International Congress Series 1288 (2006) 271–273

www.ics-elsevier.com

The Amelogenin locus displays a high frequency of X homologue failures in São Tomé Island (West Africa)

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Abstract. A multiplex STR study using the Powerplex 16 System (Promega) in 503 unrelated individuals from the island of São Tomé (Gulf of Guinea, West Africa) revealed 10 male individuals presenting only the Y homologue of the Amelogenin locus (~2%). These individuals were further typed with other commercial kits which also amplify the Amelogenin locus, namely the AmpFLSTR Identifiler (Applied Biosystems) and Y-Plex 12 (Reliagene) kits, and an X/Y genotype was only obtained with the primers used in Y-Plex 12. Although this X Amelogenin drop-out was only detected in males, this does not rule out the fact that females may also carry it. Sequencing of the X Amelogenin allele responsible for the amplification failure in these male individuals revealed a C to T substitution at position 294 of GeneBank sequence M55418, corresponding to the Powerplex 16 forward primer binding site.

Keywords: Amelogenin locus; Null allele; São Tomé Island

1. Introduction

The detection of STR allele sequence variants has become more and more common since many laboratories use commercial kits from different manufacturers, which may amplify the same loci but with distinct primer pairs. If a sequence variation is present on the annealing site of a primer, then it is expected that little or no amplification will occur, depending on which position the variation is located.

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0531-5131/
doi:10.1016/j.ics.2005.10.036

Apart from STR loci, most of the forensic genetic kits also include the Amelogenin locus for sex determination. This locus is present in both the X and the Y chromosomes and the distinction is made by size: the fragment amplified from the X chromosome is 6 bp smaller than the one from the Y chromosome. Failure to amplify alleles in the Amelogenin locus has been described before (e.g. [1–4]), mainly in cases where the Y homologue fails, which can have critical consequences in forensic casework. In this work, we report amplification failures observed on the X homologue in 10 male individuals, during a STR population study of 503 unrelated individuals from the island of São Tomé (Gulf of Guinea, West Africa) with the Powerplex 16 System (Promega). The sequence variation was detected and it is located in the Powerplex 16 forward primer binding site.

2. Material and methods

DNA samples from 503 unrelated individuals were amplified with the Powerplex 16 System (Promega) according to the manufacturers' instructions. Male samples showing no amplification of the X homologue of the Amelogenin locus ($N=10$), were further typed with the AmpFLSTR Identifiler (Applied Biosystems) and Y-Plex 12 (Reliagene) kits, also according to the manufacturers. Genotyping was carried out on an ABI 310 Genetic Analyser (AB Applied Biosystems) with the appropriate software.

For sequencing purposes, amplification of these samples was performed with the following set of primers (see Fig. 1): forward 5′-ACCTCATCCTGGGCACCCTGG (same sequence as Promega's Monoplex Amelogenin forward primer) and reverse 5′-ATCAGAGCTTAAACTGGGAAGCTG (same sequence as Promega's Powerplex 16 and Reliagene's Y-Plex 12 Amelogenin reverse primers). PCR conditions were as follows: 10 ng DNA in 25μl total reaction volume containing 2mM $MgCl_2$, 1× PCR Taq Gold buffer, 0.5 U Taq Gold polymerase (AB Applied Biosystems), 200 μM of each dNTP and 0.5 μM of each primer. PCR reaction was undertaken in a GeneAmp PCR System 2700 (AB Applied Biosystems) with a pre-incubation step at 95 °C for 7 min, 32 cycles of 94 °C/30s, 58 °C/30 s and 72 °C/30 s; 45 min final extension at 60 °C.

Protocols for elution of fragments from polyacrylamide gels and purification of PCR products are described elsewhere [5]. The sequencing reactions were done with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (AB Applied Biosystems) according to the manufacturer. Samples were run on an ABI 3100 Genetic Analyser (AB Applied Biosystems) and analysed with the appropriate software.

3. Results

During population analysis of 503 individuals from São Tomé with the Powerplex 16 System, 10 cases of male individuals showed only the Y homologue fragment of the Amelogenin locus. This

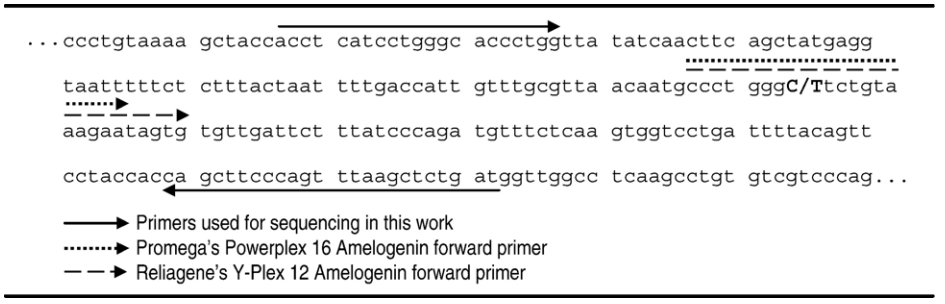

Fig. 1. Part of GeneBank sequence M55418 corresponding to the X homologue of the Amelogenin locus. The variation found is presented in bold and arrows correspond to primers as described below.

was further confirmed by typing these samples with the AmpFLSTR Identifiler kit (AB Applied Biosystems). A full X/Y genotype was only obtained with the Y-Plex 12 kit (Reliagene). Sequencing of the X fragment was undertaken with the primers described above and shown in Fig. 1, revealing a C to T substitution at position 294 of GeneBank sequence M55418 (Fig. 1). This variation was enough to prevent amplification of the X fragment with the Powerplex 16 primers, since it lies inside the forward primer binding site. However, this variation does not influence the amplification with the Reliagene's forward primer, which has the same sequence as the Powerplex 16 forward primer, but is 5 bp longer (Fig. 1). Since we do not have access to the Identifiler primer sequences and since X amplification was not obtained with this kit, we assume that the Identifiler's Amelogenin forward primer also lies in this region and probably has the same sequence as the Powerplex 16 forward primer.

Although this X Amelogenin drop-out was only detected in males, this does not rule out the fact that females may also carry it. Since women have two X chromosomes, in some instances it could be suspected that an X failure was also present in females, by simple observation of differences in electrophoregram peak heights, in comparison with XY profiles. Although we cannot objectively consider these apparent nulls in females for frequency estimate purposes, there is no doubt of its magnitude in this population. With a 2% frequency in males, it is expected that the frequency of female carriers and homozygotes will be 3.92% and 0.04%, respectively. It is also noteworthy that the previously reported frequency for an X Amelogenin null (which was estimated in Caucasians [6]) was much lower (0.3%), but the variation is different than the one reported here (present in the annealing region of Powerplex 16's reverse primer).

Failure to amplify alleles in the Amelogenin locus has been widely described mainly in cases where the Y homologue fails, which can have critical consequences in forensic casework. Cases where the X counterpart fails to amplify, as described here, are not of fundamental significance in forensic genetics, since there is no danger of a male individual being mistaken for a female one. However, this can have a different impact in other fields, such as in prenatal diagnosis of certain XY chromosome abnormalities, like XXY, using quantitative assays.

The high frequency of amplification failures already detected for either the X or Y chromosome Amelogenin locus only draws our attention more to the need for caution when applying solely the Amelogenin test for sex determination.

Acknowledgements

Financial support was granted by Fundação para a Ciência e Tecnologia, Programa Operacional Ciência, Tecnologia e Inovação (POCTI); research grant POCTI/42510/ANT/2001.

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