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Short communication

Genetic characterization of Guinea-Bissau using a 12 X-chromosomal STR system: Inferences from a multiethnic population

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ABSTRACT

A male West African sample from Guinea-Bissau (West-African coast) was genetically analyzed using 12 X chromosomal short tandem repeats that are grouped into four haplotype groups. Linkage disequilibrium was tested ($p \leq 0.0008$) and association was detected for the majority of markers in three out of the four studied haplotype clusters. The sample of 332 unrelated individuals analyzed in this study belonged to several recognized ethnic groups ($n = 18$) which were used to evaluate the genetic variation of Guinea-Bissau's population. Pairwise genetic distances (F_{ST}) did not reveal significant differences among the majority of groups. An additional 110 samples from other countries also belonging to West Africa were as well compared with the sample of Guinea-Bissau. No significant differences were found between these two groups of West African individuals, supporting the genetic homogeneity of this region on the X chromosome level. The generation of over 100 DNA West African sequences provided new insights into the repeat sequence structure of some of the present X-STRs. Parameters for forensic evaluation were also calculated for each X-STR, supporting the potential application of these markers in typical kinship scenarios. Also, the high power of discrimination values for samples of female and male origin observed in this study, confirms the usefulness of the present X-STRs in identification analysis.

1. Introduction

The X chromosome has distinct genetic features when compared to the autosomes and to the Y-chromosome. X chromosome STRs are hemizygous in males, thus behaving as a haplotype such as the Y chromosome, but in females they resemble autosomes due to recombination. Owing to the way in which it is inherited, the X chromosome is a rich resource of easily accessible genetic data, and therefore provides a unique tool for population-genetic studies [1]. X-STRs seem to be more effective than autosomal markers in the identification of female traces in male contamination [2]. In several other occasions, like case-studies involving identification of skeletons or victims of mass disasters where only remote relatives are available and females play a key role in identification, X-STRs can also be more efficient than autosomal ones [3]. The same is true in deficiency paternity cases, for example, relationship can be excluded when comparing potential half-sisters, even without the presence of both parents.

Four linkage groups of X chromosome markers are recognized in the commonly used commercial X-STR kit, the Investigator Argus X-12 kit [4]: linkage group 1: DXS10148, DXS10135 and DXS8378; linkage group 2: DXS7132, DXS10079 and DXS10074; linkage group 3: DXS10103, HPRTB and DXS10101; linkage group 4: DXS10146, DXS10134 and DXS7423. Each group of markers can be treated as a haplotype which may provide useful information for human identification, especially for paternity or other kinship testing [3]. On the other hand, it has been shown that STRs are also useful markers to infer ethnicity although the degree of correct assignment varies considerably depending on the degree of historical and ethnical relationships [5].

Guinea-Bissau is a very small western-African country, about 37000 km^2, with 1.6 million inhabitants, speaking about 25 different languages, and an equal number of recognized ethnic groups [6–8]. Animists constitute the vast majority of the population (55%) followed by Islamites (40%). Settling started around 40000 years before present [9]. Around 9000 YBP several Neolithic cultures flourished in the region bringing in peoples from both Sub-Sahara and North Africa origin. Around 4000 YBP the region received the income of Bantu peoples,

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mostly coming from the Gulf of Guinea area. In the 9th century there is a documented admixture with Berbers following the arrival of the first pastoral Peuls or Fulbe (here designated by Fula). In the 15th century most of the different groups were already in place [8,10–12]. The Balanta constitute the major ethnic group from Guinea-Bissau (30%) and have an oral tradition of being originally from East Africa (Sudan and Ethiopia) migrating to West Africa between the 10–14th centuries. During the 16th century they settled in the territory which is today Guinea-Bissau. Mandinga (Mandenka) is one if not the major ethnic group in West Africa with an estimated population of 11 million. They descend from the Mali Empire (12–13th centuries) and were originally animists later Islamized by the Fula [8,10–12]. They belong to the biggest ethnolinguistic group of West Africa – the Mandé (https://www.ethnologue.com/). This is the main ethnic group from which most of the slaves to the Americas came from. The Fula (Fulbe, Peuls) constitute today the second ethnic group in number (25%). Totally Islamized, they are a heterogeneous group, originally coming from the Sahel region. The Fula Islamized the Mandinga and both pushed all other groups towards the coastal and southern regions of today's Guinea-Bissau. Around the 17th century they already dominated the whole area [11–13]. Manjacos, Mancanhas, Papel and Brame are closely related groups (some even claim that they are just different clans from the same ethnic group) [14]. This animist group accounts for 24% of the population inhabiting coastal areas but is considered autochthonous at least since the 15th century [11]. In spite of the relatively small population, Guinea-Bissau has today a huge collection of ethnic groups, speaking different dialects and languages and most importantly with different religious beliefs and traditions (see Supplementary Figs. S1 and S2 and Supplementary table S1 for a comprehensive review).

