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Characterization and Forensic Significance of X-STRs in Mutated Autosomal STR Markers of Paternity Cases

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Abstract

Genetic markers can be defined as the sequence of DNA that has a known physical location on the chromosome. Generally, markers help to link the inherited disease with the responsible gene. In forensics, Short Tandem Repeats (STRs) are most commonly used gold standard markers that consist of repeated 2-10 base pair units. Now days, in paternity cases 20 or more autosomal STR markers are using to establish paternity or other forensic application. It is reported in many cases one or two autosomal markers of the offspring's DNA profile show different alleles which are not present in their parent's DNA profile. This might be possible due to mutation or incest paternity cases. Therefore, to confirm mutation or support to establish paternity in such cases, lineage markers viz. Y-STRs, X-STRs, and Mt. DNA are potential tools. In this article, the characterization and forensic significance of X-STRs in mutated autosomal markers of two paternity cases were discussed. X- STRs of X-chromosomes show their unique pattern of inheritance than other autosomal STRs. The X chromosome is inherited in the offspring from father and mother both. The X chromosome is inherited from mother to daughter and son; and from father to daughter only. X-STR are used to decode genetic structure in respect to the cases like parentage, human identification, kinship, and disease identification. In this paper, significant role of X-chromosomal STR analysis in mutated autosomal STRs especially in paternity cases has been discussed.

Keywords: X-STRs; Human Identification; Parentage; Kinship; Forensic significance.

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INTRODUCTION

Genomic variations can be measured and are utilized to identify individual, population genetic studies, inherited diseases are said to be genetic markers. Individuals of the population are discriminate on the basis of genetic polymorphism i.e., genetic variation in the arrangement of nucleic acid in the DNA.[1] Majorly, there are three types of STR markers viz. autosomal STR marker, X-STR marker (Table 1), and Y-STR marker. Every nucleated cell (except sperm and ovum cell) in our body has 22 pairs of autosomal chromosomes, and

one pair of sex chromosomes (Female- XX and Male- XY). Autosomal markers retain all the phonotypical specific traits, and this transmission of traits depends on the gene in autosomal chromosomes. Y-STR is taken from the Y chromosome of the male, it has a limited application compared to other STR markers as the Y chromosome is only found in males, which means the Y chromosome can only be inherited by the father. X-STRs are the repeating sequence that is present on the X chromosome.[2]

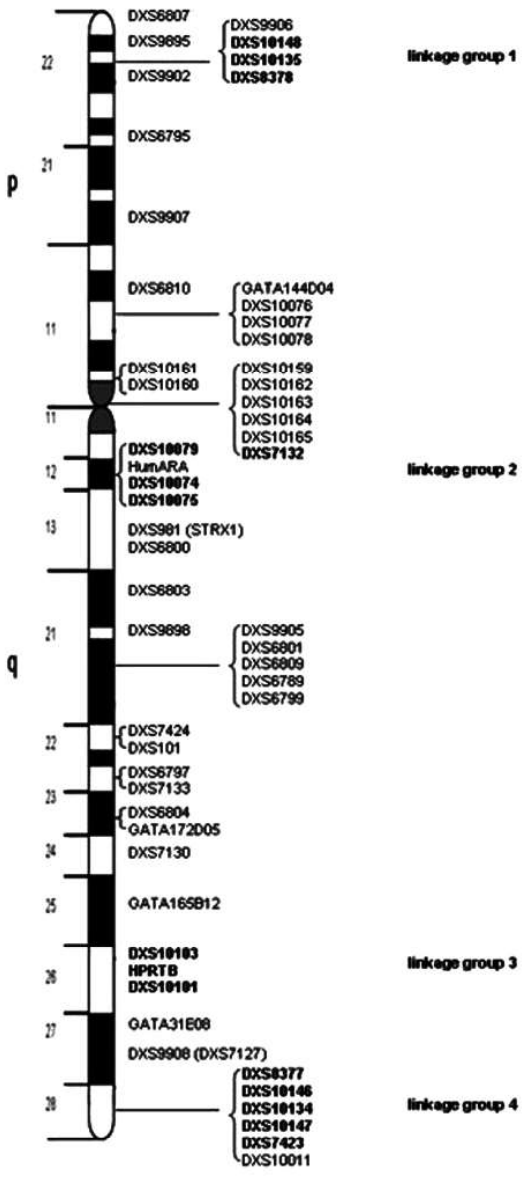
Table 1: X-STRs-Chromosomal location and Linkage group

