Journal of Scientific Research and Development (2023) Vol. 22 (1) 1-13

A bi-annual journal published by the Faculty of Science, University of Lagos, Nigeria
http://jsrd.unilag.edu.ng/index.php/jsrd

Sequence polymorphism and haplogroup distribution of mitochondrial DNA control regions HVS1 and HVS2 in Lagos State, Nigeria, population

*Iroanya Onyekachi Ogbonnaya, Fakorede Samson Taiwo, Edosa Osamudiamen Jennifer and Azeez Hadiat Ajoke

Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Akoka Yaba, Lagos Nigeria

*Corresponding Author: E-mail: oiroanya@unilag.edu.ng

(Received 21 August 2022/Revised 02 March 2023/Accepted 12 April 2023)

Abstract

Mitochondrial DNA (mtDNA) consists of circular DNA molecules with about 16.5kbp, with smaller fragments of 1200bp known as the control region. Knowledge of mtDNA variation within populations has been employed in forensic and molecular anthropology studies. This study investigated the polymorphic nature of the two hypervariable segments of the mtDNA i.e. HVS1 and HVS2, and determined the haplogroup distribution among individuals resident in Lagos, Nigeria. Peripheral blood was obtained from sixty maternally unrelated individuals. DNA was extracted and amplified using specific primers. DNA amplicons were sequenced, sequenced data were aligned and compared to the revised Cambridge Reference Sequence (rCRS) GenBank Accession number: NC_012920.1) using BioEdit 7.2 software. Results showed 61 and 52 polymorphic nucleotide positions for HVS1 and HVS2, respectively. While three indels mutation were recorded for HVS1, seven were recorded for HVS2. Transition mutations (49.2%) predominate nucleotide change was observed. Genetic diversity values for HVS1 and HVS2 were estimated to be 84.21 and 90.4%, respectively while, random match probability was 0.17% for HVS1 and 0.89% for HVS2. The study showed mixed haplogroups specific to the African (L1-L3) and the Eurasians (U and H) lineages. New polymorphic sites obtained from the study could be used for human identification purposes.

Keywords: hypervariable region, indels, mitochondrial DNA, mutation, polymorphism

---

Introduction

Mitochondrion (*pl: mitochondria*) is a double membranous organelle found in the cytoplasm of eukaryotic cells. It helps to generate energy for several cellular activities, elucidates various signaling responses, and serves as transducers and effectors in multiple processes (Tait and Green, 2012; Zapico and Ubelaker, 2013; Hameed *et al.*, 2016). The human mitochondrial DNA (mtDNA) consists of a circular genome of 16,569 bp in size (Anderson *et al.*, 1981; Shokolenko and Alexeyev, 2015; Habbane *et al.*, 2021). It is made up of 37 tightly packed genes encoding 2 rRNAs, 13 mRNAs, and 22 tRNAs (Ingman *et al.*, 2000; Helgason *et al.*, 2003). The genome comprises two regions known as the coding region and the control region. While the coding region is essential for energy generation, the control region, a smaller fragment, is a highly polymorphic region that contains three hypervariable segments (HVS) called HVS1, HVS2, and HVS3 with considerable level of variations; hence their use in molecular evolution and population studies (Lutz *et al.*, 1998; Kraytsberg *et al.*, 2004). These hypervariable regions have played significant roles in anthropological and evolutionary studies as well as in forensics (Achilli *et al.*, 2004; Hedman *et al.*, 2007; Gubina *et al.*, 2018).

MtDNA is inherited along with the mitochondria exclusively from the mother (i.e. maternal inheritance) and constitutes a source of maternal differentiation between populations (Wan *et al.*, 2004). It exhibits high population-specific polymorphism together with other genetic traits such as lack of recombination, high stability as a result of complex protein coat that protects it from degradation by restriction endonucleases (Chen *et al.*, 2008; Chemale *et al.*, 2013). Compared to nuclear

DNA, the mtDNA has a higher copy number per cell (running into thousands) and a very high mutation rate due to high infidelity in mtDNA repair mechanisms making it a good tool for human identification (Bourdon *et al*., 2014; Jankova-Ajanovska *et al*., 2014). The mtDNA has a higher mutation rate and it is more polymorphic than nuclear DNA, making the mtDNA an interesting molecular marker in evolutionary studies (Allio *et al*., 2017). The higher mutation rate usually results from insertions, deletions or transitions (Di Benedetto *et al*., 2000; Zhang *et al*., 2005), and is considered to be as high as five-fold of what happens with genetic material in the nucleus (Pfeiffer *et al*., 2005). Studying the genetic variation in mtDNA has been the main focus of population genetic and phylogenetic researchers. These variations or polymorphisms are higher in the region known as the displacement loop or the D-loop of the mtDNA (Andrews *et al*., 1999). As a result, the analysis of nucleotide variations in the D-loop has been of utmost interest in human identification, investigation of migration patterns, and origin of species (Horai *et al*., 1995; Ngili *et al*., 2012).

