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Gender determination in highly degraded DNA samples

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Abstract

Based on the method described in Stone et al.'s [Am. J. Phys. Anthropol. 99 (1999) 231] publication, a fragment of the amelogenin gene (exon 6) was shortened. In a new method, a flanked and amplified 78-bp fragment from X and Y copies of the amelogenin gene, followed by hybridization and ligation with fluorescent-labeled oligonucleotides, resulted in two gender-specific products analyzed by capillary electrophoresis. The method was tested using fresh DNA samples and DNA isolated from bones of different ages.

Keywords: Gender; Amelogenin; aDNA; Bone

1. Introduction

The main lesion of DNA occurring after death is hydrolytic modification–depurination followed by degradation to short fragments [2]. The most useful and known strategy for gender estimation is amplification of the short fragments of the amelogenin gene 106-bp of the X chromosome and 112-bp of the Y chromosome [6]. Highly sensitive known markers are DXZ3 and DYZ1 (130 and 170 bp), which are based on centromeric alphoid repeats [8] and can be comparable to mtDNA sensitivity (5000 copies for DXZ3 locus and 10–10,000 copies for mtDNA). The shortest known marker used for Y-typing is a 93-bp fragment of the SRY gene [4].

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2. Material and methods

DNA was isolated from the blood samples of 20 males and females, according to Walsh et al. [7]. From a 1-year-old fragment of skull bone, a 2-year-old tooth, two fragments of bone from the Bronze Age and a fragment of skull bone and one of long bone from the middle Neolithic, DNA was isolated using Ivanov et al.'s [1] method with slight modifications. All precautions pertaining to work with ancient DNA were taken as describe elsewhere. DNA was obtained from the bone samples from two independent extractions per specimen. Based on Stone et al.'s [5] publication, the fragment of the amelogenin gene [3] from exon 6 in the X (accession no. X14440, nucleotides 2277–2356) and Y (accession no. X14439, nucleotides 2097–2176) chromosomes was shortened. The newly designed primers AMELEXT F and AMELEXT R flank a 78-bp region which differs in seven SNPs between the X and Y copies of the amelogenin gene. The targeted sequence and the underlined primers are:

X: **CAGCCACCTCTGCCT**CCGATGTTCCCCATGCAGCCCCTGCCTCCCATGC
Y: …….A…….C……G…….C…….A..
↓ [ligation point]
X: TTCCTGATCTGAC**TCTGGAAGCTTGGCCA**
Y: ……CA……...
↑

Amplification was performed in a 15-μl reaction volume with 10 × PCR buffer II (PE Applied Biosystems), 200 μM of dNTPs, 15 mM MgCl, 1 μM of each primer and 1 U of AmpliTaq Gold Polymerase (PE Applied Biosystems). Thirty-five cycles of amplification were performed at 95 °C for 10 min, 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min. At least two separate PCRs were performed for the old samples. The hybridization and ligation were conducted in a 10-μl volume with 10 × ligation buffer, 1 mM of ATP and 0.5 U of thermostable Pfu ligase (Stratagene). Two pairs of oligonucleotides were used:

AMGLIG X ATGGCCAAGCTTCCAGAGT 6-Fam labeled
LX CAGATCAGGAAGCATGGGAGGCAGGGGCTGCATGGGGAACATC
AMGLIG Y CTGGCCAAGCTTCCAGATG Joe labeled
LY CAGATCAGGAAGTATGGGGGGCAGGGGCCGCAGGGGGAACAT

The process consisted of 25 cycles of denaturation at 95 °C and hybridization at 60 °C with subsequent overnight ligation at 4 °C. Analysis of the PCR products was performed using capillary electrophoresis on an ABI PRISM 310 genetic analyzer with ROX 350 as an internal marker of migration. The Gene Scan version 2.1 Collection software was employed (PE Applied Biosystems).

3. Results

The 78-bp blue peak for the X- and green for the Y-derived fragments were observed on the Gene Scan plots. The genders of 20 persons were correctly determined. The data obtained from the forensic and anthropological samples were consistent with those estimated by anthropologists.

4. Discussion

In this study, we described an alternative method of gender estimation in highly degraded DNA samples in cases where the routinely used method [6] failed to yield a result or gave ambiguous results. To increase of sensitivity, the flanked fragment was shortened, although after such shortening of the fragments from the X and Y chromosomes, the sensitivity usually increases but the specificity decreases. The possibility of contamination of the PCR increases due to the high sensitivity; moreover, an interpretation of the results is only possible if all controls are included and a separate series of amplifications from independent isolations are performed. The same amplification product can be detected with the SnaPshot technique when the A/C SNP in nucleotides 2339 and 2159 on the X and Y copies, respectively, are used. A reverse AMELEX R primer can be utilized for extension where G is expected for the X and T for the Y copies.

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