ALLELE FREQUENCIES AND HAPLOTYPE DIVERSITIES OF FIVE Y-CHROMOSOME SHORT TANDEM REPEAT LOCI IN A RANDOM SAMPLE OF YORUBA POPULATION IN LAGOS, NIGERIA

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Samson Taiwo Fakorede*, Khalid Olajide Adekoya, Utom-Obong Udom Akpan and Olajumoke Victoria Ogunlusi
Genetics Research Group Unit, Department of Cell Biology and Genetics, University of Lagos, Nigeria
*Correspondence author: tfakorede@unilag.edu.ng

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Abstract: Y-chromosome short tandem repeats (Y-STRs) are specialized class of short tandem repeats located on human Y (male) chromosomes and are passed unchanged (barring a mutation) from one generation to the next. They are widely used in population genetic studies, forensics, paternity and genealogical DNA testing. The non-recombining nature of these loci makes them to have high discriminatory capacities among individuals of the same population or ethnicity. This is because they are polymorphic and exhibit high mutation rates. For Y-STR data to be applicable in forensics, knowledge of the haplotype frequencies in a geographic or ethnic substructure is required. DNA was extracted from peripheral blood sample of 110 unrelated Yoruba males, who have consented to participate in the research by signing the informed consent form, using conventional method. Five Y-STR markers (DYS392, DYS393, DYS449, DYS490 and DYS576) were typed and the PCR products were resolved on 4% agarose electrophoresis. Haplotype diversity was highest in DYS449 (0.840) and lowest in DYS392 (0.406). Also, the power of discrimination was found to be highest in DYS449 and lowest in DYS392 with 85.9% and 40.7%, respectively. The study revealed that the loci under study are suitable for use in identification, discrimination and forensic applications in the Yoruba population in Nigeria.

Keywords: Discrimination, forensics, genetic diversity, haplotypes, tandem repeats, Yoruba

Introduction

The male-specific part of the human Y chromosome is widely used in forensic DNA analysis, particularly in cases where standard autosomal DNA profiling is not informative. Short tandem repeats are polymorphic DNA markers usually used for obtaining human DNA profiles in forensic investigations such as human identification, paternity testing and characterization of paternal lineages (Jobling *et al*., 1997; Rangel-Villalobos *et al*., 2001; Kayser, 2017). Polymorphic nature of Y-STR has been used in diversity studies among tribes (Yussup *et al*., 2017). The Y-chromosome short tandem repeats (Y-STRs) are linear tandemly repeat loci of about 2 – 6 nucleotide sequences found specifically on the non-recombining portion of the Y chromosome which are passed down directly from father to son unaltered, except there are mutations (Jobling and Tyler-Smith, 2003; Batini *et al*., 2015).

Y chromosome is normally found only in human males and absent in females. Since it does not participate in meiotic recombination, allelic combination of polymorphic markers on the Y chromosome, known as haplotypes, is usually inherited by male children from the father without alterations. This unique feature has distinguished the Y-chromosome STRs as a powerful tool in the forensic community and has helped researchers in understanding human genetic and genealogical history (Butler, 2007; Campbell and Tishkoff, 2010). The Y chromosome has proven to be of potential use in forensics, evolutionary studies and paternity testing because of its uniparental mode of inheritance (Chakraborty, 1985; Butler, 2006; Gao *et al*., 2015).

