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An overview of recent advances in utilizing Y-Chromosome short tandem repeat markers for forensic and population genetics applications a snapshot of recent developments in the use of Y-Chromosome short tandem repeats markers in forensic investigations and population genetics studies

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Abstract

Significant variability Y-chromosomal polymorphisms, also known as short tandem repeats (STR) sequences, are a crucial resource in forensic and population genetics. This results from their capacity to identify male-specific DNA. The Y chromosome male-specific nature renders it particularly advantageous in scenarios involving mixed male and female cells, such as in sexual assault cases and the investigation of paternal genealogical lineage. The past few years has witnessed improvements in the discovery of Y-chromosome STRs loci as well as the development of Y-STR typing kits that can analyse several Y-STR loci at once using Multiplex PCR techniques. These advancements have proven to be an indispensable tool in modern forensic casework and population genetics studies. This review summarizes the history of the evolution of Y-STR typing so far and highlights the recent developments of its application in forensic and genealogical investigations.

Keywords: Y chromosome STR, Population genetics, Forensic science, Haplotype.

Introduction

The origin of mankind has always intrigued and compelled humans towards seeking comprehensive logical explanation for the observed similarities and differences in phenotypic and socio-geographical distribution of humans around the globe. Interestingly, variations in the genetics of modern people hold the key to unravelling not only the past of mankind with respect to origin, anatomical spread and demography, but also understanding the genetics of complex diseases as well as the use of genetics in solving crime (1). Despite decades of classical genetics identifying considerable variety and differences within and between human groups, more elucidation of these differences is still required. The advent of the DNA era brought about advances in the sequencing and the analysis of the genomes of both modern and ancient peoples, in the past few decades ushered in new insights into genetic variations and answered questions related to demographic history through investigations involving three different approaches (2). “These include the autosomal chromosomes inherited from both parents, the Y chromosome passed down from fathers, and mitochondrial DNA (mtDNA) inherited from mothers. Because the Y chromosome and mtDNA are inherited from only one parent and do not undergo recombination, they are especially useful in population genetics for tracing genetic lineages back to common ancestors (2,3). “

“Within the human genome, some non-coding sections of DNA contain recurrent units of 2 to 6 base pairs, referred to as short tandem repeats (STRs). The human Y-chromosome has STRs located out of the pseudo-autosomal parts and are transmitted without recombination, through patrilineal inheritance (i.e. from fathers to male offspring). The Y-STR exhibits an average mutation rate of 3.17 x 10-3, indicating a relatively high mutation rate that contributes to Y-STR diversity and polymorphisms. These characteristics can be utilised as genetic markers in population research and forensic investigations,

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including paternity testing, identification of sexual abuse perpetrators, and the determination male victims' male relatives in catastrophes or mishaps, among various other applications (4).“

“The most common attestation to the strength of Y-STR typing in forensic investigations is the analysis of vaginal swabs from rape victims of sexual assault cases“. These swabs despite having predominantly female DNA, trace amount of male DNA can be differentially extracted, amplified, analysed and compared with those of suspects to identify the culprit(s). Furthermore, Y quantification enables the detection of male DNA below 100 pg in saliva from kissed victims, particularly when the male perpetrator possesses azoospermic semen and relatively minimal saliva is present at the crime site. Similarly, in population studies, Y-STR polymorphisms create microsatellites that are widely distributed on the Y-chromosome and can easily be scored and genotyped hence they are useful tools for the deciphering history of human population (2,4).

“Consequently, comprehending the mutation rate at Y-STR loci is essential for establishing the genetic clock in evolutionary research and for determining probability in forensic analyses. Hence, this review summarizes the history of the evolution of Y-STR typing so far and highlights the recent developments of its application in forensic and genealogical investigations. “

The role of Y-chromosomal DNA in forensics and ancestry

The Y chromosome exhibits several exceptions to conventional patterns observed in human genetics. Despite housing genes that are non-essential for human life as only males have it while females do not, about half of this chromosome is composed of tandem repeats motif i.e., satellite DNA while the remaining part holds a few genes, of which majority do not undergo recombination (5). This particular chromosome plays a crucial role in medical and forensic genetics. It differs from other genomic regions because it does not undergo recombination, which means standard genetic mapping techniques are less informative. Therefore, direct physical examination methods are necessary for its study (1,3).