S. No	Markers	Chromosomal location	Linkage group
1	DXS6807	p 22.33	-
2	DXS9895	p 22.32	-
3	DXS9906	p 22.31	-
4	DXS10148	p 22.31	X1
5	DXS10135	p 22.31	X1
6	DXS8378	p 22.31	X1
7	DXS9902	p 22.2	-
8	DXS6795	p 22.11	-
9	DXS9907	p 21.1	-
10	DXS6810	p 11.3	-
11	GATA144D04	p 11.23	-
12	DXS10076	p 11.23	-
13	DXS10077	p 11.23	-
14	DXS10078	p 11.23	-
15	DXS10161	p 11.21	-
16	DXS10160	p 11.21	-
17	DXS10159	centromere	-
18	DXS10162	centromere	-
19	DXS10163	centromere	-
20	DXS10164	centromere	-
21	DXS10165	centromere	-
22	DXS7132	centromere	X2
23	DXS10079	q 12	X2
24	HumARA	q 12	-
25	DXS10074	q 12	X2
26	DXS10075	q 12	X2
27	DXS981 (STRX1)	q 13.1	-
28	DXS6800	q 13.3	-
29	DXS6803	q 21.2	-
30	DXS9898	q 21.31	-
31	DXS9905	q 21.32	-
32	DXS6801	q 21.32	-
33	DXS6809	q 21.33	-
34	DXS6789	q 21.33	-
35	DXS6799	q 21.33	-
36	DXS7424	q 22.1	-
37	DXS101	q 22.1	-
38	DXS6797	q 22.3	-
39	DXS7133	q 22.3	-
40	DXS6804	q 23	-

41	GATA172D05	q 23	-
42	DXS7130	q 24	-
43	GATA165B12	q 25	-
44	DXS10103	q 26.2	X3
45	HPRTB	q 26.2	X3
46	DXS10101	q 26.3	X3
47	GATA31E08	q 27.1	-
48	DXS9908 (DXS7127)	q 27.3	-
49	DXS8377	q 28	X4
50	DXS10146	q 28	X4
51	DXS10134	q 28	X4
52	DXS10147	q 28	X4
53	DXS7423	q 28	X4
54	DXS10011	q 28	-

The X chromosome has a unique pattern of inheritance which is employed for kinship cases, paternity cases, cases related to missing persons, disease, and human identification.[3,4] As, X chromosome is 100 percent inherited from mother to son and daughter both; father to daughter only, and from parental grandmother to granddaughter.

During the process of meiosis, recombination occurring between two homologous chromosomes, some closely situated genes remains in linkage. Linkage can be defined as the relationship between the two loci on the same chromosome. In general terms, it can be said that if more than 50% of gametes have the same segment of parental chromosome then it can be taken into consideration that parental markers were linked. According to the rule of thumb, when the genetic distance (the distance between two markers on the same chromosome) is less than 50 cM than linkage is said to be there. Absence of linkage talks about the independent transmission of the genes in the offspring. Majorly there are four linkage groups in haploid conditions of X-STRs (Fig. 1). The most reliable method of linkage analysis is pedigree examination.[5]



X-STR markers and their linkage:

Short tandem repeats are the arrangement of two or more repeating nucleotide units. A repeat can range between 2-10 base pair units. As the sequence of these repeats differs from person to person. Therefore, by examining the sequence of repeats at distinct locations, the identity of the person by STR profile can be established6. Variants of STRs are found in autosomal and sex chromosomal X and Y chromosome. STRs are better than any other genetic markers due to the following reasons High discriminating power >0.9, Heterozygosity (Ho)

>70%, different locations on chromosomes (no closely linked loci), robustness and reproducibility of results (low artifacts), allele's length in the range of 90–500 bp (most suitable in degraded DNA), low stutters rates.

PASSING OF STR MARKERS

Markers from one generation to the next generation can be passed in four different ways.[7]

1. Markers that are located on autosomes are acquired by both father and mother. In this case, only half of the autosomal markers are present in the offspring due to the process of meiosis. Autosomal STR markers tell best about the mixed biographic ancestry.
2. Maternal linkage is only determined by the markers present in the mitochondrial DNA.
3. Paternal lineage is only determined by only markers present on the Y chromosome. The Y chromosome is passed by the father to son only.
4. Markers present on the X chromosome are passed from both father and mother to the daughter, and mother to the son. X-STR markers tell best about the mixed biographic ancestry. Particularly, disease identification, human identification, parent, kinship cases, and many more.