Sequence analysis of the hypervariable regions of the mtDNA have been reported in different populations for forensic and evolutionary applications (Lutz *et al*., 1999; Lu *et al*., 2012; Kareem *et al*., 2016). Results have been reported on paternally inherited Y-chromosome marker in Nigeria (Martinez *et al*., 2017; Fakorede *et al*., 2019). However, there is paucity of information on the sequencing of HVS markers in Nigerian populations. This study sequenced HVS1 and HVS2 fragments of the non-coding region of the mtDNA and determined the nucleotide variations and their suitability in forensics and maternal lineage investigations in Nigeria.

Materials and Methods

Sampling

Prior to sample collection, what the research entailed was explained to all the participants, and they willingly consented to participate in the study by signing an informed consent document. Blood samples were collected from a total of 60 maternally unrelated individuals resident in Lagos, Southwestern Nigeria. Specifically, individuals were sampled in and around the University of Lagos, Akoka, Yaba. All samples were code-labelled and transported to the Central Research Laboratory of the University of Lagos for DNA extraction and downstream analysis. Ethical approval for the study was obtained from the Research and Ethics Committee of the College of Medicine of the University of Lagos (Approval Number: CMUL/HREC/11/18/470) before sampling.

DNA Extraction:

Total DNA was extracted from the collected samples using the *Quick*-DNA™ Miniprep Plus DNA extraction Kit (Zymo Research, California, USA) strictly following the manufacturer's instruction manual. Extracted DNA samples were then stored at -20°C pending further analysis.

PCR Amplification of mtDNA control region

Amplification of the hypervariable regions of the mtDNA was performed using primer pair L16024/H16365 for HVS1 and L72/H340 for HVS2 in a 25 µL reaction volume consisting of 12.5 µL One*Taq* Master mix, 8.5 µL ddH_2O, 1 µL each of forward and reverse primers (Table 1), and 2 µL DNA samples. Polymerase chain reaction (PCR) amplification was carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). PCR parameters were as follows: initial denaturation at 94ºC for 1 minute followed by 35 cycles of denaturation at 94ºC for 30 seconds, annealing at 55ºC for 1 minute, extension at 72ºC for 3 minutes and a final extension at 72ºC for 10 minutes. PCR products were run on 2% agarose gel electrophoresis to ascertain successful amplification reaction prior to sequencing.

Table 1: Primers information for HVS1 and HVS2

Primer name	D-loop region	Primer sequences (5′-3′)	Primer lengths	Amplicon size (bp)
HVS1	L16024–H16365	F: CACCATTAGCACCCAAAGCT	20	411
		R: TGATTTCACGGAGGATGGTG	20	
HVS2	L72–H340	F: GGTCTATCACCCTATTAACCAC	22	397
		R: CTGTTAAAAGTGCATACCGCCA	22	

bp = base pairs; F = Forward primer; R = Reverse primer; L = Light strand; H = Heavy strand

Purification and Sequencing of PCR Products

PCR products were purified from residual primers using EXOSAP protocol. Sequencing was performed using Applied Biosystem Big-Dye™ terminator v3.1 cycle sequencing kit. The labelled products were then cleaned with the ZR-96 DNA sequencing clean-up kit and injected on the ABI 3500xL Genetic Analyzer (Applied Biosystems, Foster City, California) using POP-7 with a 50 cm array for sequencing. This procedure was done for both forward and reverse primers for sequence validation. Sequence validation was performed using FinchTV software v.1.4.0 (https://digitalworldbiology.com/FinchTV).
Sequencing was done at InqabaBiotec Laboratory, South Africa.

Analysis of mtDNA Sequences

Sequenced data were aligned and compared to the revised Cambridge Reference Sequence (rCRS) (Anderson *et al.*, 1981, GenBank Accession number: NC_012920.1) using BioEdit software. Sequence variants and haplogroups were also determined using the mitochondrial genome database, *Mitomap* (www.mitomap.org).

Genetic diversity (GD) for the sequenced fragments was calculated using the formula:
GD = $n(1-\sum x_i^2)/(n-1)$ as per Nei and Tajima (1981), where n = sample size, and x_i = frequency of the *i*-th mtDNA type.

