Values of 15 STR in Beninese population's filiation test

Simon Azonbakin[1*], Cedric Bigot[2], Marius Adjagba[3], Max Debaly[4], Raphael Darboux[5], Anatole Laleye[6]

[1,3-6]Laboratory of Histology, [2]Unity of Forenscic Medecine, [1,3-6]Reproductive Biology, [1,3-6]Cytogenetics and Medical Genetics, [1-6]Faculty of Heath Sciences, University of Abomey, Calavi, Cotonou, Benin

***Corresponding Author: Simon Azonbakin**
Email: azandeg@yahoo.fr

Abstract
The population genetic characterization is very important in medicine and particularly in medical anthropology. This characterization can be done using SNP (Single Nucleotide Polymorphism), haplotypes of the genes of the HLA system, and with STR (Short Tandem Repetition). STRs, which are small nucleotides repetition used to characterize populations, are very interesting in parentage tests. In this study, the DNA was extracted from the peripheral blood taken from 251 Beninese born from Beninese parents without any parental link. Fifteen (15) STR, including D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338, D16S539 were investigated. The amplified PCR products were separated by capillary electrophoresis on an ABI 310 XL genetic analyzer (Applied Biosystems). The diversity software version 1.9.89 was used for the calculation of allele frequencies, number of alleles per locus, heterozygosity and the Hardy-Weinberg balance test in the population. Forensic parameters such as Discrimination Power (PD), Exclusion Power (PE), Informative Content of Polymorphism (ICP) are calculated using the software R. Six loci (D21S11, D7S820, D18S51, THO1, D5S818 and FGA) out of the fifteen studied diverged from steady-state even after the bonferroni correction. The STRs used for paternity research and for forensic identification in Benin are very discriminating with a combined exclusion power in order of 0.9999 and a combined discrimination power higher than 0.9999999999. Therefore they are very informative in the filiation and identification tests of persons.

Keywords: STR, Genetic diversity, Filiation test, Forensic identification, Legal medicine, Benin.

Introduction
Benin is an African country located in the Gulf of Guinea with a population of 10 million inhabitants, spread over an area of 112,600 squares kilometers. The population is heterogeneous and made by different ethnic and socio-linguistic groups (Insae, 2016). Short tandem repetitions (STR), which are repetitive units with a very short pattern (4 base pairs) and repeated 2 to 10 times or more are used for the study of the genetic fingerprint (Budowle B. and al. 1998). These are routine markers located mostly on autosomes, used for human identification. Since the standardization of genetic testing using the STR in forensic biology, more than 1000 studies have been carried out in different populations around the world by using these markers (Butler JM, 2006). This made it possible to study the genetic diversity of several communities, to track the population migration and to confirm the legal interests of these STRs. In Benin, any study has not been carried out to evaluate the benefit of the STRs in the population. The aim of this study is to evaluate the forensic parameters such as the Power of Discrimination (PD), the Exclusion Power (EP) and the content of the polymorphism (CP) of 15 STRs within a sample of the Beninese population.

Population of study
A group of 251 people born from Beninese parents were registered by the Cytogenetics and Molecular Biology Laboratory at the Faculty of Health Sciences of Cotonou (University of Abomey-Calavi). They were all healthy, without any parental relationship with each other, from all regions of Benin, irrespective of ethnicities or languages and addressed in the filiation test.

DNA extraction and analysis of STR
The DNA was extracted from 5 ml of venous blood according to the conventional phenol-chloroform method. The DNA was amplified using KIT Quantifiler (Applied Biosystems, 2001). The different STR including: D3S1358, v WA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D19S433, D2S1338, D16S539 were amplified simultaneously and the amplification product was separated by capillary electrophoresis on an ABI 310 XL sequencer. Data from the genetic analyzer were processed by a specific software « Gene Mapper » (Applied Biosystems, Foster City, CA).

Statistical analysis
The software « diveRsity » version 1.9.89 was used to estimate allele frequencies, heterozygosity, homozygosity and the Hardy-Weinberg balance test (Prodohl PA and al. 2013). Bonferroni test was applied to adjust the results because of the multiple comparisons and also to maintain the level of the Hardy-Weinberg test at 5%. Results are considered significant for p-values below 0.003, ie 0.05 / 15(Weir, 1996). Forensic parameters such as discrimination power (PD), exclusion power (PE), informative content of polymorphism (ICP) are calculated using the R software according to John Butler (Butler, 2014).

Results
Table 1 shows the allelic frequencies of the 15 microsatellites studied in the Beninese population. From this table, it appears that the allele 12 has the highest frequency (0.452) at the D13S317 locus. The Benin population is very

diverse with values of heterozygosity ranging from 0.63 at the D13S317 locus to 0.89 at D2S1338 (Table 2).