Mt. DNA and Y-DNA are passed unchanged over generation and generation (Except mutation). Therefore, they are the best ancestry markers.

Forensic Importance of X-STRs:

In forensics, various paternity cases have a mutation at a specific autosomal STR locus, which leads to exclusion or incest paternity. Such doubtful conditions overcome with the application of X-STRs along with autosomal STRs.

CASE-1

In a case of dispute paternity, 21 autosomal STRs viz., D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391 and D2S1338 were used. All the alleles of the female child were contributed by the suspected father and mother except locus D10S1248. At this locus female child's genotypes was 14, 15; mother's genotype was 13, 14; suspected father's genotype was 16, 16. Thus in the female child's DNA profile allele 14 was contributed by the mother whereas the child's allele 15 was not contributed by the suspected father as per the Mendel's law of inheritance. This result makes it difficult to decide the parentage of a girl child. However, at this locus only a difference in one repeat, which might be possible due to mutation. So, to precise this outcome, X-STRs were also typed. As in a girl child, there are two X chromosomes, one is inherited from the mother and one is inherited from the father. Therefore, 12 X-STRs viz., DXS10103, DXS8378, DXS10101, DXS10134, DXS10074, DXS7132, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148 were genotyped. At all the typed X-STRs in the girl child, all the alleles were contributed by the mother and suspected father. Thus, in this case, with the help of X-STRs, the definite paternity of the girl child was established.

CASE 2

In another case of paternity, 20 autosomal STRs i.e., D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, D3S1358, vWA, D16S539 and CSF1PO of of Global FilerTM PCR Amplification kit was used. All the typed alleles of the male child were contributed by the suspected father and mother except locus vWA and D18S51. At locus vWA, male child's genotypes was 17, 19; mother's genotype was 17, 19; suspected father's genotype was 15, 18. Thus, in the male child's DNA profile allele either 17 or 19 was contributed by the mother and suspected father's DNA profile at this locus was not showing the alleles which is present in the DNA profile of the male child at this locus. However, at this locus allele 18 present in the suspected father's DNA profile which differ in one repeat of allele 19 that is present in the male child's DNA profile, which might be possible due to mutation. Similarly, at locus D18S51, male child's genotypes was 14, 14; mother's genotype was 13, 16; suspected father's genotype was 14, 15. Thus in the male child's allele either 14 was contributed by the suspected father; Mother's DNA profile at this locus was not showing the allele 14 which was present in the DNA profile's of the male child at this locus. However, at this locus allele 13 was present in the mother's DNA profile which differs in one repeat of allele 14 that was present in the male child's DNA profile, which might be possible due to mutation. In this case two locus vWA and D18S51 were found where allele was not sharing by suspected father and mother both as per the Mendel's law of inheritance. To rule

out this finding, Y-STR and X-STR genetic markers were also genotyped. As a male child, there are one X and Y chromosomes. X-chromosome inherited from mother and Y chromosome inherited from father. 12 X-STRs viz., DXS10103, DXS8378, DXS10101, DXS10134, DXS10074, DXS7132, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148; and 27 Y-STRs viz., DYS576, DYS3891, DYS635, DYS38911, DYS627, DYS460, DYS458, DYS19, YGATAH4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS518, DYS570, DYS437, DYS385, DYS449, DYS393, DYS439, DYS481, DYF387S1 and DYS533 were genotyped typed. All the typed Y STRs in the male child, showing same male DNA profile with the male DNA profile obtained from suspected father. The alleles of X-STRs in the male child's DNA profile were contributed by the mother. Thus, in this case, with the help of Y and X-STRs, along with autosomal STRs, the definite paternity of the male child was established.

SOFTWARE AND DATABASES SUITABLE FOR X-STR MARKERS

FamLinkX:

FamLink is the software that is used to calculate the likelihood ratio in the specific case and pedigree analysis with the help of STRs located on the X chromosomes. It is free software with easy accessibility. Daniel Kling at Norwegian Institute of Public Health, Norway, Andreas Tillmar at National Board of Forensic Medicine, Sweden, Thore Egeland at Norwegian University of Life Sciences and Petter Mostad at Chalmers University, Mathematical Sciences Sweden developed this software. This software includes easy graphical interference that makes linkage calculations easy. In association with the Lander green algorithms, this software presents numerical calculations. The current drawback of FamLink is that it does not perform correction in mutation rate. FamLink allows for unseen alleles using two different methods; normalizing, whereby all frequencies are normalized so the final sum is 1.0, and Search and Subtract, whereby the new allele frequency is subtracted from other alleles not used in the current case.[8]