Results

Different types of sequence variations were observed in the hypervariable regions in the study as shown in Table 2 for the HVS1 and Table 3 for HVS2. These included insertion and deletion as well as transition and transversion. The polymorphism in HVS1 region spanned positions 16034-16390 nucleotide sequences. There was insertion of a cytosine residue (C) at position 16034 for NG2, deletion of adenine (A) at position 16077 in NG1, and an extra thymine (T) residue at position 16199 for NG2. Results from the present study further showed different forms of mutation such as transition and transversion as well as insertion and deletion. Significant are the insertions of C/TCC, TTC and TCCC at position 310, and insertion of an extra cytosine residue at 315th position among the sampled individuals.

Table 2: Observed polymorphic positions for HVS1

Positions

	16034	16035	16039	16040	16041	16048	16071	16072	16077	16078	16080	16081	16090	16096	16097	16109	16114	16124	16126	16129	16148	16168	16172	16183	16186	16187	16188	16189	16193	16194
Anderson*	G	G	G	C	A	G	C	C	A	A	A	A	T	G	T	A	C	T	T	G	C	C	T	A	C	C	C	T	C	A
NG1	.	.	.	.	.	.	A	.	d	.	C	.	G	.	A	G	.	.	.	A	.	.	.	.	.	.	.	.	.	.
NG2	GC	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	C
NG3	.	.	.	.	.	.	A	A	.	C	.	T	.	C	.	.	.	.	C	.	.	.	C	.	T	T	.	C	.	.
NG4	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
NG5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	.	.	.	.	.	.	.	.	.	.
NG6	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
NG7	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	C	.	.
NG8	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	T	T	C	.	.	T	G	C	.	.
NG9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.
NG10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	C	.	.

	16197	16199	16205	16209	16213	16214	16218	16223	16225	16228	16229	16230	16232	16233	16234	16235	16260	16268	16276	16281	16292	16293	16294	16295	16311	16318	16319	16320	16360	16390
Anderson*	C	T	C	A	C	C	C	C	C	C	T	A	C	A	C	A	C	C	C	C	C	A	C	C	T	A	G	C	C	G
NG1	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	C	.	.	.	.	.
NG2	G	TT	A	A	.	A	A	T	A	T	A	.	A	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NG3	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	T	.	.	.	G	.	.	C	.	.	.	.	.
NG4	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	G	A	.	.	A
NG5	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	A
NG6	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
NG7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NG8	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	T	.	.	.	G	.	.	C	.	.	T	.	.
NG9	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	C	.	.	A	.	.	.	T	.	C	.	.	.	.	.
NG10	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	T	A	.	T	.	T	.	C	.	.	.	T	.

Anderson*: rCRS GenBank Accession no. NC_012920.1; A: Adenine; C: Cytosine; G: Guanine; T: Thymine; d: deletion.

Table 3: Observed polymorphic positions for HVS2

Positions Anderson* Sample ID	63	72	73	74	77	83	86	93	95	103	104	124	131	134	141	143	146	150	152	172	182	185	186	187	189	195	198	247	263	275	310	315	324	325	330	338	348	366	411	414	418
⇩	T	T	A	T	A	T	C	A	A	G	C	G	T	T	C	G	T	C	T	T	C	G	C	G	A	T	C	G	A	G	T	C	C	C	C	C	C	G	C	T	C
NG1	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	G	.	C/TTC	.	G	.	.	.	.	A	A	G	.
NG2	C	.	G	d	d	C	.	.	.	.	.	.	A	G	.	A	G	.	C	.	.	.	.	A	.	C	.	.	G	.	.	CC	.	.	.	.	.	.	.	G	.
NG3	.	.	.	.	.	.	.	.	.	.	A	A	G	.	.	.	G	.	C	G	T	T	.	.	.	C	.	A	G	.	.	CC	.	.	G	.	.	.	G	.	.
NG4	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	T	C	.	T	.	.	.	.	C	T	.	G	.	.	CC	.	T	.	.	.	.	.	.	.
NG5	.	A	G	.	.	C	.	.	.	.	.	.	.	.	.	.	C	T	C	.	T	.	.	.	.	C	T	.	G	.	.	CC	.	.	.	.	.	.	.	.	.
NG6	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	TTC	.	G	.	.	A	.	A	G	C	.
NG7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	G	.	TCCC	.	.	.	.	.	.	.	G	G	.
NG8	.	.	.	G	.	C	.	G	C	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	G	.	.	A	G	.	.	CC	.	.	.	.	.	.	.	A	.
NG9	.	.	G	.	.	.	.	.	.	A	.	A	.	.	T	.	.	T	C	.	T	.	A	.	C	C	T	A	.	A	.	CC	.	.	.	.	.	.	G	A	.
NG10	.	.	G	.	d	.	A	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.	C	.	.	G	A	.	CC	.	.	A	.	d	.	G	A	d

Anderson*: rCRS GenBank Accession no. NC_012920.1; A: Adenine; C: Cytosine; G: Guanine; T: Thymine; d: deletion

Results of the types of mutations observed at the different positions of the regions were presented in Tables 4 and 5. The result showed the presence or novelty of nucleotide change in the Mitomap.