Unlike the autosomal STRs that amplify both the male and female DNA, the Y-STR loci are specific to males, which make it useful in male related cases such as rape and assault. It has been estimated that males are responsible for 93% of violent crimes and 99% of sexual offences in England and Wales (Jobling and Tyler-Smith, 1995; Jobling *et al*., 1997; Butler *et al*., 2006). Rape and indecent assault cases have been ranked as one of the most serious crimes against persons in Nigeria (Alemika and Chukwuma, 2010). Findings from a national survey carried out in 2014 on violence against children in Nigeria confirmed one in four females reported experiencing sexual violence in childhood with approximately 70% reporting more than one incident of sexual violence. In the same study, it was found that 24.8% of females ages 18 to 24 years experienced sexual abuse prior to age 18 of which 5.0% sought help, with only 3.5% receiving any responses (WARIF, 2015). It should be pointed out however that most of these crimes go unresolved due to lack of strong scientific evidence that could match suspects to the crime. It is believed that a crime-scene sample left by a male culprit will usually be informative when analyzed with Y chromosome markers. Even in cases where there is a mixture of both male assailant's and female victim's DNAs, as in rape cases, these specific markers (loci) will exclusively give precise information of the perpetrator's DNA. Similarly, in a situation where the paternity of a male child is being contended, comparison of the Y chromosomes can be a simple and reliable way of excluding an assumed father (Marks *et al*., 2012; Larmuseau *et al*., 2014).

Various investigations of Y-chromosome STRs globally have revealed major geographical differences in terms of genetic diversities and allele and haplotype frequencies. Purps *et al*. (2015) in their global analysis of 23 Y-STRs haplotype diversities from 129 populations in 51 different countries of the world found out that samples of African ancestry were obviously genetically different from all other continental meta-populations. They however reported related magnitude of genetic distance (measured by R_{ST}) between African and other non-African metapopulations. Also, Coelho *et al*. (2008) showed that the genetic diversities among the human populations on a small Island in Sao Tome were largely due to spatial and temporal specific events. In Burkina Faso, Barbieri *et al*. (2002) traced the language differences among the different ethnolinguistic groups to a paternal ancestral line. However, Veeramah *et al*. (2010) reported that, despite the strong language differences among a Southern Nigeria state, there are little genetic differences among them.

The Bantu-speaking population from the central to southern Africa is the most studied population in terms of genetic diversity studies in Africa. These studies have been solely centred on the complex expansion of this population which seems to be uniparental or autosomal in nature (Barbujani and Colonna, 2010). Recently, there are several genetic studies in West Africa based on smaller scales. For example, Tau *et al*. (2015) reported a heterogeneous population in the distribution of 17 Y-STR loci in Sub-Saharan according to ethnicity and

geography. In the study of two Libyan villages, Ottoni *et al*. (2011) found that strong founder effects and genetic drift are the major factors responsible for the different paternal lineages found among the populations.

Despite its great prospect, the Y-chromosome is not yet well studied in Africa for its forensic characteristics compared to other continents that are less genetically diversed (Batini *et al.,* 2015; Trombetta *et al.,* 2015). Although various attempts have been made to provide data on Nigerian autosomal STRs (Awe *et al*., 2017; Akpan *et al*., 2017; Okolie *et al*., 2018), Nigeria is yet to adopt Y-STR profiling in crime investigation because there are no established Y-STR reference data for the populations owing to lack of a forensic database for comparing relevant caseworks. This study therefore aims to use DNA typing technique to screen for genetic diversity among the male Yoruba population in Lagos using five Y-chromosome markers to generate relevant forensic data suitable for human identification and discrimination in Nigerian populations.

Materials and Methods

Ethical clearance

The ethical approval for this study was obtained from the Health Research Ethics Committee of College of Medicine of the University of Lagos (Approval Number: CM/HREC/010/16/065) after thorough perusal of the aim, objectives and methodologies of the study.

Population sample

The Yorubas are one of the major ethnic groups in Nigeria. They originate and are found predominantly in the South-Western states (Oyo, Ekiti, Osun, Lagos, Ondo and Ogun) and some parts of the North Central (Kogi and Kwara states) parts of the country. They also cut across the Southern and Benin Republic. According to the 2006 population census, the Yorubas account for about 21% of Nigerian population, and are currently estimated to be more than 40 million in number representing about 40% of the estimated population. As seen in the other major ethnic groups such as the Igbo and Hausas, the Yoruba people are diverse in culture and exist as sub-tribes or clans which include the Egba, Ikale, Igbomina, Ijebu, Oyo, Ekiti, Ijesha, Yagba, Ibarapa, and many others.

