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Validation of STR system FXIIIB for forensic investigation in a population of Central Italy

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Abstract. The principle purpose of this study was to investigate the frequency distribution of the short tandem repeat system FXIIIB in a sample of individuals from Tuscany (Central Italy). Samples (204), taken from donors residing in the area of Florence, Prato and Pistoia, were analysed by PCR and vertical denaturing polyacrylamide gel electrophoresis. A sensitivity study was also undertaken and the method was optimised for the study of forensic samples.

The research resulted in the identification of 5 alleles and 11 genotypes. The system was tested for Hardy–Weinberg equilibrium and the Central Italian population was compared with populations from Northern Italy and Austria. Moreover, we introduced the use of this microsatellite in an automatic platform, in combination with other STRs in a new multiplex amplification system. An infrared automatic DNA sequencer was used to analyse this marker. The introduction of this marker in our protocol could be useful to resolve particular intriguing cases of forensic interest, as for example incest or deficitary paternity/maternity tests, in which additional loci must be investigated.

Keywords: Short tandem repeat; FXIIIB; Population studies; Central Italy

1. Introduction

The STR FXIIIB is a tetranucleotide tandem repeat system (TTTA), mapped in the region 1q31–q32.1 [1], which codifies for the coagulation factor XIIIB.

Using techniques of DNA amplification, this polymorphism can be employed in various types of analyses: loss of heterozygosity in tumours, anthropological studies of populations, linkage analysis, paternity studies and the forensic identification of various types of biological samples. A significant number of studies on FXIIIB [2–4] already exist, but no such study has ever been conducted on a population from our geographical area, so we extended the analysis to a population sample of 204 individuals residing in Florence, Prato, Pistoia area (Central Italy). The accumulated data were then compared

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with the published data regarding Caucasian population samples from Northern Italy [3] and Austria [4].

2. Materials and methods

2.1. DNA extraction and amplification

DNA was extracted using a modified saline procedure [5] or with the QIAamp Blood kit® (QIAGEN®, Germany) method, for samples from infants (blood and fragments of nail). For the forensic samples, the Chelex extraction method was used [6].

The amplifications were performed as suggested [1].

2.2. DNA detection

Exact identification of the alleles was done through comparison with a mixture of alleles included in the GenePrint™ STR System (Promega, USA), in accordance with ISFG guidelines [7], as previously described [8].

We studied the sensitivity of this polymorphic system, using K562 cell line DNA dilution (Promega).

Moreover, we introduced the use of this microsatellite in an automatic platform, in combination with other STRs in a new multiplex amplification system, as shown in another article in this book. An infrared automatic DNA sequencer (LICOR-4200, Nebraska, USA) [9] was used to analyse this marker. A new pair of oligonucleotide primers was used that produce larger fragments (252–276 bp) than original primers (169–193 bp). The amplicons were labelled using a forward oligonucleotide primer, covalently linked to a new infrared fluorescent molecule (IRDye™ 800). The alleles are displayed as familiar autoradiogram-like images with real-time detection.

3. Statistics

The statistical analysis was performed using a simple BASIC program [10]. Hardy–Weinberg formulation was calculated by comparison of observed with expected phenotypes.

The three populations were compared using two-way contingency tables.

4. Results and discussion

The study of STR FXIIIB was initially conducted on genomic DNA, using a reduced number of cycles which we routinely apply for paternity testing; later, this method was optimised for forensic studies, increasing the number of cycles of amplification to 34. In the sensitivity studies, the analysis of samples of K562 cell line DNA in various dilutions, permitted the identification of up to 0.2 ng/μl of DNA. In our laboratory, this STR was used, together with other markers, to analyse 39 forensic samples (blood, saliva, sperm and hair). Family studies confirmed Mendelian inheritance of alleles. No mutation was observed in the 41 families studied. In the population study, we observed 5 alleles, for a total of 13 genotypes. The distribution of the phenotypes is in Hardy–Weinberg equilibrium (chi-square = 5.28, *d.f.* 12; $0.90 > P > 0.95$). Polymorphic informa-

Table 1
Allele frequencies for FXIIIB in Central Italy (408 alleles), Northern Italy (238 alleles) and Austria (402 alleles)

Allele	Central Italy	Northern Italy	Austria
6	0.105	0.0378	0.114
7	0.009	0.0081	0.022
8	0.257	0.2311	0.241
9	0.225	0.2227	0.276
10	0.401	0.5000	0.341
11	0.000	0.0000	0.005

tion content was 0.688. The observed heterozygosity was 0.68, while the expected value, on the base of the genotypes observed was 0.711 ± 0.032. The chance of exclusion (CE) was 0.44 and the power of discrimination (PD) was 0.866. A statistical analysis was conducted to compare the populations from the area of Milan (Norther Italy) and from Graz (Austria), using a two-way $R \times C$ contingency table and comparing allele frequency. Allele frequency distributions in comparison with these other populations were shown in Table 1. No significant differences were observed in comparison to the Northern Italian population (chi-square = 4.52, *d.f.* 4; $0.50 > P > 0.30$) and to the Austrian population (chi-square = 4.49, *d.f.* 5; $0.50 > P > 0.30$).

In conclusion, this study confirms Hardy–Weinberg equilibrium for the STR FXIIIB. The population data can be used, therefore, for forensic studies and paternity testing.

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