Table 4: Types of mutations in the polymorphic sites of HVS1

Position	Type of mutation	Nucleotide change	Presence in Mitomap
16034	Insertion	G→GC	New*
16035	Transversion	G→T	Present
16039	Transversion	G→C	Present
16040	Transversion	C→A	New*
16041	Transversion	A→T	New*
16048	Transversion	G→C	New*
16071	Transversion	C→A	Present
16072	Transversion	C→A	Present
16077	Deletion	A→d	New*
16078	Transversion	A→C	Present
16080	Transversion	A→C	Present
16081	Transversion	A→T	Present
16090	Transversion	T→G	Present
16096	Transversion	G→C	Present
16097	Transversion	T→A	New*
16109	Transition	A→G	Present
16114	Transversion	C→A	Present
16124	Transition	T→C	Present
16126	Transition	T→C	Present
16129	Transition	G→A	Present
16148	Transition	C→T	Present
16168	Transition	C→T	Present
16172	Transition	T→C	Present
16183	Transversion	A→C	Present
16186	Transition	C→T	Present
16187	Transition	C→T	Present
16188	Transversion	C→G	Present
16189	Transition	T→C	Present
16193	Transition	C→T	Present
16194	Transversion	A→C	Present
16197	Transversion	C→G	Present
16199	Insertion	T→TT	New*
16205	Transversion	C→A	Present
16209	Transition	T→C	Present
16209	Transversion	T→A	Present
16213	Transition	G→A	Present
16214	Transversion	C→A	Present
16218	Transversion	C→A	Present
16223	Transition	C→T	Present

16225	Transversion	C→A	Present
16228	Transition	C→T	Present
16229	Transversion	T→A	New*
16230	Transition	A→G	Present
16232	Transversion	C→A	Present
16233	Transversion	A→C	Present
16264	Transition	C→T	Present
16265	Transversion	A→C	Present
16270	Transition	C→T	Present
16278	Transition	C→T	Present
16286	Transversion	C→A	Present
16291	Transition	C→T	Present
16292	Transition	C→T	Present
16293	Transition	A→G	Present
16294	Transition	C→T	Present
16295	Transition	C→T	Present
16311	Transition	T→C	Present
16318	Transition	A→G	Present
16319	Transition	G→A	Present
16320	Transition	C→T	Present
16360	Transition	C→T	Present
16390	Transition	G→A	Present

Note: Present indicates that observed polymorphism in a position was present in the Mitomap; New* indicates newly observed polymorphic positions based on this study.

Position 414 of the HVS2 recorded three forms of nucleotide change with thymine being replaced by adenine, cytosine and guanine residues among the different individuals of the studied population making it the polymorphic site. Detection of new polymorphic positions in both HVS1and HVS2 is a promising indicator of its use for identification purposes

.

Table 5: Types of mutations in the polymorphic sites of HVS2

Position	Type of mutation	Nucleotide change	Presence in Mitomap
63	Transition	T→C	Present
72	Transversion	T→A	Present
73	Transition	A→G	Present
74	Transversion	T→G	Present
74	Deletion	T→d	New*
77	Deletion	A→d	New*
83	Transition	T→C	Present
86	Transversion	C→A	Present
93	Transition	A→G	Present
95	Transversion	A→C	Present
103	Transition	G→A	Present
104	Transversion	C→A	New*

124	Transition	G→A	Present
131	Transversion	T→A	New*
131	Transversion	T→G	Present
134	Transversion	T→G	New*
141	Transition	C→T	Present
143	Transition	G→A	Present
146	Transition	T→C	Present
146	Transversion	T→G	Present
150	Transition	C→T	Present
152	Transition	T→C	Present
172	Transversion	T→G	Present
182	Transition	C→T	Present
185	Transition	G→A	Present
185	Transversion	G→T	Present
186	Transversion	C→A	Present
187	Transition	G→A	Present
189	Transversion	A→C	Present
189	Transition	A→G	Present
195	Transition	T→C	Present
198	Transition	C→T	Present
247	Transition	G→A	Present
263	Transition	A→G	Present
275	Transition	G→A	Present
310	Transition	T→C	Present
310	Insertion	T→TCCC	Present
310	Insertion	T→TTC	Present
315	Insertion	C→CC	Present
324	Transversion	C→G	Present
325	Transition	C→T	Present
330	Transversion	C→A	Present
330	Transversion	C→G	Present
338	Transversion	C→A	Present
348	Deletion	C→d	New*
366	Transition	G→A	Present
411	Transversion	C→A	Present
411	Transversion	C→G	Present
414	Transversion	T→A	Present
414	Transition	T→C	Present
414	Transversion	T→G	Present
418	Deletion	C→d	New*

Note: Present indicates that observed polymorphism in a position was present in the Mitomap; New* indicates newly observed polymorphic positions based on this study.

