International Journal of Legal Medicine
https://doi.org/10.1007/s00414-019-02243-6

POPULATION DATA

Genetic characterization of 19 X-STRs in Sierra Leone population from Freetown

Lin Lin[1,2] · Jienan Li[3] · Yize Hu[4] · Han Wang[5] · Foday Ambrose Marah[6] · Moses Moseray[7,8] · Aliye Kureshi[3] · Chudong Wang[3] · Moutanou Modeste Judes Zeye[3,9] · Lagabaiyila Zha[3,10]

Received: 12 October 2019 / Accepted: 19 December 2019

Abstract

A total of 550 individuals (265 males and 285 females) from Sierra Leone, a west-African coastal country, were genotyped using the Microreader™ 19X ID System kit. No significant deviations from the Hardy-Weinberg equilibrium were observed. A total of 250 alleles were identified with corresponding allele frequencies spanning from 0.0012 to 0.6762. PIC of the loci ranged from 0.4615 to 0.9481. The CPE, CPD_F, and CPD_M were 0.9999997856, 0.999999999999999999995774, and 0.999999999998997, respectively. The highly combined MEC_{Kruger}, $MEC_{Kishida}$, $MEC_{Desmarais}$, and $MEC_{Desmarais\ Duo}$ were achieved as 0.99999992508, 0.999999999990802, 0.999999999990836, and 0.99999998412, respectively. Genetic comparisons revealed that genetic homogeneity existed in similar ethno origin or geographic origin populations. This is a pioneering genetic investigation using the Microreader™ 19X ID System kit in the population of Sierra Leone.

Keywords Population study · X chromosome · Short tandem repeat · Sierra Leone

Introduction

Sierra Leone is a coastal country in west Africa (Fig. S1) with a total area of 71,740 km^2 and 7,075,641 individuals, according to the 2015 census. The capital and largest city is Freetown. Sixteen ethnic groups with their own languages and customs inhabit Sierra Leone, including the two largest and most influential, Mende and Temne. A total of 77% of Sierra Leone individuals are Muslim and 22% are Christian [1]. X chromosomal short tandem repeat (STR) loci combine the characteristics of uniparental and autosomal genetic markers and therefore possess highly desirable features for paternity testing, especially in deficiency paternity cases with female offspring [2]. Numerous population genetic databases

Lin Lin and Jienan Li contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00414-019-02243-6) contains supplementary material, which is available to authorized users.

✉ Lagabaiyila Zha
40409716@qq.com

1 Xiangya Hospital, Central South University, No87. Xiangya Road, Changsha, Hunan, People's Republic of China

2 Reproductive Medicine Center, Fujian Provincial Maternity and Children's Hospital, No18. Daoshan Road, Fuzhou, Fujian, People's Republic of China

3 Department of Forensic Science, School of Basic Medical Sciences, Central South University, No172. Tongzipo Road, Changsha 410013, Hunan Province, People's Republic of China

4 Wuxi Municipal Public Security Bureau, Wuxi, Jiangsu, People's Republic of China

5 Department of Clinical Diagnostic Centre, the Fifth Medical Centre, Chinese PLA (People's Liberation Army) General Hospital, Beijing, People's Republic of China

6 The GREY Bush Community Health Center, Grey Bush, Ascension-town, Sierra Leone

7 Joint Medical Unit, Freetown, Sierra Leone

8 Clinical Laboratory, 34 Military hospital, Freetown, Sierra Leone

9 Laboratory of Molecular Biology and Genetics (LMBG), University Joseph KI – ZERBO, CERBA/LABIOGENE, 01 BP 364, Ouagadougou 01, Burkina Faso

10 China-Africa Research Center of Infectious Deseases, Central South University, Changsha, People's Republic of China

of X-STR loci have been reported all over the world, while the genetic polymorphisms and forensic characterization in Sierra Leone remain unexplored. This study used the Microreader™ 19X ID System kit in a Sierra Leone population.

A total of 550 individuals (265 males and 285 females) from Freetown were included in this study. All gave informed consent. Blood samples were collected on Whatman FTA cards and kept at 4 °C. Isolation of genomic DNA was carried out using the Chelex-100 method. The multiplex PCR amplification was performed using the Microreader™ 19X ID System kit (Microread Genetics Co, Suzhou, China), including DXS6795, DXS6803, DXS6807, DXS9907, DXS7423, GATA172D05, DXS101, DXS9902, DXS7133, DXS6810, GATA31E08, DXS6800, DXS981, DXS10162, DXS6809, GATA165B12, DXS10079, DXS10135, and HPRTB, in a single PCR multiplex reaction on the GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Standard DNA 9947A and ddH_2O were used as positive and negative control respectively. PCR products were detected and separated on an ABI PRISM 3130xl Genetic Analyzer by capillary electrophoresis. The fragment sizes and genotypes were determined using GeneMapper v.3.2 software (Applied Biosystems, Foster City, CA, USA) in comparison with allelic ladders provided in the kit. Our experiments were carried out in line with the ethical guidelines of the Grey Bush Community Health Center, Ascension Town, Sierra Leone.

