GENETIC DIVERSITY OF SRI LANKANS WITH RESPECT TO THE HUMAN DNA TYPING STR MARKERS, D3S1358, D5S818 AND D8S1179

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Introduction

DNA typing is the characterization of individuals at the level of their genetic material, DNA (deoxyribonucleic acid). The DNA of every human being (with the exception of monozygotic twins) is unique. The DNA of an individual is identical whether it is extracted from hair bulbs, white blood cells, or semen. These principles of individual uniqueness and identical DNA structure within all tissues of the same body provide the basis for DNA typing.

Tandemly repeated DNA sequences in the human genome are a source of genetic markers useful for genetic studies, medical diagnostics, paternity testing and identification of individuals in forensic casework. Short tandem repeat (STR) loci are a subgroup of the highly polymorphic variable number of tandem repeats (VNTR). They are composed of tandemly repeated sequences of 1 to 6 base pairs in length and are abundant in the human genome. DNA typing based on amplification of STR loci is an effective means to overcome some of the problems encountered in forensic practice such as typing of minute amounts of DNA, highly degraded DNA or mixtures of DNA from different individuals. The use of genetic markers, such as STR, in identity testing must accompany allele/genotype frequency data from the relevant population for estimating the frequency of any particular genetic profile.

In Sri Lanka, there are eleven highly polymorphic autosomal STR loci used for human identity testing in establishing paternity as well as in forensic DNA analysis (Illeperuma, *et al*,. 2008). The allelic frequencies for those STR loci have been calculated and databases have been formed for the Sri Lankans.

The Federal Bureau of Investigation (FBI) of the United States of America has been a leader in developing DNA typing technology for use in the identification of perpetrators of violent crime. In 1997, the FBI announced the selection of 13 STR loci to constitute the core of the United States national database, CODIS (Combined DNA Index System). Only seven of the above loci are being used for DNA based paternity testing and human identification in Sri Lanka currently.

The present study is the first application of STR typing of the three CODIS loci, D3S1358 (D3S), D5S818 (D5S), D8S1179 (D8S), in a sample of Sri Lankans. The present study has evaluated the suitability of these three new loci to identify Sri Lankan individuals by DNA typing technology and formulated a preliminary allelic frequency database which can be used to express a chance match. These new sites will maximize the accuracy of human identification and paternity testing in Sri Lanka.

Aims and objectives

The overall aim of the study was to evaluate the usefulness of D3S, D5S and D8S STR loci as a tool for forensic identification and paternity testing in Sri Lanka. The specific objectives were to:

- establish a preliminary Sri Lankan population database for the test STR loci.
- estimate allele and genotype frequency data across the test loci for the Sri Lankan population.
- assess the statistical parameters which evaluate the ability of these DNA markers to distinguish between individuals, and thereby, their forensic utility

Methods

Human blood samples, representing all ethnic groups, were taken after obtaining informed written consent from unrelated Sri Lankan donors. DNA was extracted from 50 samples by Chelex-100 method (1).

Amplification of D3S, D5S and D8S STR loci was carried out in a GeneAmp 2400 (Applied Biosystems) Thermal Cycler with appropriate PCR conditions and products were visualized through Gel electrophoresis. Silver staining was carried out using DNA Silver Staining System according to the manufacturer's recommendations. Allelic ladders were constructed by mixing alleles with varying lengths obtained from a randomly selected set of individuals.

Statistical parameters of forensic importance, the power of discrimination (PD) polymorphism information content (PIC), Typical paternity index (PI) and power of exclusion (PE) for each locus were determined with the use of PowerStats computer program from Promega Corporation (Illeperuma, *et al*,. 2008; Tereba, 1999).

Results and Discussion

i) Alleles observed for the three STR loci and their frequencies in the test population (Table 1):

Alleles known for the D3S locus among humans range from 8-21. Of these, the alleles 14,15,16,17,18 were observed for D3S in the test population. Of the alleles known for the D5S locus among humans (7 – 18), the alleles observed in the test population were 9,10,11,12,13,14. The alleles known for D8S locus among humans range from 7 – 20 and in the test the test population only the 8,9,10,11,12,13,14,15,16 were observed.

The most common alleles were allele 15 for D3S (f=0.316), allele 12 for D5S (f=0.375) and allele 14 for D8S (f=0.295). An allele which occurs more than 50% frequency is not very useful in paternity or forensics. In this study, all alleles showed a frequency less than 50%.

Table 1: Alleles observed and their frequencies for the test loci.

Allele	Frequency		
	D3S	**D5S**	**D8S**
8			1.1%
9		3.1%	1.1%
10		10.4%	17.0%
11		33.3%	9.1%
12		37.5%	8.0%
13		12.5%	9.1%
14	7.1%	3.1%	29.5%
15	31.6%		17.0%
16	30.6%		8.0%
17	22.4%		
18	8.2%		
Homozygotes	38.8%	22.9%	11.4%
Heterozygotes	61.2%	77.1%	88.6%
Total Alleles	98	96	88

Allelic frequencies of the loci D3S and D5S have shown normal bell shaped distributions; D8S has a bi modal distribution. D8S locus appears the most informative, as higher the herorozygozity of a locus, higher the existence of variation, which implies a large number of alleles of significant frequencies exist in the population.

ii) Statistical parameters of forensic importance:

The Power of Discrimination (PD), Polymorphism information content (PIC), Paternity Index (PI) and Power of exclusion (PE) are listed in Table 2.

Table 2: Statistical parameters of forensic importance for the test loci.

	D3S	D5S	D8S
Power of Discrimination	0.885	0.861	0.926
Polymorphism information content	0.70	0.67	0.80
Power of Exclusion	0.306	0.546	0.768
Typical Paternity Index	1.29	2.18	4.40

The Power of discrimination expresses the probability of two random DNA profiles matching at all tested loci. The PD values for the present loci showed a high power of discrimination ranging from 0.861 to 0.926. The combined power of discrimination for the three loci was 0.9988. The significance of any match will increase, when typed with more loci that show random association with the present STR loci.

The PIC is a measure of the amount of polymorphism that a particular site has. Normally loci which have PIC values more than 0.5 are considered by the forensic community in the world to be the loci which can be used in human identification purposes using DNA typing. In this study all three loci exhibited a high PIC, indicating a high variability of alleles between individuals at those particular sites. Thus, the PIC values show that all three loci were highly informative (PIC>0.5).

The PI is a means of presenting the genetic odds in favor of paternity given the genotypes for the mother, the child and the putative father. The paternity index (PI) is a means of presenting the genetic odds in favor of paternity given the genotypes for the mother, the child and the putative father. The combined typical paternity index for the three loci was 12.37. An alternative value used in paternity analyses is the power of exclusion (PE). The combined power of exclusion for the three loci was 0.1283.

Conclusions

A preliminary Sri Lankan population database has been established for the STR loci D3S, D5S and D8S. The observed allele frequencies may be used to estimate genotype frequencies across loci in this population. After appropriate validation work, the use of these highly polymorphic STR loci will be a useful tool for forensic identification and paternity testing in Sri Lanka.

References

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