The exclusion power of the loci varies from 0.33 (for the D13S317 locus) to 0.78 (for the D2S1338 locus). According to 9 out of the 15 studied loci (except the D21S11, D7S820, D18S51, THO1, D5S818 and FGA), the Beninese population states in Hardy-Weinberg balance. The D18S51 locus appears to be the most polymorphic (26 alleles) and possesses the strongest discriminating power (0.99) as well as the informative content of the polymorphism (0.92). The D13S317 locus is the least polymorphic (7 alleles) and has the lowest discriminating power and polymorphism informative content respectively at 0.75 and 0.63. The combined exclusion power of the STRs is in the range of 0.9999, and the combined discrimination power is greater than 0.9999999999.

Table 1: Allele frequencies of 15 STR in beninese population

Allele	D8S1179	D21S11	D7S820	CSF1PO	D19S433	vWA	TPOX	D18S51	D3S1358	THO1	D13S317	D16S539	D2S1338	D5S818	FGA
5							0.036			0.004					
5.2			0.002												
5.3										0.110					
6			0.002	0.002			0.030			0.038					
6.2															
6.3										0.331					
7			0.141	0.102			0.203			0.122		0.014			
7.3										0.159					
8	0.008		0.090	0.066			0.291			0.082	0.006	0.175		0.088	
8.2			0.062												
8.3										0.066					
9	0.006		0.038	0.052	0.010		0.183			0.058	0.006	0.161		0.012	
9.2			0.237				0.002								
9.3										0.030					
10	0.020		0.261	0.232	0.102		0.171	0.002		0.002	0.014	0.229		0.060	
10.2				0.012	0.002			0.002							
11	0.066		0.120	0.222	0.114		0.080		0.002		0.265	0.187		0.183	
11.2					0.032			0.018							
12	0.149		0.038	0.254	0.243	0.006	0.004	0.026	0.010		0.452	0.163		0.402	
12.2				0.002	0.056			0.022				0.002			
13	0.323		0.004	0.048	0.189	0.014		0.016	0.040		0.185	0.058		0.229	
13.2					0.058			0.038							
14	0.261		0.006	0.006	0.100	0.052		0.018	0.191		0.072	0.008		0.022	
14.2					0.038			0.086							
15	0.112				0.010	0.225		0.064	0.249			0.002	0.004	0.002	
15.2					0.028	0.219		0.106	0.223						
15.3									0.002						
16	0.046					0.147		0.084	0.078				0.062		
16.2					0.016			0.054	0.112						
17	0.008					0.159		0.135	0.044				0.086	0.002	
17.2					0.002	0.002		0.010	0.030						
18	0.002					0.096		0.090	0.018				0.036		0.004
18.2					0.002	0.034		0.010							0.010
19				0.002		0.030		0.088	0.002				0.118		0.094
19.2								0.006							0.010
20						0.010		0.050					0.082		0.036
20.2								0.018							0.002
21						0.006		0.028					0.145		0.088
21.2								0.008							0.004
22								0.014					0.181		0.143
22.2								0.010							0.004
23													0.124		0.229
23.2															0.006
24								0.004					0.090		0.137
24.2															0.004
25													0.052		0.094
26		0.006											0.022		0.046
26.2		0.028													0.002
27		0.014													0.040
27.2		0.197													0.002
28		0.086													0.032
28.2		0.133													0.004
29		0.056													0.006
29.2		0.116													
30		0.058													
30.2		0.082													
31		0.036													
31.2		0.036													0.002
32		0.022													
32.2		0.042													
33		0.024													
33.2		0.018													
34		0.014													
34.2		0.018													
35		0.006													
36		0.008													
36.2		0.002													
46.2															0.002

Table 2: Statistical parameters of 15 STR in beninese population

Stat	D8S1179	D21S11	D7S820	CSF1PO	D19S433	vWA	TPOX	D18S51	D3S1358	THO1	D13S317	D16S539	D2S1338	D5S818	FGA
N	251	251	251	250	251	251	251	251	251	251	251	251	251	251	251
Minimum Fréq	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
A	11	21	12	12	16	13	9	26	13	11	7	10	12	9	24
Ho	0.78	0.86	0.74	0.8	0.84	0.8	0.77	0.88	0.73	0.71	0.63	0.8	0.89	0.72	0.86
He	0.79	0.9	0.83	0.81	0.86	0.84	0.8	0.93	0.83	0.82	0.69	0.83	0.89	0.74	0.88
h	0.21	0.10	0.17	0.19	0.14	0.16	0.20	0.07	0.17	0.18	0.31	0.17	0.11	0.26	0.12
HWE	0.828	0.000	0.000	0.024	0.706	0.311	0.074	0.000	0.008	0.000	0.692	0.700	0.991	0.001	0.000
PIC	0.76	0.89	0.81	0.79	0.85	0.82	0.77	0.92	0.81	0.80	0.63	0.80	0.88	0.70	0.87
PD	0.89	0.98	0.93	0.92	0.96	0.94	0.91	0.99	0.93	0.92	0.75	0.93	0.97	0.83	0.97
PE	0.56	0.71	0.49	0.60	0.68	0.60	0.54	0.75	0.48	0.44	0.33	0.60	0.78	0.46	0.71