X-STR population genetic data is still limited for some populations, in particular of African origin. We therefore aimed to present the results obtained for 12 X-STRs for a male population sample from Guinea-Bissau. The genetic variation of X-STRs complements previous studies done on the same population group using other markers namely HLAs, mtDNA, Y-Chromosome SNPs and STRs, and autosomal STRs [15–19]. Moreover the typing of specific sub-Saharan populations such as the Guinean-Bissau presented here has an obvious forensic interest in other Western countries, such as Portugal, where a significant immigration population exists representing 4% of the total population [20,21] and for which ethnic specific databases can be extremely valuable.

2. Materials and methods

2.1. Samples, DNA extraction and quantification

Blood samples were obtained from 332 unrelated and healthy male individuals from Guinea-Bissau (West African coast, Senegambia region). All individuals gave informed consent and were submitted to an interview in order to be linked to a given ethnic group (Supplementary table S1). A complete description of the Guinean ethnic groups, languages and linguistic affiliation according to major language groups, as well as religious beliefs and geographical distribution, has been published in detail elsewhere [18] but updated here [see Supplementary Figs. S1, S2]. Only individuals that could unambiguously trace the origin of their parents and ascendants back to three generations as belonging to the same ethnic group were used in the present study. Finally, 18 groups were used for the data analyses (Supplementary table S1). An additional 110 individuals (31 females and 79 males) belonging to other countries from West Africa (namely from Senegal, Gambia, Guinea, Serra Leo, Liberia, Ghana, Togo, Benin, Nigeria and Niger) were also included in this study for population comparisons. Only samples obtained with informed consent for research purposes were selected.

DNA from the Guinea-Bissau samples was extracted from whole blood samples using a traditional salting-out methodology. The other West African samples were extracted using the EZ1 Investigator kit and the EZ1 Advanced automated nucleic acid purification system according to the manufacturer's protocol (Qiagen GmbH, Hilden, Germany). DNA concentrations were estimated using the NanoDrop® 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) following the manufacturer's protocol.

2.2. DNA amplification and detection of alleles

PCR amplification of X-STR markers was performed using the Investigator® Argus X-12 kit according to the manufacturer's instructions (Qiagen GmbH, Hilden, Germany) [4]. The following twelve X-STRs were amplified: DXS10103, DXS8378, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148. These 12 loci are distributed along the X chromosome and grouped under four linkage groups: linkage group 1 comprises DXS10148, DXS10135 and DXS8378; linkage group 2 comprises DXS7132, DXS10079 and DX10074; linkage group 3 is formed by DXS10103, HPRTB and DXS10101 and finally linkage group 4 by DXS10146, DXS10134 and DXS7423. Fragment length determination and allele designation were performed using the allelic ladder, internal size standard and positive controls included in the Argus X-12 kit. Detection of amplified fragments was performed by standard capillary electrophoresis using an ABI PRISM® 3130 Genetic Analyzer (Life Technologies, Darmstadt, Germany) and analyzed with GeneMapper® ID Software v3.2.

2.3. Sequencing of X-STR alleles

A total of 105 samples were directly sequenced by traditional Sanger sequencing due to the identification of silent (null), rare/new or uncertain genotypes of some alleles such as intermediate alleles (Supplementary table S2). Sequences were obtained for the following 6 X-STRs: DXS10101, DXS10148, HPRTB, DXS10079, DXS10146 and DXS10135 (Supplementary table S2). All details regarding the sequencing method and conditions used were the same as the ones described in the sequencing study of Gomes et al. [22]. In this previous study [22], sequencing primers used for DXS10148 were the same used in the present work. Sequencing primers for the other 5 loci (DXS10101, DXS10135, DXS10146, DXS10079 and HPRTB) were either redesigned or chosen form the literature if suitable. Descriptions of all primers used for the sequencing reactions as well as references of nomenclatures used for allele assignment are listed in Supplementary table S2.

2.4. Statistical analysis

Basic genetic parameters such as allele and haplotype frequencies, gene diversities (same as the power of discrimination in males; PD_M) and linkage disequilibrium (LD) testing between loci were determined using the Arlequin 3.5.2.2 software [23]. In addition, pairwise genetic distances (F_{ST}) were also calculated among the Guinean-Bissau ethnic groups as well as with an additional population sample from West Africa using the same software tool. F_{ST} is based on the number of different alleles and was chosen as an alternative to R_{ST} which takes into account the "stepwise mutation model" behind the formation of STRs. The main reason is that the majority of X-STRs here studied have many intermediate alleles and at high frequencies. Non-consensus alleles likely originate from an insertion/deletion rather than a single step mutation and therefore R_{ST} is not appropriate. Forensic efficiency parameters such as expected probability of exclusion in trios with female offspring (PE_T); expected probability of exclusion in father/daughter duos (PE_D); power of discrimination in females (PD_F) and power of discrimination in males (PD_M) were calculated using the formulae according to Desmarais et al. [24]. In addition, the expected probability of exclusion in two half-sisters (PE_{HS}) has also been calculated according to the formula in Gomes et al. [25].