Being one of the smallest chromosomes, it has an approximate size of 60 Mb, consisting of about 30 Mb heterochromatin and about 24 Mb euchromatin, the tips of its arms contain regions known as pseudo-autosomal regions (PARs). These PARs possess sequences similar to those of the X-chromosome and are crucial for sex chromosomal matching during meiosis. The non-recombining region (NRY) of the Y chromosome is transmitted unaltered through paternal lineages, as most of it is devoid of recombination, maintaining a stable haploid state until a mutation arises at a specified locus. While using Y-chromosomal markers for identification checking may be constrained by this aspect (since unlike autosomal STRs which are unique for every individual, Y-STRs are shared by male paternal relatives); it forms the basis for Y-STR haplotypes and haplogroups that aid the tracing of paternal genealogy in population genetics studies (6).

Using variations in Y-chromosomal DNA to study human evolution raises questions about the related physical traits, as phenotypic alterations can influence evolutionary patterns shaped by natural selection rather than gene flow and genetic drift; consequently, increased understanding of the Y chromosome leads to the emergence of further questions. However, the structure and by extension, the sequence of the Y chromosome allows us to have a comprehensive picture of its organisation as well as ever increasing usefulness and application in the field of population genetic and forensic investigations (4.6).

“In the last ten years, research has shown that Y-chromosome markers, particularly the highly variable Y-STRs, are effective tools in forensic science. They are commonly used in cases such as sexual assault investigations and resolving paternity questions involving absent or unidentified male relatives. As a result, multiple Y-STR multiplex kits have been created for these purposes (5.7). “

Timeline of Y-STR discovery

Only a small number of Y-STR loci were readily available for forensic studies as recently as the middle of the 1990s. The usefulness of 13 of the accessible loci for forensic studies was evaluated through a cooperative effort. The Forensic Y User group, an association of forensic laboratories, was

founded to control the collection of population data in partially as a result of the inspiration from the (http://www.yhrd.org). Under the guidance of the Forensic Y User Group, the loci encompassed in the "minimum haplotype" or "extended haplotype" is analysed. The "minimum haplotype" consists of the duplicated locus DYS385 and the following genes: DYS393, DYS392, DYS391, DYS390, DYS389 I/II, and DYS19. The Y-STR sites associated with the "minimum haplotype" are unquestionably the most frequently employed in forensic investigations (8).

The repeated dinucleotide locus YCA II was initially combined with the loci of the minimal haplotype to form the "extended haplotype". Finding new Y-STR loci required a number of cloning as well as hybridization techniques before vast amounts of Y-chromosome sequence were made available. The most recent and very certainly final application of this strategy involved the construction of a cosmid library from stream human Y chromosomes. Subclones with repeated GATA elements were chosen using probes with the repeating elements [TATC] 10 or [GATA] 10. This research identified seven loci suitable for human identification testing. The number of tetranucleotide Y-STR loci currently known has increased (4,9).

The availability of a sizable amount of Y-chromosome sequencing facilitated the identification of novel Y-STR-loci. As the first analysis of its kind nvestigated 1.22 Mb of the Y chromosome sequence and found 18 novel STR sequences. Three of these loci—DYS439, DYS438 and DYS437—that have been included in commercial Y-STR typing systems are universally accepted for use by the forensic community. Recently 639 selected DYS439 and DYS438 as potential replacements for the enlarged haplotype's YCA II gene (10,41).

A significant number of STR loci have recently been identified from Y-chromosomal sequencing. Five loci were identified by 17 as DYS445, DYS444, DYS443, DYS442, and DYS441 carried out a more comprehensive survey that led to the discovery of 14 novel Y-STR loci, including DYS464, DYS459, DYS458, DYS456, DYS455, DYS454, DYS453, DYS452, DYS450, DYS449, DYS448, DYS447 and DYS446. The 23 Mb of euchromatic Y-chromosomal sequence scan was by far the most detailed examination (http://www.gdb.org). Table I summarizes the timeline of the discovery of new Y-STR haplotypes which brings about the availability of commercial kits that enable the investigation of new Y-STR haplotypes in research laboratories.