N-Patient's number
Minimum Allele Frequencies
A- Number of Allele
Ho-Observed Heterozygosity
He-Expected Heterozygosity
H- Homozygosity
HWE-HE-Hardy-Weinberg Heterozygote Excess test
PIC-Polymorphism Information Content
PD-Power of Discrimination
PE-Power of Exclusion

Discussion

Benin population is very diverse, with a large number of alleles on loci. The most polymorphic locus possesses 26 alleles, and 60% of the loci have at least 12 alleles. For nine (09) loci out of the 15 studied (except for D21S11, D7S820, D18S51, THO1, D5S818 and FGA), Benin population is in Hardy-Weinberg balance. The D21S11, D7S820, D18S51, THO1, D5S818 and FGA loci differed from the equilibrium state of Hardy-Weinberg after the Bonforroni correction. The reasons that could explain these deviations may be related to the sample size and the population structure. Moreover, the Benin population has several ethnic groups with different anthropological origins. This gap of the balanced state on six loci thus shows the genetic diversity in the Benin population. This ethnic mixing results in the emergence of new genetic profiles, which could explain the steady-state differences observed in the Hardy-Weinberg tests and justify that the minimum copy of alleles (minimum allelic frequency) is not reached at several loci.

Some populations with homogeneous or inbred marriages are generally in balance on almost all loci, for example in population of Guinea Bissau or on 13 out of 15 loci in Moroccan population (Goncalves, 2002 ; El Osmani 2007) . In a study in Morocco, Bouadellath et al. also validated 15 STRs in Moroccan population and obtained heterogeneity values ranging as follow, 0.7226 for the CSF10 locus, 0.8142 for the D2S1338 locus associated with a combinated –discrimination power greater than 0.9999 (Bouabdellah and al., 2008) . The probability of excluding paternity varied from 0.395 (TPOX) to 0.652 (D19S433) with a probability of excluding paternity greater than 0.9999.

Except for the differences in alleles, the results obtained by these authors are almost comparable to ours. All this showed the interest of the 15 STRs in the African Maghreb and sub-Saharan population. Our results confirmed the significant discriminating potential of these markers, with a significant value at the D18S51 locus, which is consistent with the results of Shepard and Herrara in a study of the Iranian population and H. El-Ossmani in a study carried out in Morocco (Shepard and al. 2006).

These loci have significant exclusion and discrimination powers. The values of the combined - discrimination and exclusion powers of these 15 loci close to 1, show that they are very useful for paternity investigations, individual identifications and forensic applications.

Conclusion

Our results confirmed that STRs are discriminating for filiation tests in the Beninese population. The results of this study showed that STR markers are handy in forensic and legal context. This is very useful for solving the social and moral problems associated with the search for paternity among the Beninese population and for human remain identification.

Source of Funding: None.

Conflict of Interest: None.

References

1. Applied Biosystems, USA, 2001: AmpFl STR Identifiler TM User's Manual.
2. Budowle B, Moretti TR, Niezgoda SJ, Brown BL. 1998: CODIS and PCR-basedshort tandem repeat loci: law enforcement tools. Procedings of the Second European Symposium on Human Identification; Innsbruck, Austria, June 1998 Madison, WI: Promega Corporation;73-88.
3. Butler JM. 2006. Genetics and genomics of core short tandem repeat loci used in human identity testing. *J Forensic Sci* 51:253-65.
4. Butler JM. 2014. Advanced topics in forensic DNA typing: interpretation, 1st Edition , Elsevier Academic Press , San Diego CA.
5. El Osmani H, Bouchrif B, Talbi J, El Amri H, Chafik A. 2007. La diversité génétique des 15 STR chez la population arabophone de Rabat-Salé Zemmour –Zeer, Antropo 15:(55),55-62.
6. Bouabdellah M , Ouenzar F, Aboukhalid R, Elmzibri M , Squalli D, S. Amzazi S. 2008. STR data for the 15 AmpFlSTR Identifiler loci in the Moroccan population. *Forensic Science International*: Genetics Supplement Series 1 306–8
7. Goncalves R , Jesus J, Fernandes AT , Brehm A.2002. Genetic profile of a multi-ethnic population from Guinée-Bissau (west African coast) using the new PowerPlex1 16 System kit. *Forensic Science International* 129:78–80
8. National Institute of Statistics and Economic Analysis of Benin . www.insae-bj.org.
9. Keenan K, Mc Ginnity P, Cross T F, Crozier W W and Prodohl PA. 2013. DievRsirt : An R package for the estimation of population genetics parameters and their associated errors. *Methods Ecol Evol* 2013: doi 10.1111/2041-210X.12067
10. Shepard, EM and Herrara RJ. 2006. Iranian STR variation at the fringes of biogeogrophical demarcation. *Forensic Sci Int* 158:140-8.
11. Weir BS.1996, Multiple tests. Dans: Genetic data analysis II, Sinauer Associates, USA,134:109-10.

How to cite this article: Azonbakin S, Bigot C, Adjagba M, Debaly M, Darboux R, Laleye A. Values of 15 STR in Beninese population's filiation test. *Int J Forensic Med Toxicol Sci* 2019;4(4):127-9.