Table 6 showed the summary of the polymorphism in the mtDNA regions under study. Owing to the fact that some sites contained about two or three variants, sequenced data showed 61 polymorphic positions for HVS1 and 52 for HVS2. The recorded mutation for each sample differed in type, position and number. There were 45.9% and 40.4% mutations resulting from transversions for HVS1 and HVS2, respectively. There were five indels for the regions under study, comprising of 2 insertion mutations for HVS1, and 3 for HVS2. There were registered five deletions, one in the HVS1 and four in the HVS2 regions. The majority of the positions considered in the study exhibited only one type of nucleotide change.

Table 6: Summary of polymorphism in the mtDNA regions

Nucleotide change	HVS1	HVS2
A→C	6	2
A→G	4	4
A→T	2	0
C→A	10	6
C→G	2	3
C→T	16	5
G→A	4	8
G→C	3	0
G→T	1	1
T→A	3	3
T→C	6	7
T→G	1	6
No. of polymorphic sites	61	52
Total transitions	30	24
Total transversions	28	21
Insertions	2	3
Deletions	1	4

Table 7 showed the haplogroup distribution of the HVS1 and HVS2 control regions in the sampled Nigerian population. This showed the extant L haplogroup for African population with the U and H haplogroups of the Eurasians.

Table 7: Predicted haplogroups for studied individuals.

	Haplogroups:	
Sample ID	HVS1	HVS2
NG1	U4a	L3f
NG2	G3a	L2a
NG3	L1b	L1b
NG4	L2c1a	L2c
NG5	L2b1a	L2b
NG6	U7b2	L3d
NG7	H11a	H1b
NG8	L0a	L0a
NG9	L1c	L0d
NG10	L2a1ab	L1c

Discussion

Mitochondria are generally regarded as the powerhouse of the cell, providing energy for most cellular activities. They participate in the regulation of apoptosis and calcium storage, and are the major players in the electron transport chain. mtDNA has been documented to be an important tool in evolution and population genetics studies as well as in forensic investigation. Hence, mtDNA has proved to be an essential genetic marker that can be used for forensic and human evolutionary investigations.

The present study was aimed at determining the diversity of HVS1 and HVS2 regions of the human mitochondria in the Nigerian population. The study identified sequence variations in the mitochondria region of maternally unrelated individuals from the West African State. Mutations observed in this study were mostly the result of nucleotide substitutions (i.e. transition and transversion, with transition mutation having a higher percentage). The observed deletions and insertions were in agreement with the mutation types reported in the HVS2 region by Hameed *et al.* (2015). The dominance of transition mutation over transversion was reported in other studies (Brown *et al.*, 1982; Yang and Yoder, 1999). Our study showed a high level of variation in the D-loop region of the mitochondria which can be a consequence of several factors such as natural selection, mutation, or even marriage (Susanti *et al.*, 2017). Our results showed three novel transversion mutations for HSV2 at C104A, T131A and T134G. An insertion of an extra thymine residue at position 16199 of HVS1 (T→TT) was also observed and a cytosine was added to the guanine residue at position 16034 (G→GC).

Overall, transition mutations were found to be slightly higher than the other types of mutations recorded in the study. This accounts for 47% of the nucleotide substitutions observed, while transversions (43%), insertions (5%) and deletions (5%) were observed with significantly lower frequency. The transition mutations were mainly pyrimidine substitutions with a high frequency of C→T nucleotide change which was in agreement with the study of Lutz *et al.* (1998). The high frequency of transition and transversion mutations recorded in the present study could be an indication of spontaneous mitochondrial DNA mutation resulting from base mispairing during DNA replication as previously reported (Thomas and Beckenbach, 1989). The calculated GD values of 84.21% for HVS1 and 90.40% for HVS2 differed from the genetic diversities obtained in the regions from other populations which may be attributed to our relatively small sample size. Hedman *et al.* (2007) reported genetic diversity values of 0.959 and 0.970 for HVS1 and HVS2 for Finnish population, Kareem *et al.* (2015) had 96.5% genetic diversity value for HVS1 in Iraq, while Lutz and colleagues (1998) obtained 99% diversity in Germany.