The chosen loci have repetitive elements ranging in size from 3 to 6 bp. Since dinucleotide repeating elements are known to cause PCR stutter artifacts, these loci were avoided. Using this technique, 475 potential Y-STR loci were found, of which 45 had already been identified. 281 loci were successfully designed with in silico PCR primers (http://www.gdb.org). Among them, 166 primer sets produced male-specific amplicons, and 139 loci were identified as polymorphic in a cohort of 8 males representing various binary-marker haplogroups. This innovative sequence-based approach has greatly increased the number of Y-STR loci accessible for forensic, paternity and phylogenetic testing (11). From Table I, it can be inferred that the Y-STR markers were identified through individual research groups and different time intervals which adds to the pool of already known markers. Interestingly, nearly all Y-STR markers were discovered within the period of a decade (i.e. 1992 to 2002); with majority of these markers identified in 2002, mostly by (5,8-10,11) with 6 and 9 markers respectively. Examples of organizations involved in this field are the European DNA Profiling Group (EDNAP), the Scientific Working Group on DNA Analysis Methods, and the International Society for Forensic Genetics (ISFG).

Table I: Timeline of Y-STR haplotypes discovery

S/N	Markers	Year of discovery
1	DYS19	1992
2	YCAII a/b, DXYS156, YCAIII a/b (DYS4l3), YCAI a/b	1994
3	DYS389I/II, DYS393, DYS392, DYS391, DYS390	1996
4	DYS426, DYF371, DYS425	1996
5	DYS388, DYS288	1997
6	DYS385 a/b	1998
7	A7.2 (DYS461), A7.1 (DYS460), H4, A10, C4	1999
8	DYS439, DYS438, DYS437, DYS436, DYS435, DYS434	2000
9	DYS442, DYS441	2001
10	DYS445, DYS444, DYS443	2002
11	DYS462	2002
12	DYS464 a/b/c/d, DYS463, DYS459 a/b, DYS458, DYS456, DYS455, DYS454, DYS453, DYS452, DYS450, DYS449, DYS448, DYS447, DYS446	2002
13	DYS468-DYS596 (+129)	2002
14	DYS597-DYS645 (+50)	2003

The most commonly used markers are DYS19, DYS 393, DYS 389I/II, DYS 392 and DYS 391 as well as DYS 385 a/b. A multi-copy marker's two copies are identified as "a/b" if there are two of them. The total number of markers accessible is computed taking into consideration the primer pair utilized to generate them as well as the end products generated. Retrieved from; (Butler, 200

The Multiplex PCR system

The utilization of techniques that enable the investigation of several loci at once is crucial to forensic genetics. Multiplex PCR methods, which amplify two or more target sequences simultaneously, are commonly used to achieve this. Multiplex PCR provides multiple benefits over uniplex PCR and has been used to analyze polymorphisms, deletions, and mutations. The ability to amplify and evaluate a large number of loci quickly as well as the fact that less PCR reagent and template DNA are eventually required, forms the two major benefits of the multiplex PCR system (10-12).

Systems capable of simultaneously amplifying up to 21 markers in a single reaction have been developed, enabling the generation of substantial genetic data even from minimal quantities of template DNA. Multiplex PCR system development often involves a variety of difficulties. When there are multiple primer sets present, the likelihood of producing non-specific artefacts increases because DNA target sequences frequently do not amplify uniformly. Not only does this reduce the effectiveness of the PCR, but it also makes it more challenging to analyze the outcomes. To create a multiplex PCR system where all target sequences are reliably and equitably amplified, a significant amount of tuning is needed. Multiple strategies have been employed to create multiplex PCR typing systems (13). While there are distinctions between each strategy, there are also some commonalities. Designing suitable and targeted primer sets is perhaps the most crucial step in multiplex creation. It is crucial that all primer pairs are made with nearly identical melting temperatures since they must all operate under the same set of physical conditions (14).

