POPULATION STUDIES OF THE Y-CHROMOSOME OF LOCI DYS390, DYS391 AND DYS393 IN BRAZILIAN SUBJECTS AND ITS USE IN HUMAN IDENTIFICATION

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ABSTRACT

Human identification can be accomplished by several technical procedures, especially by the comparative analysis of dental documents. Recent advances in molecular biology have now widened the scope for human identification and defined the protocols for the collection of biological material. Allele patterns of a population are particularly useful and they should be verified since they vary in different populations. We have studied the frequency of the alleles in three STR loci of the Y chromosome (DYS390, DYS391 and DYS393) in a group of Brazilian caucasian subjects. Results presented alleles 21, 22, 23, 24, 25 and 26 in locus DYS390, alleles 8, 9, 10, 11 12 and 13 in STR DYS391 and alleles in STR DYS393 were 11, 12, 13 14 and 15. The highest frequencies were 24 (0.46), 11 (0.37) and 13 (0.45). Each of the three STR systems had a PE (power of exclusion) of 0.6764 (DYS390), 0.5988 (DYS391) and 0.6136 (DYS393), The combination of the three STR systems revealed a PE of 0.9498, suggesting that this data can help in human identification.
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INTRODUCTION

Modern technology is being used extensively in forensic analysis to produce scientifically reliable data to serve as acceptable evidence. The search for the best evidence has been fundamental in supporting criminal investigation and the law, and thus contributing to the high quality of justice. Physical evidence such as the dental profile from dentists' records of patients has proved to be a very useful tool in identification but many are incomplete or absent, sometimes because the victim's dentist cannot be identified or located. In developing countries this is a common problem because changing dentist is frequent in these populations. Clark,[1] analyzing 10 mass disasters in Great Britain, where dental records were used, came to the same frustrating conclusion.

This limitation and others have stimulated the search for new and improved human identification techniques.[2] Blood groups defined by antigens on the surface of red cells is a popular technique but is not discriminatory enough because of the high frequency of recurrence of blood groups in the population. DNA typing on the other hand has replaced ABO, Rh and Lewis blood group tests, as well as a variety of enzymes such as phosphoglucomutase (PGM), esterase D (EsD), glyoxylase 1 (GLO 1), erythrocyte acid phosphatase (EAP), adenosine deaminase (ADA), adenylate kinase (AK), carbonic anidrase (CAII), peptidase A (PepA) and glucose-6-phosphate dehydrogenase (G6PD) that had been routinely used.[3] Restriction Fragment Length Polymorphisms (RFLP) was the first system used in human identification, known as DNA fingerprinting, but the most revolutionary advance was in DNA amplification by PCR of specific hyper-variable regions, called VNTR (variable number of tandem repeat) and STR (short tandem repeat). These regions have been explored with increased interest because they are very polymorphic and give good discrimination. DNA amplification can be applied to very small amounts of material, decomposed tissues, bone, tooth and

other organic materials found at disaster or crime scenes such as blood, saliva and hair.[4,5] In the past 5 years STR from Y chromosome has been used to identify a rapist or murderer in a sexual abuse case.[6,7] There are many advantages in using Y chromosome loci and most importantly, that female DNA does not interfere with the analysis.

An important aspect of population studies is to compile a database of allelic frequencies using DNA analysis, which can assist in the calculation of statistical probabilities[8-16] for forensic applications or studies of evolution. In Brazil there is no study describing Y chromosome STR loci with allelic frequency to use as reference in creating an index of probability.

The object of this study was to set up a statistical database for a local population. Three STR loci from Y chromosome DYS390, DYS391, DYS393, in the population of São Paulo, Brazil, were analyzed.

MATERIALS AND METHODS

DNA extraction

DNA was obtained from white blood cells of 100 Caucasian individuals living in São Paulo, SP, Brazil, using the salting out procedure of Salazar *et al.*[17] DNA from saliva was obtained from 5 women as a negative control group using the technique described by Hochmeister et al.[18]

DNA amplification using Polymerase Chain Reaction

Based on the sequence from the Y-STR haplotype reference database,[14] primers for DYS390, DYS 391, DYS 393 were designed. Of each pair of primers described below, one was labelled with Cy5 at 5' end*.