Analysis of sequenced data resulted in predicted haplogroups for the population to be U, G, L, and H. This result is in tandem with what has been reported for African populations by previous studies (Salas *et al.*, 2002; Lott *et al.*, 2013) with some exceptions. While haplogroup L is one of oldest large haplogroups predominant in the African continent, especially in the sub-Sahara Africa, and consists of subgroups L0, L1, L2, L3, L4, L5, L6 and L7 of which L1-L3 have been reported in West African populations (Salas *et al.*, 2002; González *et al.*, 2006; Maji *et al.*, 2009), U and H have been classified as major haplogroups in the Eurasians (Mitchell *et al.*, 2014). The presence of two of the predominant European haplogroups i.e. U4a and U7b2 in our study might be as a result of gene flow from European populations. In contrast, Maca-Meyer *et al.* (2003) have reported U6b lineage in Algerian and Morocco, North Africa and West African countries of Nigeria and Senegal. The absence of macrohaplogroups L4-L6 in our finding was not surprising. Previous studies have shown that they (particularly L4 and L5) have been reported to exhibit high prevalence in East Africa (Maca-Meyer *et al.*, 2003; Brandstätter *et al.*, 2004; Castrì *et al.*, 2009) while L6 is more common in Northern Africa (Poloni *et al.*, 2009). A similar prevalence of L1-L3 haplogroups to our study was reported by Fendt *et al* (Fendt, 2012) in Ghana which might be attributed to gene flow between the two West African countries.

Conclusion

The present study indicates that HVS1 and HVS2 sequencing is a promising tool for human evolutionary studies, population and forensic genetics investigations as well as molecular anthropology in our population. The genetic diversity analysis showed that these sequences are polymorphic enough for identification purposes. Furthermore, the haplogroup distribution

revealed a likely gene flow between the Nigerian population neighbouring West African countries and the Eurasians, which could be attributed to intermarriages.

References

Achilli, A., Rengo, C., Magri, C., Battaglia, V., Olivieri, A., Scozzari, R., Cruziani, F., Zeviani, M., Briem, E., Carelli, V., Moral, P., Dugoujon, J. M., Roostalu, U., Loogvali, E. L., Kivisild, T., Bandelt, H. J., Richards, M., Villems, R., Santachiara-Benerecetti, A. S., Semino, O. and Torroni, A. (2004). The molecular dissection of mtDNA haplogroup H confirms that the Franco-Cantabrian glacial refuge was a major source for the European gene pool. *The American Journal of Human Genetics* **75**(5), 910-918.

Allio, R., Donega, S., Galtier, N. and Nabholz, B. (2017). Large variation in the ratio of mitochondrial to nuclear mutation rates across animals: implications for genetic diversity and the use of mitochondrial DNA a molecular marker. *Molecular Biology and Evolution* **34**(11): 2762-2772.

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J., Staden, R. and Young, I. G. (1981). Sequence and organization of the human mitochondrial genome. *Nature* **290**(5806): 457-465.

Andrews, R. M., Kubacka, I., Chinnery, P. F., Lightowlers, R. N., Turnbull, D. M. and Howell, N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nature Genetics* **23**(2): 147.

Bourdon, V., Ng, C., Harris, J., Prinz, M. and Shapiro, E. (2014). Optimization of Human mt DNA Control Region Sequencing for Forensic Applications. *Journal of Forensic Sciences* **59**(4): 1057-1063.

Brandstätter, A., Peterson, C. T., Irwin, J. A., Mpoke, S., Koech, D. K., Parson, W. and Parsons, T. J. (2004). Mitochondrial DNA control region sequences from Nairobi (Kenya): inferring phylogenetic parameters for the establishment of a forensic database. *International Journal of Legal Medicine* **118**(5): 294-306.

Brown, W. M., Prager, E. M., Wang, A. (1982). Mitochondrial DNA sequences of primates: tempo and mode of evolution. *Journal of Molecular Evolution* **18**: 225-239.

Castrì, L., Tofanelli, S., Garagnani, P., Bini, C., Fosella, X., Pelotti, S., Paoli, G., Pettener, D. and Luiselli, D. (2009). mtDNA variability in two Bantu-speaking populations (Shona and Hutu) from Eastern Africa: Implications for peopling and migration patterns in sub-Saharan Africa. *American Journal of Physical Anthropology* **140**(2): 302-311.

Chemale, G., Paneto, G. G., Menezes, M. A. M., De Freitas, J. M., Jacques, G. S., Cicarelli, R. M. B. and Fagundes, P. R. (2013). Development and validation of a D-loop mtDNA SNP assay for the screening of specimens in forensic casework. *Forensic Science International: Genetics* **7**(3): 353-358.

Chen, F., Deng, Y., Dang, Y., Zhang, B., Mu, H., Yu, X., Li, L., Yan, C. and Chen, T. (2008). Genetic polymorphism of mitochondrial DNA HVS-I and HVS-II of Chinese Tu ethnic minority group. *Journal of Genetics and Genomics* **35**(4): 225-232.

