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The highly discriminating Y-STR DYS464: a reasonable extension of the minimal Y-STR haplotype?

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Abstract. DYS464 is a multi-copy STR system with four positions on the Y chromosome (DYS464a, b, c, and d) which was recently identified and characterized. The aims of our study were to perform a population study, to estimate the mutation-rate and an extensive sequence analysis in order to confirm the nomenclature. Fourteen different alleles were found with allele lengths varying from 9 to 19 repeats. All alleles were cloned and sequenced. A total of 54 different genotypes were identified in 135 men corresponding to a gene diversity (GD) of 0.97. This value is much higher than those of other Y-STRs. DYS464 has the same discrimination capacity as the combination of the five Y-STR loci with the lowest gene diversity of the Y-STR core set. The mutation-rate estimate based on the 70 meioses analyzed amounts to 2.86×10^{-2}.

Keywords: Y chromosome; Y-STR; DYS464; Minimal haplotype; Mutation-rate

1. Introduction

A number of new Y-STRs have been characterized and studied in different populations during the last years. As a small set of the most informative Y-STRs can distinguish Y-chromosomal lineages more efficiently than a large set of less informative loci, it is advantageous to focus on the most diverse group of Y-STR loci. Recently, Redd et al. [1] identified and characterized the multi-copy marker DYS464, showing four copies on the Y-chromosome. A comparison of up to 36 Y-STR loci proofed DYS464 to be the most polymorphic Y-STR yet described.

Abbreviations: STR, short tandem repeat; minHT, minimal haplotype; GD, gene diversity; HD, haplotype diversity.

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2. Materials and methods

Blood samples were obtained from 205 male Caucasians from Tirol (Austria). This population sample comprises 135 unrelated men (fathers) as well as 70 sons (paternity index>1000). The DNA was isolated using the QIAmp DNA Blood miniKit (Qiagen, Hilden, Germany).

The primer sequences and the PCR conditions used for the amplification of DYS464 can be found in Ref. [1]. The Y-STRs of the minHT were amplified as in Ref. [2]. Separation of the PCR products was carried out on an ABI 3100.

Selected DNA samples were amplified with unlabeled primers as described above. The PCR products were cloned in the pCR®4-TOPO® vector and sequenced (for details, see Ref. [3]).

Following the ISFG guidelines on the nomenclature of duplicated Y-STRs [4], the alleles are listed in ascending order and are separated by a hyphen. The gene diversity (GD) and the haplotype diversity (HD) are computed as described in Ref. [5].

3. Results and discussion

DYS464 shows a variable repeat block, namely $(CCTT)_n$ as well as five additional non-variant (CCTT) repeat motifs. The general repeat structure is: $(CCTT)_n \ldots (CCTT)_2 \ldots (CCTT)_3 \ldots (CCTT)_4 \ldots (CCTT)_2 \ldots (CCTT)_2$.

In the variant alleles, the tetramer repeat is interrupted by a CTT motif. Two subtypes of allele 14.3 (A and B), differing in the position of the CTT motif within the variable repeat block, were found. Subtype A has the structure $(CCTT)_3CTT(CCTT)_{11}$ and subtype B $(CCTT)_7CTT(CCTT)_7$. According to this classification, all sequenced 15.3 alleles can be designated as subtype B with the structure $(CCTT)_7CTT(CCTT)_8$. Fifty-four different DYS464 genotypes were identified in 135 men, 57% of these being unique. The three most frequent genotypes were 15–17 (8.2%), 12–15–16 (7.4%) and 15–16–17 (7.4%). The most common genotype containing a variant allele (14–15.3–16–17) was found with a frequency of 3.7%. The Y-STR core set enabled us to differentiate 110 paternal lineages. By adding DYS464, it was possible to distinguish 122 haplotypes, 114 (85.7%) of these being unique. The cumulative haplotype diversity of all nine STR loci (minHT + DYS464) was 0.9983. This is a similar value as found for a 19- and a 14-locus Y-STR haplotype [5,6]. DYS464 has the same discrimination capacity as the combination of the five Y-STR loci of the core set with the lowest gene diversity. On the other hand, a combination of the three most diverse loci (DYS464, DYS385 and DYS390) has the same capacity to distinguish paternal lineages compared with the complete minimal haplotype (eight loci).

We analyzed DYS464 in 70 meioses from confirmed father/son pairs and identified two mutations. The mutation-rate estimate amounts to 2.86×10^{-2} (95% CI 3.5×10^{-3}–9.95×10^{-2}). This value is approximately 10 times higher than the average mutation-rate estimate for Y-STRs and 3 to 4 times higher than the mutation-rate of DYS390, which is the locus with the highest mutation-rate known so far [7]. Because of the limited number of observed male germ line transmissions, stochastic sampling effects cannot be excluded.

4. Conclusions

Our results confirm that DYS464 is the most polymorphic Y-STR described (GD = 0.97). The cloning and sequencing of the alleles shows that the variability is

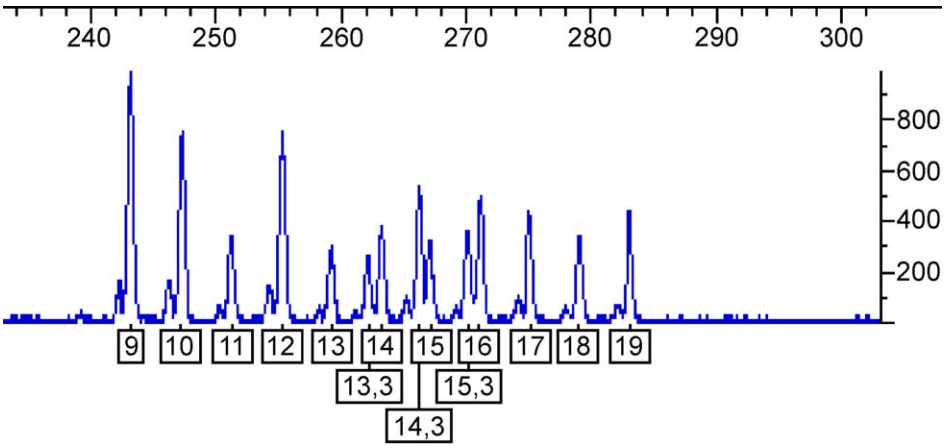

Fig. 1. Electropherogram for the DYS464 allelic ladder. All alleles were cloned and sequenced.

attributed to the first repeat block only and allows for the creation of an allelic ladder in order to meet the ISFG recommendations on forensic analysis using Y-STRs [4] (Fig. 1). We believe that DYS464 is mostly practical for high-throughput screenings and could be successfully applied for mass screenings, e.g. in connection with rape cases or mass disasters.

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