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SHORT COMMUNICATION

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Detection of additional structural variation at the FES/FPS system and population data from S. Tomé e Príncipe and North Portugal

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Abstract Comparative analysis of heteroduplex patterns on the STR FES/FPS system led to the detection of a new non-consensus allele 10 in the African population of São Tomé e Príncipe. Automated sequencing confirmed a T→C substitution at position 177 (1stT of the 6th repeat) which was exclusively found in haplotypic combination with base A in the previously described polymorphic position 34. The new substitution was not detected in a sample from North Portugal. Sequence analysis revealed a triplet of inverted bases, from positions 101 to 103, relative to the sequence described in the GeneBank (Accession No. X06292). This work confirms the capacity of heteroduplex analysis in the detection of DNA structural microvariation and emphasises the complementary utility of manual system analysis and semi-automated techniques for a full characterisation of the genetic variability of STRs.

Key words FES/FPS · New non-consensus allele · Heteroduplex analysis · Population genetics

Introduction

FES/FPS is a well studied STR system currently used in paternity testing and forensic identification as well as in anthropological genetics (Hammond et al. 1994; Klintschar 1995; Iwasa et al. 1997). Until now, variation ranging from 7 to 15 consensus ATTT repeats and a single A→C substitution at position 34 in the 5′ flanking region has been reported (Möller et al. 1994).

As for most STRs used in forensic analysis, semi-automated techniques are routinely applied for FES phenotyping. Although this methodology offers some advantages over manual systems, it is not able to detect structural microvariation not affecting the size of DNA fragments. In this paper we present the results of the FES allele characterisation by heteroduplex analysis in the populations from S. Tomé e Príncipe (Western Africa) and North Portugal.

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Material and methods

Blood was collected from unrelated adults by venipuncture or from the umbilical cord of unrelated newborn infants. DNA was extracted from whole blood using the method of Lareu et al. (1994). PCR amplification was performed with the set of primers described by Polymeropoulos et al. (1991) using the following conditions: initial denaturation 95 °C – 2 min; 35 cycles at 94 °C – 30 s, 54 °C – 1 min, 72 °C – 1 min; final extension 72 °C – 5 min. PCR mixtures, electrophoretic conditions and visualisation of DNA fragments were as described in Luis and Caeiro (1995). Genotyping was made by side-by-side comparison with sequenced allelic ladders. Allele nomenclature followed the recommendations of the DNA Commission of the ISFH (1997).

For heteroduplex analysis, samples were denatured for 5 min at 95 °C and allowed to cool to room temperature. Samples from assumed homozygous phenotypes were previously mixed with a different kind of homozygous sample. For automated sequencing, after electrophoresis allele bands were cut individually from the gel and eluted in 50 µl of TE, frozen (–20 °C, 30 min) and thawed (65 °C, 15 min) 3 times, amplified as described and purified with Microspin S-200 HR columns (Pharmacia). The purified products were submitted to dideoxy cycling sequencing using the ABI PRISM Dye termitator kit of Perkin Elmer. Sequence analysis was performed on an ABI Prism 377 DNA Sequencer using the Data Collection Software 377–18. Hardy-Weinberg equilibrium was assayed by an exact test using the statistical software package GENEPOP (Raymond and Rousset 1995).

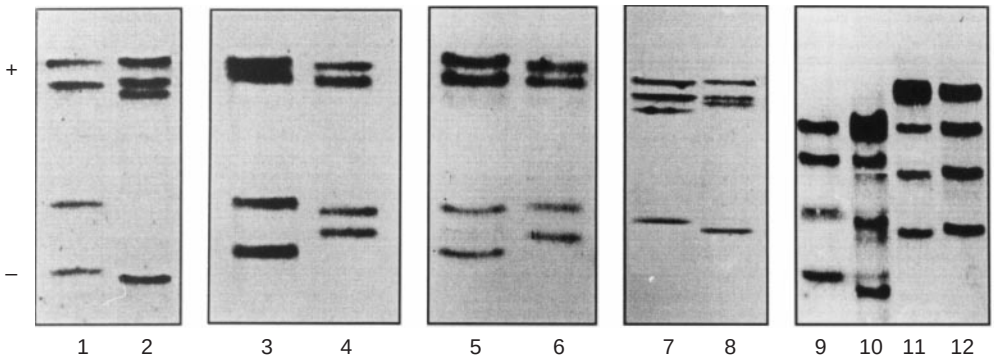

Fig. 1 Different types of heteroduplex patterns observed in some genotypes of FES/FPS. Lanes 1: 8–10 T(177), 2: 8–10 C(177), 3: 9–10 T(177), 4: 9–10 C(177), 5: 10 T(177)-11, 6: 10 C(177)-11, 7: 10 C(177)-12, 8: 10 T(177)-12, 9:10 T(177)-13, 10: 10 C(177)-13, 11: 7–10 T(177), 12: 7–10 C(177)