Di Benedetto, G., Nasidze, I. S., Stenico, M., Nigro, L., Krings, M., Lanzinger, M., Vigilant, L., Stoneking, M., Paabo, S. and Barbujani, G. (2000). Mitochondrial DNA sequences in prehistoric human remains from the Alps. *European Journal of Human Genetics* **8**(9): 669-677.

Fakorede, S. T., Adekoya, K. O., Akpan, U. U. and Ogunlusi, O. V. (2019). Allele frequencies and haplotype diversities of five Y-chromosome short tandem repeat loci in a random sample of Yoruba population in Lagos, Nigeria. *FUW Trends in Science and Technology Journal* **4**(2): 577-581.

Fendt, L., Röck, A., Zimmermann, B., Bodner, M., Thye, T., Tschentscher, F., Owusu-Dabo, E., Gobel, T. M. K., Schneider, P. M. and Parson, W. (2012). MtDNA diversity of Ghana: a forensic and phylogeographic view. *Forensic Science International: Genetics* **6**(2): 244-249.

González, A. M., Cabrera, V. M., Larruga, J. M., Tounkara, A., Noumsi, G., Thomas, B. N. and Moulds, J. M. (2006). Mitochondrial DNA variation in Mauritania and Mali and their

genetic relationship to other Western Africa populations. *Annals of Human Genetics* **70**(5): 631-657.

Gubina, M. A., Babenko, V. N. and Voevoda, M. I. (2018). Polymorphism of Mitochondrial DNA in Population of Siberian Tatars from Barabinsk Forest Steppe. *Russian Journal of Genetics* **54**(6): 717-731.

Habbane, M., Montoya, J., Rhouda, T., Sbaoui, Y., Radallah, D. and Emperador, S. (2021). Human mitochondrial DNA: Particularities and diseases. *Biomedicines*. **9**(10): 1364-1374.

Hameed, I. H., Abdulzahra, A. I., Jebor, M. A., Kqueen, C. Y. and Ommer, A. J. (2015). Haplotypes and variable position detection in the mitochondrial DNA coding region encompassing nucleotide positions 10,716–11,184. *Mitochondrial DNA* **26**(4): 544-549.

Hameed, I. H., Jebor, M. A., Ommer, A. J., Abdulzahra, A. I. and Yoke, C. (2016). Haplotype data of mitochondrial DNA coding region encompassing nucleotide positions 11,719–12,184 and evaluate the importance of these positions for forensic genetic purposes in Iraq. *Mitochondrial DNA Part A* **27**(2): 1324-1327.

Hedman, M., Brandstätter, A., Pimenoff, V., Sistonen, P., Palo, J. U., Parson, W. and Sajantila, A. (2007). Finnish mitochondrial DNA HVS-I and HVS-II population data. *Forensic Science International* **172**(2-3): 171-178.

Helgason, A., Hrafnkelsson, B., Gulcher, J. R., Ward, R. and Stefánsson, K. (2003). A populationwide coalescent analysis of Icelandic matrilineal and patrilineal genealogies: evidence for a faster evolutionary rate of mtDNA lineages than Y chromosomes. *The American Journal of Human Genetics* **72**(6): 1370-1388.

Horai, S., Hayasaka, K., Kondo, R., Tsugane, K. and Takahata, N. (1995). Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. *Proceedings of the National Academy of Sciences* **92**(2): 532-536.

Ingman, M., Kaessmann, H., Pääbo, S. and Gyllensten, U. (2000). Mitochondrial genome variation and the origin of modern humans. *Nature* **408**(6813): 708-713.

Jankova-Ajanovska, R., Zimmermann, B., Huber, G., Röck, A. W., Bodner, M., Jakovski, Z., Janeska, B., Duma, A. and Parson, W. (2014). Mitochondrial DNA control region analysis of three ethnic groups in the Republic of Macedonia. *Forensic Science International: Genetics* **13**: 1-2.

Kareem, M. A., Abdulzahra, A. I., Hameed, I. H. and Jebor, M. A. (2016). A new polymorphic positions discovered in mitochondrial DNA hypervariable region HVIII from central and north-central of Iraq. *Mitochondrial DNA Part A* **27**(5): 3250-3254.

Kraytsberg, Y., Schwartz, M., Brown, T. A., Ebralidse, K., Kunz, W. S., Clayton, D. A., Vissing, J. and Khrapko, K. (2004). Recombination of human mitochondrial DNA. *Science* **304**(5673): 981-981.

Lott, M. T., Leipzig, J. N., Derbeneva, O., Xie, H. M., Chalkia, D., Sarmady, M., Procaccio, V. and Wallace, D. C. (2013). mtDNA variation and analysis using MITOMAP and MITOMASTER. *Current Protocols in Bioinformatics* **44**(1): 1-23.

