Pattern Analysis of Short Tandem Repeats Allele Frequencies among the Population of Khuzestan Province, South of Iran

Seyed Farzad Hosseini 1, Mahdi Bijanzadeh 2*, and Elham Modheji 1

1. Department of Forensic Medicine, Khuzestan Legal Medicine Organization, Ahvaz, Iran
2. Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract

**Background:** The basis of genetic fingerprinting and DNA profiling in forensic laboratories is the use of Short Tandem Repeats (STRs) according to local and ethnic genetic characteristics.

**Methods:** Forensic parameters and allele frequencies for 15 autosomal STRs in 100 unrelated individuals from Khuzestan province, south Iran were determined. PCR was carried out for amplification of STRs and GeneMapper ID software was used for genotyping and allelic analyzing.

**Results:** The Power of Exclusion (PE) varied between 0.332 (TPOX) and 0.768 (FGA). With exception of the THO1 (0.020), TPOX (0.014) and D18S51 (0.003), other STRs showed no deviation from the Hardy-Weinberg equilibrium (p>0.05).

**Conclusion:** Out of 15 STRs, 12 repeats seemed to be more useful and more powerful tools in identity and paternity determination for our studied population. Variation in our data analysis revealed that effective use of these 15 STR loci in forensic cases needed to be localized by collection and analysis of population data from the general population.

**Keywords:** DNA fingerprinting, Forensic sciences, Genotype, Polymerase chain reaction

Introduction

The law organizations have greatly benefited from current improvements in the genetic micro and nanotechniques. The advanced forensic laboratories outfitted by bunch of biological evidence from a crime scene to the guilty can reliably exclude falsely accused individuals. In the past three decades, numerous advances in genetic technologies have occurred and most of them included the development of DNA testing and especially Polymerase Chain Reaction (PCR)-based typing methods 1,2.

The basis of genetic fingerprinting and DNA profiling is that twins are the only individuals who have identical copies of the human genome. Of course, the human genome is almost the same in everybody, although it contains many polymorphisms, positions where causes different nucleotide sequence to occur in every member of the population. These polymorphisms include Restriction Fragment Length Polymorphisms (RFLPs), Short Tandem Repeats (STRs), and Single Nucleotide Polymorphisms (SNPs), that can occur within genes as well as in intergenic regions, and altogether there are millions of these polymorphic sites in the human genome. The more powerful technique of DNA profiling is the use of STRs, a short sequence, 1-13 nucleotides in length, which is repeated several times in a tandem array 3. Although for many years the importance of using specific STR for human identification, forensic DNA casework, missing persons, mass disasters, monitoring needle sharing, monitoring transplants, military casualties and so on has been accepted, for detection of their impact for each population, their forensic parameters specially Hardy-Weinberg equilibrium should be calculated 4.

In the population, generally, there might be as many as ten different versions of a particular STR, each of the alleles characterized by a different number of repeats. In DNA profiling, the alleles of a selected number of different STRs are identified. This can be achieved quickly and with very small amounts of DNA by PCRs. The size of the band or bands that are seen in the agarose gel electrophoresis indicate the allele or alleles present in the DNA sample that has been tested. Two alleles of an STR can be present in a single DNA sample because there are two copies of each STR, one on the chromosome inherited from the mother and one on the chromosome from the father 2. Khuzestan province in southwest of Iran has wide ethnical distribution including Fars, Arab, Lur and so on. This study was performed to analyze STR genotypes, report their allele frequencies and evaluate their application among the
population of Khuzestan province in Iran.

**Materials and Methods**

**Samples and DNA extraction**

Blood samples were collected under informed consent, from 100 healthy unrelated men and women (men: 65, women: 35) belonging to three ethnic groups (Arab: 39, Lur: 26, Fars and others: 35) living in Khuzestan province. The research conformed to the provisions of the declaration of Helsinki in 1995 (as revised in Edinburgh 2000) and ethical approval was obtained from the ethics committee of Khuzestan Legal Medicine Organization and informed consent was obtained from all participants. Genomic DNA was extracted by using dried blood samples on FTA cards according to the manufacturer's instructions. Laboratory procedures were carried out in genetic laboratory of Khuzestan Legal Medicine Organization.

