Application of a Y-linked tetranucleotide repeat polymorphism to paternity cases.

E. Trabetti, A. Casartelli, P.F. Pignatti.

Institute of Biological Sciences, University of Verona, Italy.

Abstract. The polymorphic microsatellite Y-27H39 was analyzed in a large Italian population sample by the polymerase chain reaction amplification technique and a simple method of native polyacrylamide gel electrophoresis followed by ethidium bromide fluorescence. Five alleles were distinguished in 202 males, with frequencies ranging from 1% to 51%. In 52 father-son pairs, selected among 94 disputed paternity cases, previously tested with different autosomal VNTR and microsatellite regions, the Y-27H39 polymorphism confirmed all the 27 attributions and 19 of the 25 exclusions, indicating 76% informativity in Y chromosome exclusion testing.

Introduction. Many highly informative human microsatellite polymorphisms with a motif length of 1-5 bp have been described to date, and most of them are detected by PCR analysis (1). We have used several microsatellites (2) and other markers (3,4) in the analysis of Italian population samples. In order to expand the informativity to sex determination we have now introduced a Y specific chromosome marker. The microsatellite Y-27H39 is based on the repetition of a GATA motif and was recently discovered on the short arm of the human Y chromosome (5).

Materials and Methods. All subjects studied were coming from several Italian regions. In all father-son pairs, the paternity was previously established by autosomal VNTR and microsatellite PCR determinations (2). DNA was prepared from peripheral

blood samples, according to standard protocols. The PCR reactions were carried out according to the conditions previously described (6). Native 10% 25-cm long polyacrylamide gel electrophoresis and ethidium bromide staining (2) of the PCR products were employed to analyze the Y-27H39 locus.

Results. Allele frequencies for the Y-27H39 locus were first determined. Among the 202 unrelated Italian males studied, 5 different alleles were detected: A, B, C, D, E, with frequencies of 0.07, 0.51, 0.31, 0.10, 0.01, respectively. The size interval and alleles detected were similar to what previously reported for the Brazilian (6) and German (7) populations. The frequency distribution in our sample differs: allele A is significantly less frequent and allele C is more frequent in our population sample.

80 father-son pairs with established paternity from among the 202 individuals were analyzed with the Y-27H39 polymorphism. The inheritance of the same allele was confirmed in all cases.

To determine the informativity of the Y specific locus in paternity testing we performed a retrospective analysis with Y-27H39 polymorphism in 52 Italian father-son pairs, selected among 94 disputed paternity ceses, which we previously studied with different autosomal VNTR and microsatellite regions (2). In all the 25 attribution cases there was allelic concordance, as expected. For the exclusion cases, 19/25 were confirmed: a 76% informativity rate.

Conclusion. The number of Y-27H39 alleles, their frequency distribution in the Italian population and the informativity derived from our paternity data, indicated that this is a good marker for individual and sex typing. The PCR technique and the non isotopic detection advantages allow this system to be used for several purposes, expecially for forensic case work with minute biological samples.

Acknowledgements. This work was partially supported by the Italian National Research Council target projects "Genetic Engineering" and "Biotechnology and Bioinstrumentation".

References:

(1) Weber JL (1990) Human DNA polymorphisms based on length variations in simple-sequence tandem repeats. Genome Analysis 1: 159-181.

(2) Trabetti E, Galavotti R, Pignatti PF (1993) Genetic variation in the Italian population at five tandem repeat loci amplified in vitro: use in paternity testing. Molecular and Cell Probes 7: 81-87.

(3) Gasparini P, Trabetti E, Savoia A, Marigo M, Pignatti PF (1990) Frequency distribution of the alleles of several variable number of tandem repeat DNA polymorphisms in the Italian population. Hum Hered 40: 61-68.

(4) Gasparini P, Mandich P, Novelli G et al. (1991) Forensic applications of molecular genetic analysis: an Italian collaborative study on paternity testing by the determination of variable number of tandem repeat DNA polymorphisms. Hum Hered 41: 174-181.

(5) Roewer L, Epplen JT (1992) Rapid and sensitive typing of forensic stains by PCR amplification of polymorphic simple (gata)n repeats. Forens Sci Int 53: 163-171.

(6) Santos FR, Pena SDJ, Epplen JT (1993) Genetic and population study of a Y-linked tetranucleotide repeat DNA polymorphism with a simple non-isotopic technique. Hum Genet 90: 655-656.

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