APPLICATION OF THE STR ANDROGEN RECEPTOR (HUMARA) POLYMORPHISM TO PATERNITY CASES

Caenazzo L., Ponzano E., Crestani C., Bonan G., Cortivo P.

Istitute of Legal Medicine, University of Padova, V. Falloppio 50, 35121 Padova, Italy

INTRODUCTION

The human Androgen Receptor gene located on chromosome X (Xcen-q13) contains an high polymorphic trinucleotide repeat (acg)n in the coding region of the first exon (1). The polymorphism, was first described by Sleddens (2) for its usefulness in the diagnosis of Androgen Intensitivity Syndromes. The Androgen Receptor polymorphism was studied by Edwards et al. (3,4) for personal identification pourposes.
In order to valuate the possibility of application of this system to forensic casework of our laboratory we have studied a population sample and a group of paternity with femeale son.

MATERIALS AND METHODS

The sample consists in 123 unrelated individuals living in Veneto (Italy): 56 female and 67 male.
DNA was extracted in according to a non-organic procedure. Each sample containing: 10 ng of template DNA, 1 U Taq polymerase (Pharmacia), 1 μM each primer, 200 μM each nucleotide, 1X PCR Buffer (Pharmacia), diluted with distilled water to a final volume of 25 μl,were amplified in GeneAmp 2400 PCR System.
Primer sequences and amplification parameters are in according to Hammond et al. (5) with minor modification:
5' TCCAGAATCTGTTCCAGAGCGTGC
5' GCTGTGAAGGTTGCTGTTCCTCAT
95°C 45 sec, 64°C 45 sec, 72°C 45sec 28 cycles.
PCR amplified DNA samples were separated in a non denaturanting vertical polyacrylamide gels 12% 25 cm long. Running time: 12 hours 20 mA.
The bands were visualised by silver staining and classified by comparation with an homemade allelic ladder.
This system was succesfully co-amplified, with HumTH01 with the same conditions.

RESULTS AND DISCUSSION

Allele frequencies for the HUMARA were first determined. Among the Veneto sample 8 different alleles in the range 250-310 bp were detected as reported in table 1. The alleles number is less to that found by Edwards (4) this could be due to the sample dimension, and there are little discrepances in the number of repeats of the alleles in comparison to the study of Hammond (5), for this reason we don't called our alleles with the number of repeats but from A1 to A8.
To evaluate the usefulness of this marker in paternity testing we studied 52 family trios with female son previously studied with convetional and VNTR systems. In all 26 attribution cases there was allelic concordance as expected. For the others 26 exclusion cases, 18/26 were confirmed, this correspond to a 70% informativity rate.
In conclusion these preliminary results, suggest that HUMARA can be valuable, additional marker for paternity disputes relatively to the parental origin of the X chromosome.

Table 1. Allele frequency of HUMARA system

allele	frequency ± S.E.	allele	frequency ± S.E.
A1	0.0218 ± 0.009	A5	0.2363 ± 0.027
A2	0.0953 ± 0.060	A6	0.1172 ± 0.020
A3	0.1281 ± 0.021	A7	0.0535 ± 0.031
A4	0.3418 ± 0.030	A8	0.0059 ± 0.004

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