**PCR and genotyping**

For amplification of 15 autosomal STR (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA), PCR procedure was performed according to the manufacturer's instruction (AmpFISTRIdentiﬁer™, Applied Biosystems, Foster City, CA, USA), by using the AmpFISTRIdentiﬁer PCR Ampliﬁcation kit. PCR products electrophoresis was performed on an ABI 3500 Genetic Analyser 8-capillary array system (Applied Biosystems, Foster City, CA). 3500 series data collection version 1.0 software was used for data collection. For genotyping and allelic calls, GeneMapper ID software version 3.2 (Applied Biosystems, USA) was used by comparison of samples proﬁle with allelic ladder included in the kit.

**Statistical analysis**

Arlequin software version 3.5 was used to calculate allele frequencies and to obtain expected heterozygosity (He), observed heterozygosity (Ho) and probability value (p-value) of Hardy-Weinberg equilibrium. PowerStats v1.2 (Promega Corporation) software package was used to obtain statistical parameters of forensic interest including MP, matching probability; PD, power of discrimination; PIC, polymorphic information content; PE, power of exclusion and PI, paternity index.

**Results**

Forensic parameters and allele frequencies of 15 autosomal STR loci (D8S1179, D21S11, D7S820, CSF-
Hosseini SF, et al

Discussion

A set of autosomal STR loci were analyzed in a mixed ethnic population group in Khuzestan province, in order to obtain a genetic characterization of south Iran. Several local studies on molecular genetics loci have been reported in different parts of the world. Hammond et al presented a PCR-based DNA-typing method using 13 unlinked STR loci from unrelated individuals presenting to a Houston area blood bank. They reported that this valid statistical analysis is generally simpler than similar analysis of RFLP-VNTR results. Two levels of analysis have been devised using two sets of 12 STR loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820) and 21 STR loci (the former set plus D9S926, D1S2010, D13S767, D14S-

Conclusion

Analysis of autosomal STR loci can reveal heterogeneity of geographically and culturally related individuals and different populations, thus, it might be possible to better differentiate populations through the procedure. Today, STRs are used for crime forensic identity, paternity and corpse identity in many related laboratories, while for effective use of these 15 STR loci in forensic cases, it is needed to collect and analyze population data from the general population region in which the crime has happened and adjust them by local characteristics. In order to contribute in construction of STR profiling of the different ethnic groups in geographical areas, similar study with the present population can be useful.

Table 2. Forensic parameters for 15 autosomal STR loci in a population sample of Khuzestan province

| Loci       | MP  | PD  | PIC  | PE  | Ho  | He  | PIC  | PD  |
|------------|-----|-----|------|-----|-----|-----|------|-----|
| D8S1179    | 0.083 | 0.107 | 0.090 | 0.090 | 0.148 | 0.063 | 0.043 |
| D21S11     | 0.917 | 0.893 | 0.910 | 0.810 | 0.932 | 0.937 | 0.957 |
| D7S820     | 0.74  | 0.71  | 0.73  | 0.73  | 0.64  | 0.79  | 0.84  |
| CSF1PO     | 0.409 | 0.419 | 0.350 | 0.573 | 0.403 | 0.569 | 0.727 |
| D3S1358    | 1.6  | 1.63 | 1.41 | 2.33 | 1.58 | 2.31 | 3.73 |
| TH01       | 31.3 | 30.6 | 35.4 | 21.4 | 31.6 | 21.6 | 13.4 |
| D16S539    | 68.8 | 69.4 | 64.6 | 78.6 | 68.4 | 78.4 | 86.6 |
| D21S138    | 63.3 | 64.9 | 68.4 | 75.0 | 75.1 | 75.3 | 82.0 |
| D19S433    | 75.0 | 75.1 | 75.2 | 75.3 | 75.4 | 75.5 | 75.6 |
| vWA        | 80.6 | 80.7 | 80.8 | 80.9 | 81.0 | 81.1 | 81.2 |
| TPOX       | 79.3 | 79.4 | 79.5 | 79.6 | 79.7 | 79.8 | 79.9 |
| D18S51     | 80.6 | 80.7 | 80.8 | 80.9 | 81.0 | 81.1 | 81.2 |
| D5S818     | 80.6 | 80.7 | 80.8 | 80.9 | 81.0 | 81.1 | 81.2 |
| FGA        | 93.3 | 93.4 | 93.5 | 93.6 | 93.7 | 93.8 | 93.9 |