DYS390
Primer A: TAT ATT TTA CAC ATT TTT ggg CC
Primer B: TgA CAg TAA AAT gAA CAC ATT gC

DYS391
Primer A: CTA TTC ATT CAA TCATAC ACC CA
Primer B: gAT TCT TTg Tgg Tgg gTC Tg

DYS393
Primer A: gTg gTC TTC TAC TTg TgT CAA TAC
Primer B: AAC TCA AgT CCA AAA AAT gAg g

The reaction conditions were set up using 10mM of each nucleotide (dNTPs), 80nM of each primer, 2mM MgCl2, 50 mM KCl, 10 mM TRIS-HCl, and 2,5 U of Taq DNA polymerase to a final volume of 25 µL. A thermocycler model PTC-100** was programmed for 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 2 min with a final extension of 72 °C for 10 min. These PCR conditions were used for the three STRs.

Fragments identification by electrophoresis

The ALF Express System¥ was utilized for fragment analysis in a 20% polyacrylamide gel using denaturing conditions. The electrophoresis separation and analysis of fragments were evaluated using a program from the ALF express system fragment manager. A ladder from Pharmacia Biotech¥ (ALF Express Sizer™ 50-500, cat. # 27-4539-01) was used as a pattern to determine the allelic size.

The PCR products were diluted with 40% of a loading dye (100% formamide and 0.05% Dextran blue 2000, and 10 ml were applied to a 20% polyacrylamide gel (acrylamide/bis:19/1) in 1X TBE buffer and electrophoresed at 500 volts, 60mA at 55°C, for 2 hours. Peak patterns were analysed using the ALF running gel software (ALFwin Instrument Control Version 2.00)¥ and fragment manager software (Allele Locator).¥

Statistical analyses

The exclusion index for each locus and the combination of three STRs was then calculated by $P(Y) = 1 - \sum(Pi)^2$.and $A = 1 - (1\text{-}PE_1).\ (1\text{-}PE_2).\ (1\text{-}PE_n)$ respectively,[13] and for population differentiation we used the Raymond & Rousset[16] test.

RESULTS AND DISCUSSION

Six different alleles were found in STRs DYS390 and DYS391, and in DYS393 there were five alleles (Table 1). Alleles 21, 22, 23, 24, 25 and 26 were

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Table 1: *Allele distribution of DYS390, DYS391 and DYS393 (n=100)*

DYS390	Allele	21	22	23	24	25	26
	Length (bp)	203	207	211	215	219	223
	Frequency	0.02	0.15	0.29	0.46	0.07	0.01
DYS391	Allele	08	09	10	11	12	13
	Length (bp)	275	279	283	287	291	295
	Frequency	0.01	0.05	0.16	0.37	0.34	0.07
DYS393	Allele	11	12	13	14	15	
	Length (bp)	115	119	123	127	131	
	Frequency	0.05	0.42	0.45	0.07	0.01	

discovered in locus DYS390, the most frequent one was 24, the locus DYS391 has shown alleles 8, 9, 10, 11, 12, 13. Allele 11 was the most frequent. Allele distributions in locus DYS393 were 11, 12, 13, 14 and 15 and the most frequent was number 13. The observed frequencies ranging in the spectrum previously described [8,9,11,13,14] showed apparent differences in the allele distribution. Comparing the loci found in the Brazilian population with the ones described in New Guinea/Australia[8,9,14] there was a significant difference ($p < 0.001$),[16] suggesting that the data base for statistical analysis can modify the PE results in different populations. However, it is necessary to increase the number of studies to create a representative database in order to confirm these preliminary results.

In Brazil there are people from different ethnic origins including Europeans, Asians, Africans and Amerindians and we used the general classification of Caucasian described by the Brazilian Institute of Geography and Statistics (IBGE – Instituto Brasileiro de Geografia e Estatística).[19] In further studies we intend to study other ethnically diverse Brazilian populations in order to create a more representative database for application in forensic analysis.

Employing a positive and negative control in each reaction enabled us to validate the amplification parameters used for the Y chromosome in this study. However, the absence of amplification of the negative control in all experiments validates the primers used as a choice in forensic cases. Further, a positive control with a known allele in each PCR reaction and gel was run.

The calculated individual PE[13] for each locus was 0.6764, 0.5988 and 0.6136 for DYS390, DYS391 and DYS393 respectively. The combined PE result of 0.9498 shows that these three STRs provide important data that can be used in conjunction with other STRs for forensic analysis, as an initial distinction between suspects of crimes such as rape cases or in human bitemark, as most victims are females and the male specific Y chromosome STRs have been shown to be a powerful tool in solving these cases.

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