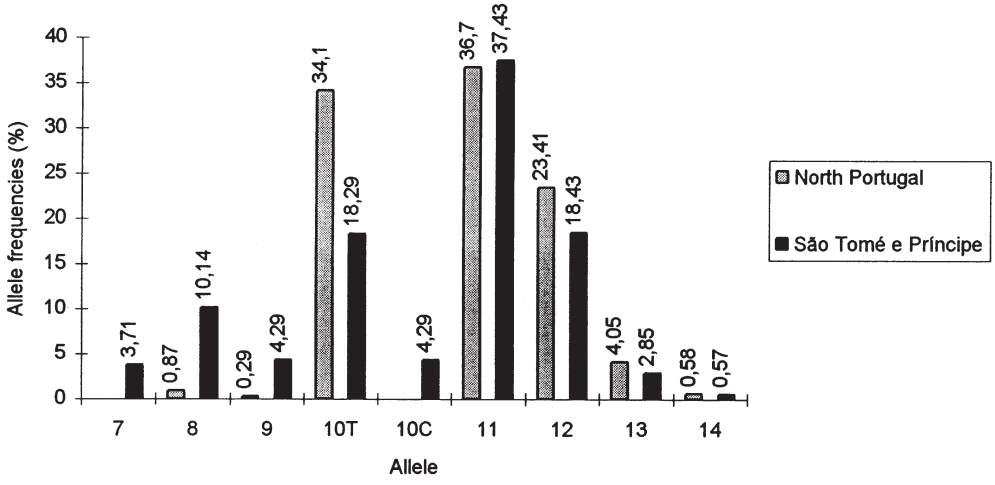

Fig. 2 Allele frequencies for FES/FPS in North Portugal and São Tomé e Príncipe

Results

Comparative analysis of heteroduplex mobility patterns in the São Tomé e Príncipe population sample revealed that whenever allele 10 was present, two distinct patterns of heteroduplexes were found for each genotype (Fig. 1). Sequence analysis of alleles from 12 individuals exhibiting the less common heteroduplex patterns, systematically revealed a T→C substitution at position 177 defining the non-consensus allele 10C(177) with the structure $(ATTT)_5A\mathbf{C}TT(ATTT)_4$. Alleles from 21 individuals presenting the most common heteroduplex patterns were also sequenced showing the presence of 10 consensus repeats – allele 10T(177). The newly detected substitution, that reached a frequency of 4.3%, was invariably associated with adenine at position 34 and never with cytosine. In the northern Portuguese sample no similar heterogeneous patterns were detected.

Allele frequency profiles for FES in both populations are shown in Fig. 2. The observed genotype distributions did not deviate significantly from Hardy-Weinberg expectations (p = 0.674, s.e. ± 0.008 for São Tomé e Príncipe; p = 0.263, s.e. ± 0.009 for North Portugal). In the course of this study 66 FES alleles chosen at random were sequenced, either from North Portugal or São Tomé e Príncipe. In all of these the sequence CTT was detected from base 101 to 103, which is not in accordance with the TTC inverted sequence described in the GeneBank (Accession No. X06292).

Discussion

In this work we confirmed the well known discriminating power of heteroduplex analysis concerning structural microvariation (Barros et al 1994) by the detection of a new point substitution in FES system that subsequent sequencing confirmed to be a T→C at position 177. However the previously described A→C(34) substitution could not be detected by the same analytical strategy, suggesting that the position and/or the kind of substitution is crucial for heteroduplex differentiation. The strong haplotypic association of bases C(177) and A(34) in allele 10 suggests that the T→C(177) transition presumably had a more recent origin then the ancestral allelic configuration –10A(34)- where the mutation occurred.

All sequenced alleles revealed a triplet of inverted bases relative to that described in the GeneBank. Since the existence of a TTC→CTT polymorphic inversion does not seem very likely, this result points to a more careful revision of the FES sequence.

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