Discussion

A set of autosomal STR loci were analyzed in a mixed ethnic population group in Khuzestan province, in order to obtain a genetic characterization of south Iran. Several local studies on molecular genetics loci have been reported in different parts of the world. Hammond et al presented a PCR-based DNA-typing method using 13 unlinked STR loci from unrelated individuals presenting to a Houston area blood bank. They reported that this valid statistical analysis is generally simpler than similar analysis of RFLP-VNTR results. Two levels of analysis have been devised using two sets of 12 STR loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820) and 21 STR loci (the former set plus D9S926, D1S2010, D13S767, D14S-

Conclusion

Analysis of autosomal STR loci can reveal heterogeneity of geographically and culturally related individuals and different populations, thus, it might be possible to better differentiate populations through the procedure. Today, STRs are used for crime forensic identity, paternity and corpse identity in many related laboratories, while for effective use of these 15 STR loci in forensic cases, it is needed to collect and analyze population data from the general population region in which the crime has happened and adjust them by local characteristics. In order to contribute in construction of STR profiling of the different ethnic groups in geographical areas, similar study with the present population can be useful.
Acknowledgement
We are grateful to the Legal Medicine Organization of Khuzestan for funding and Ahvaz Jundishapur University of Medical Sciences for scientific support this research project. The authors thank Hossein Jafari, Msc for software statistical analysis.

Conflict of Interest
The authors declare that they have no conflict of interest.

References
1. Butler JM, Buel E, Crivellente F, McCord BR. Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. Electrophoresis 2004;25(10-11):1397-1412.
2. DuVall JA, Roux DL, Thompson BL, Birch C, Nelson DA, Li J. et al. Rapid multiplex DNA amplification on an inexpensive microdevice for human identification via short tandem repeat analysis. Anal Chim Acta 2017;980: 41-49.
3. Brown SM, Hopkins MS, Mitchell SE, Senior M, Wang TY, Duncan R, et al. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [Sorghum bicolor (L.) Moench]. Theor Appl Genet 1996;93(1-2):190-198.
4. Jäger AC, Alvarez ML, Davis CP, Guzmán E, Han Y, Way L et al. Developmental validation of the MiSeq FGx Forensic genomics system for targeted next generation sequencing in forensic DNA casework and database laboratories. Forensic Sci Int Genet 2017;28:52-70.
5. Dobbs LJ, Madigan MN, Carter AB, Earls L. Use of FTA gene guard filter paper for the storage and transportation of tumor cells for molecular testing. Arch Pathol Lab Med 2002;126(1):56-63.
6. Kutanan W, Kitpipit T, Phetpeng S, Thanakiatkrai P. Forensic STR loci reveal common genetic ancestry of the Thai-Malay Muslims and Thai Buddhists in the deep Southern region of Thailand. J Hum Genet 2014;59(12): 675-681.
7. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 2005;1:47-50.
8. Tereba A. Tools for analysis of population statistics. Profiles DNA 1999;2:14-16.
9. Hildebrand EC, Torney DC, Wagner RP. Informativeness of polymorphic DNA markers. In: Cooper NG, editor. The human genome project: deciphering the blueprint of heredity. California: Palace Press; 1992. p. 99-102.
10. Hammond H A, Jin L, Zhong Y, Thomas Caskey C, Chakraborty R. Evaluation of 13 short tandem repeat loci for use in personal identification applications. Am J Hum Genet 1994;55(1):175-189.
11. Bosch E, Calafell F, Pérez-Lezaun A, Clarimón J, Comas D, Mateu E, et al. Genetic structure of north-west Africa revealed by STR analysis. Eur J Hum Genet 2000;8(5): 360-366.
12. Hedjazi A, Nikbakht A, Hosseini M, Hoseinzadeh A, Hosseini SM. Allele frequencies for 15 autosomal STR loci in Fars province population, southwest of Iran. Leg Med (Tokyo) 2013;15(4):226-228.
13. Stanciu F, Stoian JM, Popescu OR. Population data for 15 short tandem repeat loci from Wallachia region, south Romania. Croat Med J 2009;50(3):321-325.