Primer sequences must also be chosen to reduce primer-primer interactions. Furthermore, it is critical that primers are specifically tailored to the target gene on the Y chromosome. Avoid using primers that have many sequence similarities to homologs on the X chromosome or other regions of the genome (9,11). In uniplex PCR operations, synthesised primer pairs can be evaluated to determine if they yield male-specific amplicons of the anticipated size. Primer pairs that exhibit optimal performance in uniplex reactions can be combined in multiplex reactions, while monitoring the relative efficacy of each set (12,15). Multiplex typing systems should be tuned until they reach a particular level of performance. High standards for typing and analysis are upheld by a number of regulatory entities. “These include the European DNA Profiling Group (EDNAP), the Scientific Working Group on DNA Analysis Methods, and the International Society for Forensic Genetics (ISFG) These groups establish standards for the application and verification of multiplex PCR typing technologies (16). “

Some examples of the validation exercise include:

Establishing the sensitivity and consistency of the typing system using recently prepared and preserved DNA.

Demonstrating that the results are similar regardless of the type of tissue out of which DNA was extracted.

Demonstrating that the systems produce consistent results across multiple laboratories.

Demonstrating that the system functions well when applied to samples that are similar to those commonly faced in forensic studies.

This means that DNA taken from body fluids that have been combined with commonly found chemicals should also be successfully typed. A system's ability to analyse samples with mixed female and male DNA mixes should be investigated, as well as its impact of environmental conditions including humidity, UV and temperature. Numerous Y-STR multiplex PCR typing systems have been developed and validated based on key forensic and population genetics criteria. These systems vary in the number and combination of STR loci they target, depending on their intended application. Basic forensic kits typically include a core set of markers such as DYS19, DYS392, DYS389I/II, DYS390, DYS391, DYS393, and DYS385a/b, which provide essential discriminatory power for routine

casework.

To enhance resolution, especially in closely related male lineages, more advanced kits expand this panel to include additional loci such as DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS460, DYS481, DYS533, DYS570, DYS576, DYS635, DYS449, DYF387S1, and Y-GATA-H4. These expanded systems—exemplified by platforms like PowerPlex® Y23 and AmpFlSTR® Yfiler® Plus—have become widely adopted due to their improved sensitivity and capacity to analyze complex or degraded samples.

In population studies, particularly within East Asian populations, customized or regionally developed kits such as AGCU Y24 Plus PCR, SureID® PathFinder, Goldeneye® 26Y, Goldeneye® Y-PLUS, and DNATyper™ Y29 are commonly employed. These kits often include loci not present in Western commercial kits, such as DYS388, DYS444, DYS447, DYS522, DYS527, DYS645, and various Y-InDel markers. Some kits also incorporate triallelic or multi-copy loci to further increase discriminatory power.

It is worth noting that the Yfiler® kit by Applied Biosystems remains among the most widely used systems globally for population genetic analyses. In contrast, studies conducted in China tend to utilize a broader variety of domestic kits, reflecting the specific research goals and regional genetic diversity. Currently, STR typing in forensic and population genetics routinely employs either a 23-locus or a 27-locus panel, depending on the depth of analysis required and the geographic or ethnic focus of the study (11,17).

Forensic application

In standard forensic DNA studies, Y-SNPs and STRs represent two separate Y-chromosome polymorphisms can be applied either separately or combined for various analyses. PCR could only reliably genotype a tiny subset of Y-STRs, especially-in-the early years of routine-Y-chromosome-research. The commonly used set of Y-STR markers includes the tetra-nucleotide repeats DYS385, DYS19, DYS389-II, DYS389-I, DYS390, DYS393, and DYS391, along with the tri-nucleotide repeat marker DYS392. These eight loci are incorporated in most popular Y-STR multiplex kits such as PowerPlexY, PathFinderPlus, PowerPlexY23, Yfiler, GoldenEye, Argus-Y-28, YfilerPlus, STRtyper-27, YfilerPlatinum, and AGCUY37. (16-18).

The availability of several rapidly mutating (RM) Y-STR loci is also of particular significance in forensic analysis. The incorporation of some of these RM-Y-STRs in Y-STR genotyping/sequencing kits is gradually gaining some traction after being first disclosed in 2012. One has a considerably better likelihood of being able to tell between closely related male family members with these RM-Y-STRs ,which are obviously a huge benefit in some forensic scenarios (19). The majority of the multiplex-Y-STR-CE kits now on the market enable the routine genotyping of 15–30 Y-STR loci. However, this will change with the development of massively parallel-sequencing- (MPS), which enables the simultaneous genotyping of anywhere between a few tens and a few hundred Y-STR loci (17,19).

