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Evaluation of the forensic usefulness of the separate analysis of DYS385a and DYS385b in an Austrian population sample

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Abstract. The duplicated Y-STR DYS385 is one of the most informative markers on the Y-chromosome. It consists of two copies—DYS385a and DYS385b—that are located close to the inner borders of palindrome P4. The application of standard PCR protocols for DYS385 results in the simultaneous amplification of both copies. Hence, an explicit assignment of the alleles to their loci is not possible which causes a loss of information. Recently, a PCR strategy was published that allows the separate analysis of DYS385a and b, which we followed with modifications in our study on 133 unrelated Caucasian men from Tirol (Austria). Additionally all markers of the minimal haplotype (including DYS385) were amplified using standard PCR protocols. The non-allele-discriminating amplification strategy for DYS385 allowed the discrimination of 27 different genotypes (GD = 0.8616). By the separate analysis of the two DYS385 copies, this number increased to 34 (GD = 0.9185). However, the cumulative haplotype diversity of the complete minimal haplotype was 0.9951, regardless of the amplification strategy for DYS385. This indicates that in the context of the minimal haplotype, the separate analysis of DYS385a and b has no or only a marginally positive effect on the differentiation of paternal lineages.

Keywords: Y-chromosome; DYS385; Y-STR; Population study

1. Introduction

The analysis of polymorphic short tandem repeat (STR) markers that are located in the male specific region of the Y-chromosome has proven to be of great forensic relevance in special cases where the highly informative autosomal STRs are not applicable. However, the discrimination power of Y-STRs is lower than that of their autosomal counterparts since Y-chromosomal markers discriminate between different paternal lineages but not between individuals that belong to the same patriline. Furthermore, the analysis of the

Abbreviations: bp, base pair(s); GD, gene diversity; STR, short tandem repeat.

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system with the highest gene diversity (GD) in the minimal haplotype—DYS385—does not utilise the full information content of this duplicated marker when conventional PCR strategies are used because both copies are amplified simultaneously, which makes the assignment of the alleles to the loci DYS385a and DYS385b impossible.

Recently, Kittler et al. [1] published a PCR strategy for the separate analysis of both copies of DYS385. The two DYS385 loci 'a' and 'b' lie on the long arm of the Y-chromosome in palindrome P4 close (~ 450 bp) to the proximal and distal inner borders that separate the duplicated arms form the interspersed unique spacer sequence [2]. This enables the design of allele-specific primers that hybridize to the spacer sequence. Using these primers in combination with a nondiscriminating primer that lies in the duplicated fragment allows the separate amplification of both DYS385 loci.

The major aim of this study was to specify to which extent the separate analysis of both DYS385 copies improves Y-STR haplotyping.

2. Materials and methods

2.1. DNA samples

Blood samples were obtained from 133 unrelated Caucasian men from Tirol (Austria). DNA was extracted using the QIAmp DNA Blood miniKit (Qiagen, Hilden, Germany) and quantitated by UV absorbance measurement.

2.2. Primers and PCR conditions

The PCR conditions and sequences of the primers that were used for the amplification of the Y-STRs comprising the minimal haplotype (DYS19, DYS385, DYS392, DYS393, DYS389 I and II, DYS390 and DYS391) were taken from Parson et al. [3].

The two allele-discriminating primers dys385a and dys385b were designed with the Primer Express 1.5 software (AB, Applied Biosystems, Warrington, UK) using the sequences of the clones RP11-143C1 (GenBank accession number AC007379) and RP11-569J3 (GenBank accession number AC022486), respectively.

For the separate amplification of the two DYS385 copies, either the primer dys385a or dys385b was used at a concentration of 300 nM in combination with the primer DYS385.2B (300 nM; 5′ -CCAATTACATAGTCCTCCTTTC-3′ , [4]). Thermal cycling comprised an initial denaturation step of 95 °C for 10 min followed by 30 cycles of 95 °C for 15 s, 54 °C for 1 min and 72 °C for 2 min.

The obtained PCR products had a length of approximately 700–780 bp. One-microliter aliquots of the first-round PCR product were subjected without further manipulations to semi-nested second-round PCR amplifications using the primers DYS385.A (5′ -FAM-AGCATGGGTGACAGAGCTA-3′ , [5]) and DYS385.2B at a concentration of 200 nM each. Thermal cycling was performed after an initial denaturation at 95 °C for 10 min for 7 cycles comprising 95 °C for 15 s, 58 °C for 1 min and 72 °C for 1 min and a final extension step of 72 °C for 60 min. The fluorescent labeled amplification products were separated by capillary gel electrophoresis on an ABI Prism 3100 DNA Analyzer (AB) under standard conditions and data analysis was performed with the software packages GeneScan Analysis Version 3.1 and Genotyper Version 2.5 (both AB).

3. Results

With the conventional PCR protocol, 27 different DYS385 genotypes were found in our population sample (GD = 0.8616). The genotype with the highest frequency was 11–14 (34.59%). The separate analysis of DYS385a and DYS385b increased the number of different genotypes to 34 (GD = 0.9185). The most common allele combination was DYS385a–DYS385b 14–11 (frequency = 23.31%), followed by DYS385a–DYS385b 11–14 (frequency = 11.28%). All samples that yielded only a single band when the conventional amplification strategy was used produced two bands of identical lengths when DYS385a and DYS385b were analysed separately. The discrimination capacity of the complete minimal haplotype was not affected, regardless of the strategy for the amplification of DYS385. With both PCR protocols, we could distinguish between 109 haplotypes corresponding to a cumulative haplotype diversity of 0.9951.

4. Conclusions

Our results show that the separate analysis of the two DYS385 loci improves the power of discrimination of this single marker when compared to the results obtained with the conventional nondiscriminating amplification strategy. However, in the forensically more relevant context of the minimal haplotype, the separate analysis of DYS385 had no positive effect on the differentiation of paternal lineages in our study. The gain of information due to the separate analysis of DYS385a and b becomes redundant by the analysis of the other markers included in the minimal haplotype.

Further, in the first round of amplification of the allele-discriminating PCR protocol for DYS385a and DYS385b, amplicons with lengths ranging from ~ 700 up to ~ 780 bp are obtained. Therefore, this method is not applicable for the typing of samples that contain only highly degraded DNA.

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