Sample collection

Peripheral blood samples were obtained from 110 unrelated male individuals from South Western Nigeria at the University of Lagos Medical Centre, all the participants met the inclusion/exclusion criteria which include that the participant must be between 18–65 years old, be of Yoruba ethnicity, both parents must also be of Yoruba ethnicity. Also, each of the participants read and signed the informed consent after the aim and procedures of the study have been satisfactorily explained to them. The blood samples were collected in EDTA bottles and mixed well to avoid clotting. Each of the donors was questioned to ascertain their ethnicity before sample collection.

DNA isolation and quantitation

DNA was isolated from collected blood samples as described by Iranpur and Esmailizadeh (2010). To determine the quality and quantity of the isolated DNA samples and their suitability for PCR amplification, a UV spectrophotometric analysis was carried out on the samples using an Eppendorf BioPhotometer plus spectrophotometer (Eppendorf, Hamburg, Germany). To further ascertain their purity and integrity, the DNA samples were resolved on 1% agarose gel against a 20 bp ladder.

Polymerase chain reaction analysis

The five Y-STR markers were separated into two in-house multiplexes: Multiplex I comprise of the DYS449, DYS490 and DYS576 loci, while Multiplex II contains the DYS392 and DYS393. The loci information is presented in Table 1. The reactions for the two multiplexes were performed in a 20 µL final reaction volume as shown in Table 2. Polymerase chain reaction amplification was carried out using an Edvocycler thermocycler (Thermo Fisher Scientific, Massachusetts, USA) under the following thermal cycling conditions;

Table 1: Characteristics of the five Y-STR loci studied

Locus Accession No.	Primer Sequence (5'→3')	No. of alleles[a]	Genbank
DYS392[b]	AAAAGCCAAGAAGGAAAACAAA AGACCCAGTTGATGCAATGT	3	G09867
DYS393[b]	GTGGTCTTCTACTTGTGTCAATAC AACTCAAGTCCAAAAAATGAGG	3	G09601
DYS449[c]	CCTGGAAGTGGAGTTTGCTGT TGGAGTCTCTCAAGCCTGTTCTA	8	AC051663
DYS490[c]	CTGAGCTGAGATCACGCC ACGATATGAAAAAGCAGTATGTCCT	5	AC019058
DYS576[c]	TTGGGCTGAGGAGTTCAATC GGCAGTCTCATTTCCTGGAG	4	AC010104

[a] Based on results obtained from this study; [b]Gusmão and Alves, 2007; [c]Butler *et al*. (2006).

Table 2: PCR Amplification Mix

Reaction Mixtures	Multiplex I	Multiplex II
PCR Master Mix	10.0 µL	10.0 µL
Forward Primer	0.6 µL	0.4 µL
Reverse Primer	0.6 µL	0.4 µL
Template DNA	5.0 µL	5.0 µL
Nuclease-free sterile water	3.8 µL	4.2 µL
Reaction volume	20 µL	20 µL

Multiplex I: pre-incubation for 11 min at 95ºC and 1 min at 96ºC, followed by 10 cycles of 30 secs at 94ºC, 30 secs at 58ºC, 45 secs at 70ºC; plus 22 cycles of 30 secs at 90ºC, 30 secs at 56ºC, 45 secs at 70ºC and a final incubation step of 60ºC for 60 min.

Multiplex II: pre-incubation for 10 min at 95ºC, followed by 30 cycles of (1 min at 95ºC, 1 min at 55ºC, 1 min at 72ºC); 60ºC for 45 min and final hold at 25ºC.

The PCR products were then resolved on 4% agarose gel electrophoresis ran at 70V for 45 min, pre-stained with 2 µL ethidium bromide. The DNA was visualized under the UV transilluminator and the allele sizes were estimated by comparing with 20 bp DNA ladder.