The history of Y-SNP screening and its implementation is considerably more complex. Various research groups utilised six distinct haplogroup nomenclatures and a diverse array of screening procedures when applying Y-SNP polymorphisms for population genetic objectives (5,11,15,20). Fortunately, this nomenclatural uncertainty was resolved into the nomenclature we still use today by using the mtDNA example. There is yet no standard, straightforward, sensitive, and generally acknowledged Y-SNP screening method, unlike Y-STRs, for which CE-based multiplex PCR tests are frequently employed. Numerous proof-of-concept investigations have shown that hundreds to thousands of Y-SNPs may be correctly genotyped; hence, this situation could soon evolve further. Hybridization capture followed by MPS methodologies and tools to effectively retrieve Y-SNP information from whole-genome digital sequencing data sets have also been created in addition to all these targeted approaches. It is reasonable to anticipate that these methods for bulk screening will advance quickly (20-21).

Nonetheless, SNP analysis is still a potent instrument in a variety of specialized applications, including ancestry informative markers, mtDNA, and phenotypic trait prediction. Due to their genetic stability, SNPs are advantageous for paternity testing and large-scale identification of catastrophe victims, making their use in high-throughput analysis of

damaged samples noteworthy. Mini STRs are said to compete effectively in the space of degraded samples (23)

Comparatively speaking, SNPs are less polymorphic than STRs. Therefore, more SNPs would have the same amount of power as discriminative or random matching probabilities. Thirteen to fifteen STRs will be up against between forty and sixty SNPs, according to estimates (16-22). In clinical genetic initiatives, SNP typing typically takes into account thousands of SNP loci for a very small number of samples. A sustainable source of DNA is required for further efforts, as several loci do not provide significant findings and must be excluded from the final set of data to be analysed. In the end, attempts to directly compare the evidence with the suspect lead to data loss, which is unacceptable (8-10,11,16).

The lineage share indicates that certain Y-STR isolates are more closely related than ancestral lineages, elucidating the shared Y-STR haplotype within the population. The adoption of new Y-STRs to examine forensic caseworks is motivated by two key factors. The capacity to resolve Y chromosomal haplotypes is strengthened by this additional tool, and it also helps that all of these repeats are tetra-, penta-, and hexa-nucleotide repeats. The larger repeat motifs, or "STRs," are helpful for clarifying mixed evidences (19,23,24). Research indicates that the genetic diversity of Y-STRs may fluctuate according on the population sample examined. Geographical population distribution may also affect Y-STR diversity.

Application in population studies

Due to the Y-Chromosome's non-recombination characteristics, certain mutations are inherited across generations, making them identifiable. Because of this, the Y chromosome is one of the most valuable tools for investigating population history and understanding worldwide migration patterns. Scientists designated distinct haplogroups to populations based on their genetic disparities through the analysis of Y-chromosome-associated markers (25). Numerous genetic researches now support the idea that humans first emerged in Africa, which is supported by ideological and theological studies (22-25). Surnames in several groups were discovered using Y-chromosome linkage investigations. Due to the fact that surnames are particular to a certain population in a particular geographic area, these studies map out the people migratory patterns. The data indicate that 60% of the British and Irish populations possess the identical Y-chromosome haplotype (25,26). The relationship between Y-haplogroups and surnames has to be explored in other populations as well since it will not only reveal the connection between different historical events but also aid in the development of databases for locating the origin of populations and migratory patterns (27). Some population studies conducted using different Y-STR markers are presented in Table II. The Table highlights the successes achieved from the utilization of Y-STR markers in different population studies, to evaluate relatedness between ethnic groups and/ or countries. Measurement of genetic distances, haplotype frequencies as well as haplogrouping helped in the identification of genealogical origins, migratory patterns and genetic divergence between the populations under study.

