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Forensic applications of Y chromosomal properties

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1. Introduction

A Y chromosome is possessed by a normal human male and is absent in normal human female. Around 200 genes are present on it which is responsible for determining the male-specific characters like testis development early in the embryogenesis [1].

It is around 60 Mbs in length and consists of only 2% of total human genome. Various genetic studies revealed that at the distal portion of the short arm and long arm of Y chromosome two pseudoautosomal regions: PAR1 and PAR2 are present, respectively [2]. These regions have homology to X chromosome and X/Y crossing over frequently occurs here during male meiosis. Around 95% region of Y chromosome does not go through recombination and is transmitted intact via paternal inheritance. It remains in a haploid state and is termed as non-recombining Y (NRY) or Male-specific region (MRY) [2,3].

Due to NRY non-recombining ability, it is often used in population genetics, evolutionary studies and in anthropology [4]. Currently, two classes of NRY markers are being used in population studies and Forensic sciences. One marker is short tandem repeats on Y chromosomes (Y-STRs) whose allele combination at multiple loci on one Y chromosome gives rise to Y-STR haplotypes [5]. These Y-STRs have significant importance in forensic sciences because they are highly polymorphic [6], smaller in size (~100–400 bp) [7] and they have the ability that multiple Y-STRs could be typed in single PCR reaction [8]. Other marker is single nucleotide polymorphism on Y chromosome (Y-SNPs) whose allele combination at multiple SNPs gives rise to NRY haplogroup [5]. These NRY haplogroups are geographically specific [9] and have huge potential to aid forensic scientists in getting an idea about the geographic location of the culprit through the circumstantial evidence recovered from crime scene.

In order to utilize Y chromosome in different applications of forensics, its Y-STR profile is generated through various commercially available kits which amplify certain number of loci present of Y chromosome. Yfiler® kit is being used to generate the profile which targets 17 markers, simultaneously. Now a days, its improved version Yfiler® Plus kit is being used who has higher discrimination power and targets 27 loci at a time [10]. Other than that, Yfiler® Plus PCR Amplification Kit and AmpFLSTR® Yfiler® PCR Amplification Kit (Life Technologies, CA, USA) are currently being used to obtain complete Y-STR profiles [10,11].

This review concentrates on providing the overview of the utilization of Y chromosome in different areas of forensic sciences including the identification of rapist to the body identification from mass disasters to the finding of the true paternity. So, the aim of this review is to pen down the utilization of various Y chromosomal characteristics in different aspects of forensic sciences.

2. Major applications of Y-chromosome in forensic casework

With the intention of ensuring justice, forensic investigators are performing DNA fingerprinting to generate genetic profiles. Inclusion or the exclusion of the evidentiary sample obtained from the crime scene for paternity testing or missing person identification solely depends upon the similarities and differences between the genetic profile generated from the evidentiary samples and reference samples. In forensic casework where questioned genetic profile failed to show match to any of the available reference sample, the match probability from population database is evaluated. Like mitochondrial DNA [12], Y chromosome in this regard has proven quite assistive and has shown remarkable contribution in various fields of forensic sciences.

2.1. Sexual assault cases

Y chromosome holds an excellent potential in identifying the real culprit from male/female mixed sample, sample mixtures with multiple contributors and even from oligospermic or azoospermic perpetrators sample [13]. Initially, autosomal STRs were used for the DNA analysis of mixed forensics samples due to their ease of use and presentation of vast information. Commercially available kits, such as PowerPlex™ 16 (Promega, MA, USA), AmpFlSTR® SGM Plus™ (Applied Biosystems, USA), or AmpFISTR® Profiler Plus™ (Applied Biosystems, USA) are generally used for the DNA analysis of samples, including the amelogenin locus for the identification of assailant [14]. But they have their short-comings as below certain ratio they are unable to generate genetic profile and if the ratio of male cells is less then female cells (i.e. 1:25 – 1:50), only female part will be detected [15]. Samples with the

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admixture of different body fluids like saliva/vaginal secretion mixture, saliva/saliva mixture or fingernail scraping containing cells of both female victim and male perpetrator also make autosomal STR analysis difficult [16–18]. Profiling through autosomal STRs also fail when semen sample is old (> 48 h) or contains very low copy number of sperms. Moreover, in case of contamination or degradation which is the phenomena often occurs with biological evidence sample, the scientists have to face the failure in obtaining the autosomal STR profile [19].

As evidentiary samples are of limited quantities, so, in order to save time and wastage of samples, Y-STR analysis is preferred over autosomal STR as they are considered more efficient in detecting the Y chromosome from samples containing less than 5% of sperms [20]. They are also reported to be more efficient in generating full or partial genetic profile of the rapist from very low quantity sample through the panel of tetrameric Y-STR with the average repeat unit between 10 to 30 [21]. These markers' discriminatory power is considerably high in all major meta-populations and have low mutation rate. Through the approval of International Society of Forensic Genetics (ISFG), a panel of 17 (DYS19, DYS390, DYS385ab, DYS392, DYS391, DYS393, DYS438, DYS437, DYS448, DYS458, DYS456, DYS463, DYS389 I and II and YGATAH4) thoroughly evaluated markers is being used for investigation purposes [21,22]. Commercially available kits also use 9 and 17 Y-STR markers which can efficiently detect 0.1 ng DNA. For instance, PowerPlex Y system which used 12 markers, successfully generated results from the swabs obtained from sexual assault cases which were initially affirmed negative for semen [23]. With the help of these markers, a profile could also be generated from male cells detected in an old sample. Moreover, with the advent of technology and knowledge scientists are now able to generate Y-STRs profile from samples collected after 6–9 days of intercourse [15,24]. For this purpose, Hanson and Ballantyne [24] developed a pre-amplification system termed as Y-chromosome-targeted pre-amplification (Y-TPA) which pre-amplifies the multiple Y-STR loci via nested PCR approach. The amplified product can further be amplified by using commercially available next generation Y-STR amplifications kits like AmpFlSTR® Yfiler® Plus (Life Technologies, CA, USA) or PowerPlex® Y23 System (Promega, USA).