GenoProof 3

GenoProof 3 is software for analyzing complex forensic DNA mixtures. This system involves four steps: raw data analyses, creating projects, performing calculations, and quality control expert results. This software analyzes the markers present on autosomal chromosomes, X chromosomes, and Y chromosomes. This system helps in the investigation related to kinship cases, statistical calculations, and reports of generations. It is preferred over FamLink because it can account for the mutation rate, linkage of rare alleles, silent allele, sub-populations, linkage groups, and their calculations. followed by visualization of family relations via family tree to conduct sibling identification with the help of different tests like the monozygotic test, avuncular test.[9]

Brazilian Genetic Database of Chromosome X:

Brazilian genetic database is a freely available website available in Portuguese and English. It was developed for compiling the whole Brazilian database of X-STRs. This database also presents other information like physical and genetic locations, allelic structures, mutation rates, nomenclatures, likelihood ratio. The Brazilian database was published in the journal PubMed.[10]

Rutgers combined linkage physical human genome map:

It is the high-resolution genetic map developed by Mortise that includes polymorphic markers in the publicly available genotypic database. These genotypic databases are combined with CEPH and deCODE pedigree. It also includes information regarding SNP's and sequences. The location of these markers is confirmed with the help of a recombination database. This data is incorporated in the systematic form in the web based linkage mapping server.[11]

KITS FOR X-STR MARKERS

Investigator Argus X-12 qs amplification kit:

This kit is widely used to solve cases or kinship. It includes the installation of QS i.e. quality sensors to regulate the internal performance of the PCR reaction. Co-amplification of 12 X-chromosomal markers from 4 linkage groups and autosomal marker D21S11, it is a Well-established 5-color setup (Matrix B5). Quality Sensor and autosomal alignment marker added as valuable quality control features, Improved typing results of samples carrying common mutations, faster and more robust amplification to further streamline the workflow, more convenient reaction setup for manual and automated sample handling, optional

direct amplification capability to complete existing workflows for reference sample are some features of the kit.[12]

GHEP-ISFG-X-Decaplex:

It is a collaborative work of Spanish, Portuguese, and ISFG groups. They typed 10 X-STRs in a single reaction, including DXS8378, DXS9902, DXS7132, DXS9898, DXS6809, DXS6789, DXS7133, GATA172D05, GATA31E08, and DXS7423. Using the C-decaplex all the participating laboratories typed their reading of 200 samples (100 male and 100 female) in the system.[13]

AGCU X19 STR Kit

In 2016 genetic polymorphism of X-STR was investigated by this kit. Mainly this kit is used to study population genetics between the two populations. The 19 X-STRs multiplex system is a PCR based amplification kit that facilitates simultaneous amplification of 19 X-chromosomal STR loci (i.e., DXS7423, DXS10148, DXS10159, DXS6809, DXS7424, DXS8378, DXS10164, DXS10162, DXS7132, DXS10079, DXS6789, DXS101, DXS10103, DXS10101, HPTRB, DXS10075, DXS10074, DXS10135 and DXS10134). Eleven loci were extensively used in an Investigator Qiagen Argus X-12 (DXS7423, DXS10148, DXS8378, DXS10162, DXS7132, DXS10079, DXS10103, DXS10101, HPTRB, DXS10074 and DXS10135). 19X-STRs multiplex system is a robust and reliable amplification means to facilitate forensic and human identification testing.[14,15]

Goldeneye 17 X Kit

16 X-chromosomal STR-loci viz. DXS6795, DXS9902, DXS8378, HPRTB, GATA165B12, DXS7132, DXS7424, DXS6807, DXS6803, GATA172D05, DXS6800, DXS10134, GATA31E08, DXS10159, DXS6789, and DXS6810 employed for forensic application.[16]

MiseqFGxTM FORENSIC GENOMICS

The MiSeqFGxTM provides data in reading count and the CE in relative fluorescence units (RFU), so the two-output data cannot be directly compared to one another. 7 X-chromosomal STR-loci viz., HPRTB, DXS7132, DXS7423, DXS8378, DXS10074, DXS10103, and DXS10135 used along with other genetic markers.[17]

CONCLUSION

X-STRs are good genetic tool to decode genetic structure in respect to the cases like parentage, human identification, kinship, and disease identification. In this paper, significant role of X-chromosomal STR analysis in mutated autosomal STRs.

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