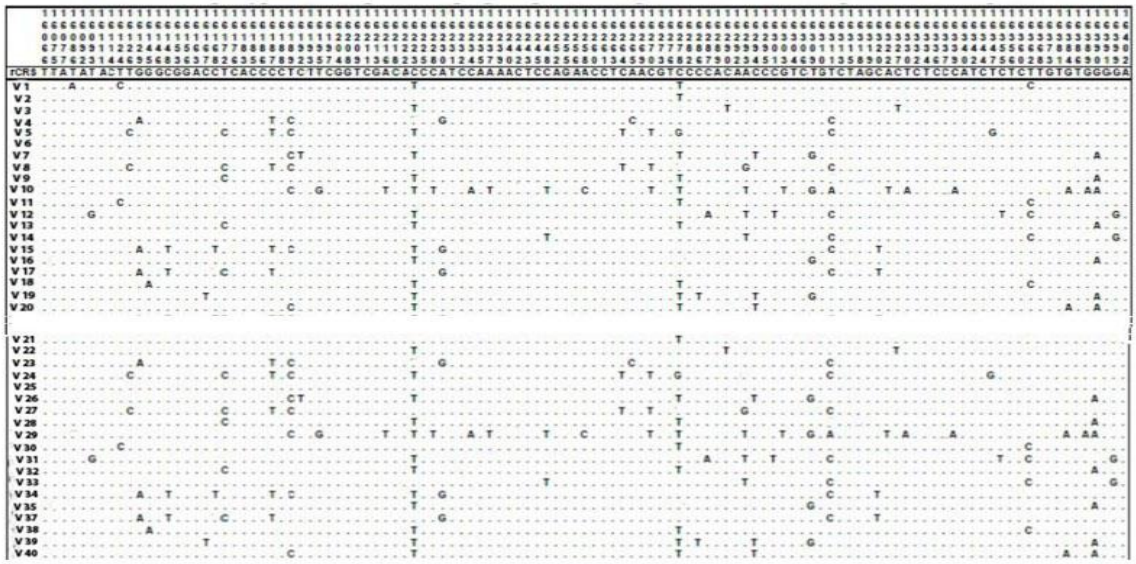

Table-4: HVS-II Sequence Polymorphisms defining mtDNA Haplotypes

HAPLOGROUP	HVS-II POLYMORPHISM MOTIF	ASHOLIO	ATAKAR	*HpaI*	*MboI*
L0a1	73T 150C 228A 263G 315T	1		+	-
L0a1	73A G150C 195G 263T 312C		3	+	-
L0a1	204C 263C 309T 315G	1		+	-
L0a1	73T 146A 152G 195C 309A 345C	2		+	-
L0a1	73C 151T 152T 263G 295	1		+	-
L1a	63T 152G 182T 186A 189C 195T 247A 263C 297G 316G		1	+	-
L1a	73T 150T 263G 315A	1		+	-
L1a	47G 143A 150A 263G 309G 315C		3	+	-
L1a	263T 309C 315G		1	+	-
L1a	C118G 146G 152T 263G 298T 315C	2		+	-
L1a	73C 150G 195G 263G 295T 315T 316C	1		+	-
L1b1	73A 146C 152T T263G 315A		1	+	-
L1b1	263C 309A 315A		4	+	-
L1c	73A 152C 263A 295C 309C 315C	3		+	-
L2a	55T 263A 309A 329A 335G		2	+	-
L2c	73A 152C 263C 315C		1	+	-
L2b	45T 152G 263G 315C	4		+	-
L2d	73C 143G 146C 152G A182G 189G 195C 263C 309T 315T	1			+
L3b	73A 263C 315T	2		-	+
L3d	T73C 150C 263A 315A		5	-	+

Transitions are indicated by the nucleotide position followed by a nucleotide and transversions are indicated by a nucleotide prefix and suffix. A plus (+) indicates a site gain, and a minus (-) indicates a loss of restriction site for the restriction enzymes.

Table-5: HVS-II diversity indices for the Asholio and Atakar ethnic groups

Population	n	Segregating sites (S)	Haplotypes (h)	Haplotype diversity (HD)	Sd (HD)	Ave pairwise differences (K)	Nucleotide diversity(π)	Sd(π)
Asholio	20	62	13	0.9431	0.020	9.45	0.024	0.006
Atakar	20	46	14	0.9640	0.015	7.56	0.030	0.010

Table-6: AMOVA table showing analysis of populations grouped according to major language groups

Source of variation	D.F	Sum of squares	Components of variation	Percentage of variation
Among groups	2	52.432	0.20746*	4.56
Among populations within groups	8	69.654	0.13513	2.41
Within populations	221	1634.51	4.96502	92.53
Total	231	1923.43	5.30761	