Table II: Selected studies using Y-STR markers for population genetics investigations

S/No	Study	Population	N	Sample type	Y-STR loci	Major findings
1	(28)	Iranian	1353	Blood	17	The study developed a machine learning model using Y-STR data, offering a reliable decision-support tool for forensic identification and regional haplogroup assignment.
2	(29)	Central Kazakh	112	Buccal Swab	23 Y-STR-12 X-STR	Identified haplotypes enhance the Y-chromosome reference database for Kazakhstan, supporting population genetics, forensic science, and genetic genealogy research.
3	(30)	Luzhou Han (China)	408	Blood	24 Y-STR 3Y-InDels	Identified high diversity and discrimination; better resolution for related males; Y-InDels add stability.

4	(31)	Qatar	379	Buccal Swab	23	The study showed diverse paternal lineages and gene flow in Qatar. It identified distinct tribal and regional genetic groups. Findings support forensic and medical genetics.
5	(32)	Iranian	1,097	Blood	17	The study found high haplotype diversity with 1,353 unique haplotypes. It demonstrated strong discrimination power useful for genetics population and forensic applications.
6	(33)	Iraqi Kurdistan	36	Blood	17	Significant genetic diversity was observed in Kurdish and Arab populations in Iraqi Kurdistan using eight Y-STR markers. Genetic analysis showed clear differentiation between the two groups. The results provide valuable data for forensic applications in the region.
7	(34)	Iraqis	178	Buccal swab	17	Genetic relationship between Central Iraq and other Arabs. Strong genetic similarity with Kuwaiti, Yemeni and Saudis.
9	(35)	Middle Euphrates Iraqis	144	Blood	5	It identified 62 haplotypes, with higher diversity in the Commoners group than the Hashemites. This shows genetic variation and good discrimination power in both groups.
10	(36)	Malaysians	59,266	-	15, 3, 7	The study was prepared in Malaysia as database for Malaysian population.
11	(37)	Arabs in United Arab Emirates	436	Blood	27	Abnormal alleles were found in UAE population and in the other populations in different loci.
12	(38)	Japanese	567	Buccal swab	17	Japanese individuals sharing common surnames revealed both clustered lineages and unique haplotypes, reflecting a combination of deep ancestral origins and more recent paternal line diversification within surname groups.
13	(39)	Naga tribes, India	203	Buccal swab	23	A unique genetic makeup is evident, as demonstrated by the neighbor-joining tree.
14	(40)	Soorani Kurds	162	-	17	The Kurdish population exhibited close genetic affinity with Asian groups, while showing the greatest genetic divergence from European and African populations.

Conclusion

This review highlights the brief history and recent Advancements in the application of Y-STR markers in forensic and population studies. With advancements in the field of forensics, Y-STRs with fast mutations that exhibit significant mutation rates have been discovered. New Y-STR markers were also found as a result of the Human Genome Project generated a substantial volume of DNA sequence data and introduction of bioinformatics techniques. In addition to being an effective tool for studying the development of human populations, the Y-chromosome is crucial in forensic investigations like sexual assault cases, paternity testing, and the burning of human remains. The Y-STR typing can be used to prove a blood relationship, solve tricky paternity issues, offer a quick way to rule out motherless paternities, and identify victims of large-scale tragedies. Genealogical research employ Y-STR markers characterised by elevated mutation rates, whereas paternity or kinship assessments utilise Y-STR markers with diminished mutation rates. These markers can be used to compare and contrast individuals' genetic make-up. As a result, it can be used to determine contacts between populations existing in different places and to determine their origin. Y-STR typing kits as forensic tools for solving challenging cases have been improved over time. These kits are being used in several genealogy

investigations on various populations worldwide. Additionally, greater study of various populations utilizing Y-STR markers is required in order to build a Y-STR database for use in forensics as well as other applications.

For prospects in the future, additional Y-STR polymorphic markers may be added to kits which can be utilized to more precisely define Y lineage. High mutation Y-STR markers are ideal for paternal lineage investigations, whereas low mutation Y-STR markers are better for kinship searching or paternity testing. Scientists must find more indicators that might be used for paternal lineage as well as forensic investigations like kinship searching and paternity testing in order to solve the problem of high or low mutation. Additionally, as the number of markers rises, more advanced statistical tools will be required to assess them effectively.

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