Statistical analysis

Allele sizes were scored using GelAnalyzer software and the frequencies were estimated by direct counting method. The power of discrimination for each marker which is equivalent to gene diversities (GD) was calculated using the formula: $1 - \sum fi^2$ where f represents the frequency of the *i*th allele (Nei, 1987). Other statistical parameter of forensic importance and the Hardy-Weinberg Equilibrium (HWE) were generated using the GenAlEx Software v6.502 (Peakall and Smouse, 2012).

Results and Discussion

The allele frequencies and gene diversities for the loci in 40 Nigerian Yoruba males are as shown in Table 3. The number of alleles ranged from three (DYS392, DYS393) to eight (DYS449). Seventeen different alleles were found in the population with allele 16 present in three (DYS393, DYS490 and DYS756) of the five loci under investigation. Gene diversity values range from 0.407 in DYS392 to 0.859 in DYS449. Also, haplotype diversities range from 0.406 to 0.840. Also, haplotype diversity was highest in DYS449 (0.840) and lowest in DYS392 (0.406) (Table 4). Power of discrimination was found to be highest in DYS449 and lowest in DYS392 with 85.9 and 40.7%, respectively.

Table 3: Allele frequencies and gene diversities of Yoruba males (N=40)

Alleles	DYS392	DYS393	DYS449	DYS490	DYS576
11	0.125				
12	0.750			0.375	
13	0.125			0.250	
14		0.111		0.125	
15		0.444		0.125	
16		0.444		0.125	0.250
17					0.250
18					0.250
19					0.250
24			0.182		
26			0.182		
27			0.091		
28			0.091		
32			0.091		
33			0.091		
35			0.091		
36			0.182		
GD	0.407	0.593	0.859	0.750	0.750

Table 4: Statistical parameters of the loci under study

Parameter	DYS392	DYS393	DYS449	DYS490	DYS576	Mean
Na	3	3	8	5	4	4.6
Ne	1.684	2.455	6.25	4.00	4.00	3.856
I	0.736	0.965	1.887	1.494	1.386	1.321
Ho	0	0	1	1	0	0.4
He	0.406	0.593	0.84	0.75	0.75	0..672
F	1	1	-0.19	-0.333	1	0.501
HD	0.406	0.593	0.840	0.750	0.750	0.668
HWE	16.00*	18.00**	17.50ns	6.67ns	12.00ns	

Na: Number of alleles; Ne: Effective number of alleles; I: Shannon index; Ho: Observed heterozygosity; He: Expected heterozygosity; F: Fixation index; HD: Haplotype diversity; HWE: Hardy-Weinberg equilibrium test; ns= not significant, * $p < 0.01$, ** $p < 0.001$

From Table 4, it can be deduced that DYS449 has the highest number of alleles of 8 while DYS392 and DYS393 have combined number of 3 alleles. Number of effective ranges from 1.684 in DYS392 to 4.00 in DYS490 and DYS576. Hardy-Weinberg Equilibrium (HWE) is found to be significant in DYS392 and DYS393 at $p < 0.01$ and $p < 0.001$, respectively.

This study was aimed at ascertaining the diversity of five Y chromosome loci (DYS392, DYS393, DYS449, DYS490 and DYS576) in a Nigerian Yoruba population resident in Lagos and the potential of the loci to discriminate the individuals of the population. These loci were carefully selected because studies have revealed that they are highly polymorphic and have high mutation rates. DYS449, one of the most polymorphic single-copy Y-STRs, and DYS576 are considered as fast mutating Y-STRs with great potential in discriminating male lineages (Ballantyne *et al*., 2010; Ballantyne *et al*., 2012). Such loci that are rapidly mutating are worthwhile in forensic community as a result of their high mutation rates, estimated to be about seven times higher than the widely used YTSR loci, and can differentiate even between closely related male individuals (Ballantyne *et al*., 2010). Also, they have been used to successfully resolve Y-STR haplotypes in different populations of the world such as the African, African-American and Asians among many other populations of the world (Redd *et al*., 2002; Butler *et al*., 2006; Gao *et al*., 2015).