*P < 0.001 Fixation indices: F_{ST} (the variance among subpopulations relative to the total variance): 0.0423, F_{SC} (the variance among subpopulations within groups): 0.0343, F_{CT} (the variance among groups relative to the total variance): 0.0216

AMOVA describes the partitioning of genetic variation (the average distance between randomly chosen haplotypes or alleles) into within and among population components. The results of the analysis are given in Table-6. The major language group model was adopted and the analysis reveals little variation among the populations (4.56 %) and among-populations within groups (2.41 %). The greatest amount of the variation (92.5 %) was found within the populations. The low fixation indices (FSC: 0.0343, FST: 0.0423 and FCT: 0.0216) further demonstrates this little variation. The fixation indices and the variance values here are statistically significant (P<0.001) based on the probability of observing the same or lower measure for each statistics.

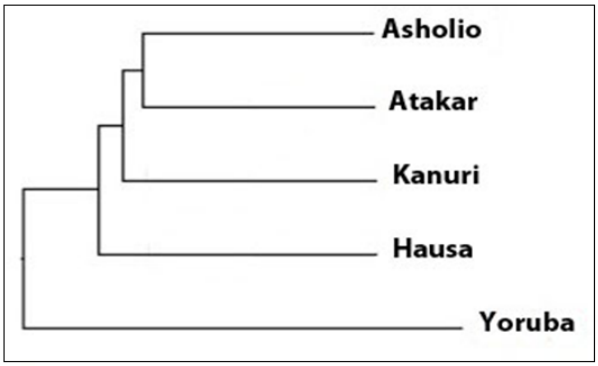

Fig-3: Neighbour-Joining (NJ) tree showing the relationship between the study population and other ethnic groups

The Neighbour-Joining (NJ) tree (Figure-3) was constructed for the study populations based on allele frequencies converted into Kimura-2p distances. This method uses the stepwise addition and star decomposition model rather than the cluster analysis to produce the nodes on a tree as against the taxa. The extracted tree is un-rooted and it consists of five major branches indicative of existing genetic variation. The clusters conform to the geographical pattern of an existing genetic differentiation. The hierarchical topology of the tree in Figure-3 suggests the absence of an evolutionary root and branch lengths are less informative. The Asholio and Atakar forming a monophyletic clade, appear more closely related. They both exhibit a shorter genetic distance with other west African ethnic group like Kanuri than the more distant Yoruba node.

Table-5 compares this study's diversity values with those from [36, 37]. Their high haplotype diversities closely matched those from other West African populations. The study populations had lower haplotype diversities than other west African populations, with the Atakar having the least haplotype diversity. The Tajima"s D statistic showed negative values for all the populations. Compared to the West African groups, the Bajju had higher number of sequence segregating sites. Generally, the populations from the present study and the comparative populations appear to share similar characteristic. During man migration to new geographic regions, genetic mutations were occurring and by selection developing into new haplogroups. Each haplogroup behaves as an independent evolutionary lineage. Climatic and environmental conditions of the different regions of the world contributed to the spread or establishment, by drift or gene flow of these haplogroups. The older the haplogroup, the greater its geographical distribution. Part of the haplogroups has a geographical distribution restricted to some parts of the globe, allowing the construction of their geographical or ethnic origin. Men, when they left their genetic markers, migrated to different continental regions about 60,000 years ago. These marks are still possible to be seen today by the gradient of allelic frequency distribution [38]. From phylogeographic studies, and the mapping of the frequencies of genetic markers, a picture could be created that suggests where and when the ancestors of modern man have moved. These migratory flows a small group of Africans left descendants to occupy the furthest points of land [38]. This study investigated the major haplogroup types, the presence of a genetic structure, statistical relationship between the ethnic groups, language family, geography and genetics and also gene flow among the Asholio and Atakar ethnic groups. In order to understand the expansion of populations in West Africa, mitochondrial DNA haplotypes from Asholio and Atakar ethnic groups were analyzed. Macrohaplogroup L, of African populations was found, however, the genetic distances measured by FST between the two ethnic groups was 0.003, that is, they are similar. These results suggest that there is no great difference between the two ethnic groups in southern Kaduna by mitochondrial DNA analysis.

CONCLUSION

The following deductions can be made about the maternal line genetic composition of the population groups included in the study. First, various levels of admixture from West African groups are present in the Asholio and Atakar groups. Secondly, the Asholio group is similar to the Atakar group and might represent remnants of another extinct hunter-gatherer group that were displaced by the Asholio-speaking expansions and became associated with the Atakar. Thirdly, there is a distance-based genetic relationship between the Asholio and Atakar groups. Fourth, the haplogroup distribution between the Asholio and Atakar groups is similar, this clusters with, and is similar to other West African ethnic groups.

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