Lu, Y., Wang, C., Qin, Z., Wen, B., Farina, S. E., Jin, L., Li, H. and the Genographic Consortium (2012). Mitochondrial origin of the matrilocal Mosuo people in China. *Mitochondrial DNA* **23**(1): 13-19.

Lutz, S., Weisser, H. J., Heizmann, J. and Pollak, S. (1998). Location and frequency of polymorphic positions in the mtDNA control region of individuals from Germany. *International Journal of Legal Medicine* **111**(2): 67-77.

Maca-Meyer, N., González, A. M., Pestano, J., Flores, C., Larruga, J. M. and Cabrera, V. M. (2003). Mitochondrial DNA transit between West Asia and North Africa inferred from U6 phylogeography. *BMC Genetics* **4**(1): 15.

Maji, S., Krithika, S. and Vasulu, T. S. (2009). Phylogeographic distribution of mitochondrial DNA macrohaplogroup M in India. *Journal of Genetics* **88**(1): 127-139.

Martinez, B., Catelli, L., Romero, M., Okolie, V. O., Keshinro, S. O., Carvalho, E. F., Vullo, C. and Gusmão, L. (2017). Forensic evaluation of 27 y-str haplotypes in a population sample from Nigeria. *Forensic Science International: Genetics Supplement Series* **6**: e289-e291.

Mitchell, S. L., Goodloe, R., Brown-Gentry, K., Pendergrass, S. A., Murdock, D. G. and Crawford, D. C. (2014). Characterization of mitochondrial haplogroups in a large population-based sample from the United States. *Human Genetics* **133**(7): 861-868.

Nei, M. and Tajima, F. (1981). DNA polymorphism detectable by restriction endonucleases. *Genetics* **97**(1): 145-163.

Ngili, Y., Ubyaan, R., Palit, E. I., Bolly, H. M. and Noer, A. S. (2012). Nucleotide mutation variants on D-Loop HVS1/HVS2 mitochondrial DNA region: studies on Papuan population, Indonesian. *European Journal of Scientific Research* **72**(1): 64-73.

Pfeiffer, I., Voelkel, I., Brenig, B., Hoberg, E. P., Lichtenfels, J. R. and Rickard, L. G. (2005). 2187601. Phylogenetics of the European Dahomey miniature cattle based on mitochondrial D-loop region DNA sequence. *Animal genetics* **36**(2): 179-181.

Poloni, E. S., Naciri, Y., Bucho, R., Niba, R., Kervaire, B., Excoffier, L., Langaney, A. and Sanchez-Mazas, A. (2009). Genetic evidence for complexity in ethnic differentiation and history in East Africa. *Annals of Human Genetics* **73**(6): 582-600.

Salas, A., Richards, M., De la Fe, T., Lareu, M. V., Sobrino, B., Sánchez-Diz, P., Macaulay, V. and Carracedo, Á. (2002). The making of the African mtDNA landscape. *The American Journal of Human Genetics* **71**(5): 1082-1111.

Shokolenko, I. N. and Alexeyev, M. F. (2015). Mitochondrial DNA: A disposable genome?. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. **1852**(9): 1805-1809.

Susanti, R., Iswari, R. S., Fibriana, F. and Sari, R. I. (2017). Mitochondrial D-loop sequence variation among Central Javanese duck in Indonesia. *Acta Scientiarum. Animal Sciences* **39**(4): 449-456.

Tait, S. W. and Green, D. R. (2012). Mitochondria and cell signalling. *Journal of Cell Science* **125**(4): 807-815.

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics* **105**(2): 437-460.

Thomas, W. K. and Beckenbach, A. T. (1989). Variation in salmonid mitochondrial DNA: Evolutioinary constraints and mechanisms of substitution. *Journal of Molecular Evolution* **29**(3): 233-245.

Wan, Q. H., Wu, H., Fujihara, T. and Fang, S. G. (2004). Which genetic marker for which conservation genetics issue?. *Electrophoresis* **25**(14): 2165-2176.

Yang, Z. and Yoder, A. D. (1999). Estimation of the transition/transversion rate bias and species sampling. *Journal of Evolution of Evolution* **48**: 274-283.

Zapico, S. C. and Ubelaker, D. H. (2013). mtDNA mutations and their role in aging, diseases and forensic sciences. *Aging and Disease* **4**(6): 364-380.

Zhang, Y., Xu, Q., Cui, H., Cui, Y., Lin, H., Kim, K. and Lee, J. (2005). Haplotype diversity in mitochondrial DNA hypervariable region I, II and III in a Korean ethnic group from northeast China. *Forensic Science International* **151**(2-3): 299-301.