Male identification using Y-chromosomal STR polymorphisms

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INTRODUCTION

The overall paucity of Y chromosome polymorphisms (Malaspina et al. 1990) refers also to a widely used class of sequence variants, the short tandem repeat (STR) polymorphisms (Tautz 1989). STR polymorphisms appear to occur less frequently on the Y chromosome compared with autosomes (Spurdle and Jenkins 1992). Only one tetrameric simple repeat polymorphism mapped to Yp (27H39/DYS19: Roewer et al. 1992) and three dimeric (YCAI, YCAII, YCAIII: Mathias et al. 1994) have been described so far. These STRs show moderate levels of polymorphism and are used for routine forensic as well as for anthropological applications (Roewer and Epplen 1992; Roewer et. al. 1993; Gomolka et al. 1994; Mathias et al. 1992).
We now demonstrate that these four together with four other polymorphic Y-chromosomal STRs (DYS385, DYS389I/II, DYS390, DYS391) can be used to construct highly discriminative Y haplotypes. As a result most of the unrelated males in a population can now be distinguished using eight different STRs.
Since the non-pseudoautosomal (male-specific) part of the Y chromosome is -like mt DNA- uniparental inherited, Y chromosomes actually represent ancient patrilineages. Provided a low mutation rate at the respective Y loci these paternal lineages trace back to the male ancestors of a recent local population. Our study includes the STR-based Y-haplotyping of father/son pairs to give a rough estimation of the mutation rate of the applied STR systems.

MATERIALS AND METHODS

DNA was extracted from blood samples of 70 randomly chosen male individuals of German ancestry according to standard procedures. The eight STR loci were additionally studied in 41 father/son pairs, whose paternity had been positively confirmed by conventional and DNA analysis.
The amplification primers of the eight Y-chromosomal STR

- 27H39LR/DYS19 (Roewer et al. 1992)
- YCAI, YCAII, YCAIII (Mathias et al. 1994)
- DYS385 (GDB Id. G00-316-257)
- DYS389 (GDB Id. G00-367-936)
- DYS390 (GDB Id. G00-367-957)
- DYS391 (GDB Id. G00-367-966)

applied in this study are designated according to the Genome Data Base (GDB) entries or to the published sequences. PCR conditions and primer sequences were optimized to allow co-amplification (multiplexing) of up to four loci in one PCR. Amplifications were carried out using one fluorescently labeled primer which enables laser-induced detection of the PCR products after electrophoretic separation. For allele-sizing the fluorescent labeled PCR products were run together with in-lane size standards on an ALF™ DNA Sequencer (Pharmacia Biotech, Freiburg, Germany). The allele lengths were ascertained with the ALF Fragment Manager™ 1.1 Software. To designate the STR-based Y haplotypes the consecutive locus-specific alleles are temporarily named according to their length with the shortest allele found at each locus defined as allele 1.

RESULTS AND DISCUSSION

To establish an efficient male identification system using STR-based Y-chromosomal haplotypes we have analysed in detail four published and four other tetrameric Y-chromosomal STR polymorphisms recently entered into the Genome Data Base. Analysis of male and female DNA confirmed that the latter STR polymorphisms (DYS385, DYS389, DYS390, DYS391) are exclusively male specific. The systems DYS385, DYS389, YCAI, YCAII, YCAIII are supposed to consist of two homologous male specific loci with different repeat lengths. For these duplicated loci we choose a simple designation of the two male-specific alleles according to their lengths ignoring their origin from either of the two loci. This proposal appears favourable for practical reasons, as long as the exact localisation and structure of these homologous loci is still unknown. The lengths of the co-amplified repeat units at the locus DYS389 differ by more than 100 base pairs which allow a definite allocation of the alleles to a locus DYS389I and a locus DYS389II.

The number of alleles/allele pairs detected at the eight STR loci varied between 2 (YCAI) and 24 (DYS385). At the loci YCAI, YCAII and YCAIII described by Mathias et al. (1994) several new alleles were found in this study, whereas for the locus 27H39/DYS19 the number of alleles and their distribution published earlier for a German population sample (Gomolka et al. 1994) was confirmed. The allele lengths at the loci differ between 124 bp (YCAI/allele 1) and 405 bp (DYS385/allele 13). After sequential PCR-typing of the whole set of eight STR systems simply encoded Y-chromosomal compound haplotypes were constructed. With the exclusion of two males which both carry the same haplotype all Y chromosomes of the unrelated 70 German males assembled in this study can be differentiated by the eight STR loci.

For all STRs applied in this study holandric transmission was confirmed. For 41 father/son pairs which were typed for the respective loci we established full-size haplotypes. All Y haplotypes were found to be inherited regularly with the exception of one father/son pair with slippage mutations occured at DYS389II and DYS390.

CONCLUSION

The main fields of application of the proposed Y chromosome STR-based haplotyping are:

1. the forensic identification of male DNA (Roewer and Epplen 1992) preferably in rape cases with male/female stain mixtures (combined gender/identity testing)
2. paternity analysis preferably for deficiency cases with a male offspring (Chakraborty 1985) and
3. the analysis of migration, settlement or mating structure of human populations in historic (rather than evolutionary) time spans (Roewer et al. 1993).

For those applications the proposed sequential multilocus PCR typing of eight different Y-chromosomal STRs provides a simple, reproducible and sensitive method.

REFERENCES

Chakraborty R (1985) Paternity testing with genetic markers: are Y-linked genes more efficient than autosomal ones? Am J Med Genet 21: 298-305

Gomolka M, Hundrieser J, Nürnberg P, Roewer L, Epplen JT, Epplen C (1994) Selected di- and tetranucleotide microsatellites from chromosomes 7, 12, 14, and Y in various Eurasian populations. Hum Genet 93: 592-596

Malaspina P, Persichetti F, Noveletto A, Iodice C, Terrenato L, Wolfe J, Ferraro M, Prantera G (1990) The human Y chromosome shows a low level of DNA polymorhism. Ann Hum Genet 54: 297-305

Mathias N, Bayes M, Tyler-Smith C (1994) Highly informative compound haplotypes for the human Y chromosome. Hum Mol Genet 3: 115-123

Roewer L, Arnemann J, Spurr NK, Grzeschik KH and Epplen JT (1992) Simple repeat sequences on the human Y chromosome are equally polymorphic as their autosomal counterparts. Hum Genet 89: 389-394

Roewer L, Epplen JT (1992) Rapid and sensitive typing of forensic stains by PCR amplification of polymorphic simple repeat sequences in case work. Forensic Sci Int 53: 163-171

Roewer L, Nagy M, Schmidt P, Epplen JT, Herzog-Schröder G (1993) Microsatellite and HLA class II oligonucleotide typing in a population of Yanomami Indians. In: Pena SDJ, Chakraborty R, Epplen JT, Jeffreys AJ (eds) DNA Fingerprinting: State of the Science. Birkhäuser, Basel Boston Berlin, p 221

Spurdle AB, Jenkins T (1992) The Y chromosome as a tool for studying human evolution. Current Opinion in Genetics and Development 2: 487-491

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17: 6463-6471

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