The allele frequencies obtained from this study revealed that locus DYS449 is the most diversed with 8 alleles, and loci DYS392 and DYS393 the least diversed with 3 alleles each, which might be as a result of mutation. Our study also revealed that four of the five loci investigated in this, except DYS392 (0.407) showed gene diversity values between 0.5 and 0.8, indicating that these loci are highly polymorphic in the studied population. These findings corroborated that of Imad *et al*. (2013) which showed low gene diversity for the DYS392 locus in Iraqi populations. However, Rangel-Villalobos and colleagues (2001) reported a lowest gene diversity value for DYS393 among six Y-STR loci studied in a Mexican population.

Despite their relatively low gene diversities/discriminatory capacities, these two loci (DYS392 and DYS393) are still included in many Y-STR forensic testing kits because their discriminatory capacities are enhanced by addition of other polymorphic markers and increased population size which assist in haplotypes resolution and increased discriminatory capacities (Rodig *et al*., 2008; Vermeulen *et al*., 2009). Hanson and Ballantyne (2007) established a correlation between single locus diversity of some Y-STRs and their ability to enhance the multi-locus diversity of core loci in a multiplex. The differences in the gene diversities may be explained by the sizes of the alleles found in each of the loci as loci with more alleles tend to have high gene diversity value, and perhaps the genetic structure of the population. It should be stated, however, that the lower diversity for the locus DYS392 has to be investigated further with increased sample sizes to be able to establish whether this is peculiar to the marker or the Yoruba population does actually possess low diversity at DYS392 locus.

Owing to the fact that large genetic differences have been shown to exist between the African- American population and indigenous Africans, there is need for the establishment of separate reference database suitable for forensic application in African populations. Furthermore, the high haplotype diversity obtained from this study for three Y-STRs DYS449, DYS490 and DYS576 is in agreement with what has been reported in previous studies of Butler *et al*. (2006) in their study of 27 Y-STR alleles frequencies among U.S. Caucasian, African American, and Hispanic populations indicating these loci are highly diversed in most populations. The high discriminatory power in terms of gene diversities, and haplotype values observed for the loci DYS449, DYS490 and DYS576 are indications that these loci are highly polymorphic and suitable for distinguishing males and male-ancestries in the studied population. Other statistical parameters obtained from this study including the fixation and Shannon indices

indicate the loci are highly genetically diversed and suitable for use in identity testing for the population under study.

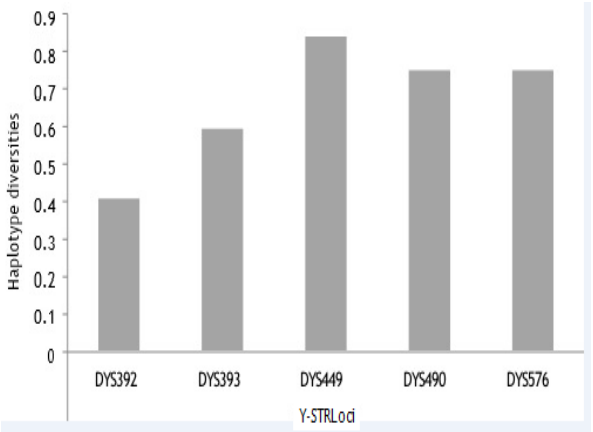

Fig. 1: Haplotype diversities of the loci

From Figure 1, the loci are shown to be highly diversed with haplotype diversity seen to be highest in DYS449 and lowest in DYS392, respectively.

Conclusion

Although a preliminary investigation, the study however showed the loci under study can successfully discriminate between individuals and could be included for paternity testing and forensic case works in Nigerian population.

Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

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