Despite having benefit of successfully generating male sex chromosome profile from scarce samples, Y-STRs are still inefficient in distinguishing Y-profiles from samples containing more than one source of Y chromosome. In such cases, only one large peak is generally obtained [21]. To get the distinguish profiles in such cases forensic scientists have conducted experiments to do profiling with the help of both Y and autosomal markers. PowerPlex® Y23 is generally preferred for such cases [19,20,25]. In a recent study in Brazil, the use of combined approach of a Y chromosomal/autosomal STR analysis was reported to genotype samples from sexual assault cases through PowerPlex® Y23. Out of which, Y-STR profiles added 24% informative data and is reported important in the assessment of match probabilities in identifying serial rapists and perpetrators [25]. Similar study was conducted in Germany in which combined approach was 97% successful in generating profiles from assault samples while only 8% Y profiles were generated via sole genotyping of Y chromosome [13]. Single use of Y chromosomal and autosomal genotyping has some extensive benefits but combinatory approach is more beneficial as it provides quick results and ensures the retrieval of maximum information.

2.2. Paternity testing

In paternity testing, the alleged father is decided on the basis of the interpretation of similarities and the dissimilarities of the genetic marker loci. If the differences between the putative father and the offspring are observed at the genetic marker loci then it is attributed to non-biological paternity and brings about the exclusion of the genetic paternity. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) [26] are two techniques mostly used for paternity testing today. But these techniques have some limitations as if the biological father is not present or deceased then scientists are unable to perform these tests. Unlike these techniques, Y chromosome STR or SNP analysis can be done even in the absence of alleged father [27]. In such cases, father's close relatives like father or brother can be the source of Y chromosome. Hence its property of remaining same generation after generation can be used for the characterization of the paternal lineage.

Various Y-STR kits are developed over the time to precisely distinguish non-paternal loci from paternal ones in cases of forensic concern [28,29]. But these commercially available kits are facing a principle limitation as the targeted loci mutates at moderate rate over generations (i.e. few mutations per locus per 1000 generation ($\mu \sim 10^{-3}$)) [30]. Due to which the related men belonging to same paternal lineage are not differentiated and test indicates false negative results. Moreover, the capability of Y-STR set to distinguish unrelated men from various paternal lineages is dependent on the population background [31]. So, to overcome such outcomes, more powerful Y-STR kits are being developed which targets more loci as compared to their parent kits. For instance, PowerPlex® Y Kit (Promega) contains 12 Y-STRs [32]; AmpFlSTR® Yfiler PCR Amplification kit (Life Technologies) contains 17 Y-STRs [28] and PowerPlex® Y23 Kit (Promega) contains 23 Y-STRs which are developed recently [29]. Despite adding more loci, the problem of mutations over generations still exists. So, even the outcomes from these kits do not fully authenticate the results for the cases of legal matters.

In 2010, forensic scientists began searching for the way out to this dilemma [33] and started estimating the mutation rate of these loci individually with the help of binomial hierarchical Bayesian model [34] and reported 13 Y-STR markers (DYF387S1, DYF399S1, DYF403S1a/b, DYF404S1, DYS449, DYS518, DYS526b, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627) with higher mutation rate, roundabout few mutations per locus per 100 generations and named these markers as rapidly mutating Y-STRs (RM Y-STRs) [33,35]. 93% of Y-STR markers show the mutation rates between 1×10^{-4} and 1×10^{-3} while RM Y-STRs display mutation rates between 1.19×10^{-2} and 7.73×10^{-2} [33] estimated through the Bayesian paradigm [34] with a Poisson distribution $P(k > 0) = 1 - P(k = 0) = 1 - e^{-Nm}$ (where N represents the number of markers and m shows the average mutation rate of the markers) [36,37].

Afterwards in 2012, Ballantyne and colleagues, studied their suitability in 604 males belonging to 51 worldwide populations to distinguish unrelated men and reported the successful separation of 98.5% of males [35]. The success of this study was also reported in an endogamous population [38] like Pakistan which is highly endogamous. Out of 572 men, RM Y-STR successfully differentiated 96.2% of men while Y-filer was able to differentiate 89.1% of males [39].

Its efficacy was also studies in 14,644 males from different 111 worldwide populations. These 13 RM Y-STRs completely individualized approximately 99% or 12,272 unrelated males [38]. The success of separation of unrelated males is also reported in Italian population [40] and Chinese Han Population [41].

2.3. Missing persons identification

DNA forensic analysis of circumstantial evidence has proven extensively prolific for the personal identification from civil and criminal investigations. After the success in identifying living persons in cases like sexual assault and paternity testing, the efforts of scientists shifted towards the identification of missing personals from mass disaster, missing soldiers or war victims from mass graves via the DNA based identification [39,42,43]. As nuclear genome is feeble and in such cases it is mostly found degraded, scientists chose mtDNA for identification which is stable and quite informative in this regard. But this method of identification is expensive and time-consuming. Furthermore, if maternal reference sample is not available then this method is