Allele frequencies were analyzed by the counting method in Microsoft Excel 2013. Polymorphism information content (PIC), heterozygosity (HET), power of exclusion (PE), power of discrimination in females (PD_F), power of discrimination in males (PD_M), and mean exclusion chance (MEC_{Kruger}, $MEC_{Kishida}$, $MEC_{Desmarais}$, and $MEC_{Desmarais\ Duo}$) based on allele frequencies were calculated using the online calculation tool on the ChrX-STR.org 2.0 database (http://www.chrx-str.org) [3]. Exact test of the Hardy-Weinberg equilibrium (HWE) in female samples, exact test of linkage disequilibrium (LD) in male samples, and likelihood ratio test of LD in female samples were calculated using the ARLEQUIN software version 3.5. The coefficient of gene differentiation based on F_{ST} genetic distance [4] was compared with populations from Africa, Latin America, Europe, and Asia using the POPTREE2 software [5] and visualized as a Neighboring-Joining tree.

As no significant differences in allele distributions between male and females were found for the 19 loci, samples were pooled and allele frequencies and the forensic statistic parameters are presented in Table S1. A total of 250 unique alleles were observed. DXS10135 was the most informative locus (PIC = 0.9481) with 60 unique alleles and DXS6807 was the least informative (PIC = 0.4615) with 8 alleles. Novel off-ladder alleles were observed at HPRTB (11.3), DXS10135 (42, 42.2), DXS10079 (12.3, 12.2), DXS10162 (11), DXS981 (9), DXS6803 (7), and DXS6795 (17) in our study.

All 19 X-STR loci in female samples were in Hardy-Weinberg equilibrium (Table S2) after Bonferroni correction ($p < 0.05/19 = 0.0026$). The PE, PD_F, and PD_M varied from 0.1778 to 0.8989, 0.7113 to 0.9953, and 0.4887 to 0.9503, respectively. The CPE, CPD_F, and CPD_M were 0.9999997856, 0.99999999999999999995774, and 0.999999999998997, respectively. The high combined MEC_{Kruger}, $MEC_{Kishida}$, $MEC_{Desmarais}$, and $MEC_{Desmarais\ Duo}$ were achieved as 0.99999992508, 0.999999999990802, 0.999999999990836, and 0.99999998412, respectively.

For a significance level of 0.00029 (after the Bonferroni correction for 171 comparisons) in the exact test of linkage disequilibrium (LD) between all pairs of loci in male samples, a significant p value ($p < 0.0001$) was obtained only for DXS101 and DXS7423 (Table S3). Generally, the significant p value may be associated with random genetic drift, founder effect, mutations, natural selection, and recent population admixture events or stratifications [6]. Considering the physical distance of DXS101 and DXS7423 is 48.16 Mb (Table S4) from the ChrX-STR.org 2.0 database (http://www.chrx-str.org) [3], we think that the sampling error is the reason we detected the linkage disequilibrium. Thus, further population analysis would be necessary to clarify precise LD relationships about these loci. For the same significance level of 0.00029, no significant p values were found in the likelihood ratio test in female samples (Table S5). Based on the existent data and these two tests, we can consider that all X-STR loci in the Microreader™ 19X ID System kit are independent of each other, which could maximize the combined polymorphism and diversity index and increase the combined power of individual discrimination and the combined mean exclusion chance level of this kit.

FST genetic distance was tested based on 6 overlapped X-STR loci to measure a genetic differentiation among other populations (Table S6). A Neighboring-Joining tree was constructed to evaluate the phylogenetic relationships between Sierra Leone and 18 other populations. Two main clusters were grouped and African countries formed the upper cluster (Fig. S2). It is worth noting that Sierra Leone was first grouped with southern Sahara Desert countries (Malawi, Equatorial Guinea, Uganda) and then northern Sahara Desert countries (Morocco and Tunisia), which indicated that the population migration and social integration process between the north and south sides of the Sahara Desert were impeded by the desert itself. The results of the bottom cluster countries were generally in accordance with their biogeographic distributions.

In conclusion, our study is pioneering for the use of Microreader™ 19X ID System kit in Sierra Leone. The results suggest that the Microreader™ 19X ID System kit is highly polymorphic and informative and can be considered to be a powerful tool in complex forensic casework, kinship analysis, and human identification in the Sierra Leone population. Population comparisons generally mirror their historical relationships and geographic location on the world scale.

Acknowledgments This study was financially supported by the National Natural Science Foundation of China (NSFC, no. 81871533), Natural Science Foundation of Hunan Province (2017JJ3422), Postdoctoral Science Foundation of Central South University (229795), and Shanghai Key Laboratory of Forensic Medicine Open Project (KF1815).

Compliance with ethical standards

Our experiments were approved by the Ethics Committee of The Grey Bush Community Health Center, Ascension-town, Sierra Leone.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Statistics Sierra Leone (2015) Sierra Leone 2015 Population and Housing Census Enumerator's Manual, pp 16–19
2. Szibor R (2007) X-chromosomal markers, past, present and future. Forensic Sci Int Genet 1:93–99
3. Szibor R, Hering S, Edelmann J (2006) A new web site compiling forensic chromosome X research is now online. Int J Legal Med 120: 252–254
4. Latter BDH (1972) Selection in finite populations with multiple alleles III Genetic divergence with centripetal selection and mutation. Genetics 70(3):475–490
5. Takezaki N, Nei M, Tamura K (2009) POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. Mol Biol Evol 27(4):747–752
6. Chakravarti A (1999) Population genetics: making sense out of a sequence. Nat Genet 21:56–60