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Forensic Applications of Markers Present on the X Chromosome

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Abstract

The X chromosome is one of the two sex chromosomes found in humans and other mammals. It plays a crucial role in determining an individual's sex and contains genetic information that can be useful in forensic and human identity testing. Unlike autosomal DNA, which is inherited from both parents, the X chromosome is inherited differently in males and females, making it useful in certain types of analyses. In forensic investigations, the X chromosome can be used to determine the sex of an individual, which can be useful in identifying potential suspects or victims. Additionally, X chromosome analysis can be used to link evidence samples to a particular individual or to exclude individuals as potential contributors of the evidence. This can be particularly useful in cases where the evidence sample is a mixture of DNA from multiple individuals. In human identity testing, the X chromosome can be used in situations where other types of DNA analysis are not possible or inconclusive. For example, in cases where a potential parent is unavailable for testing, analysis of the X chromosome can be used to determine if a child is likely to be their biological offspring. Similarly, in cases where traditional autosomal DNA analysis is inconclusive, X chromosome analysis can be used to provide additional information about the biological relationship between individuals. However, there are some limitations to the use of X chromosome analysis in forensic and human identity testing. One limitation is that it is not as informative as autosomal DNA analysis, as it contains less genetic information. Additionally, the inheritance patterns of the X chromosome can be complex, particularly in cases where there are multiple generations involved. Therefore, X chromosome analysis should be interpreted in conjunction with other types of DNA analysis and other forms of evidence to ensure accurate and reliable results. Overall, the use of X chromosome analysis in forensic and human identity testing can provide important information in certain situations, particularly where traditional DNA analysis is not possible or inconclusive. As such, it is an important tool in the fields of forensic science and human identification.

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1. Introduction

1.1. Human X chromosome

Human cells contain a total of 46 chromosomes, comprising 44 autosomes and 2 sex chromosomes. The sex chromosomes differ between males and females, with males possessing one X chromosome and one Y chromosome (X: ~155Mb, Y: ~57Mb) while females have two X chromosomes. This difference in sex chromosomes results in a distinct

genome between male and female cells. Moreover, the presence of two X chromosomes in females leads to approximately 100 Mb more DNA than in males [1]. The X and Y chromosomes evolved from a pair of autosomes, but out of the more than 600 genes on the Y chromosome, only 17 are functional after being transferred to the X chromosome. Interestingly, the X chromosome has also been involved in retrogenes donation and reception. Autosomal copies of genes originally located on the X chromosome play a crucial role in preventing its inactivation during meiotic sex-chromosome inactivation (MSCI) in spermatogenesis [2]. The majority of genes on the X chromosome are not related to sex. In females, only one X chromosome is needed to express the same number of X-linked genes as in males who have only one X chromosome. During early embryonic development, each female cell randomly chooses to inactivate either the maternally or paternally derived X chromosome, ensuring that only one X chromosome is active in each cell [3]. The X chromosome is larger and contains more genes than the Y chromosome, with about 160 Mb of genomic DNA and over 1200 expressed genes. These genes account for 5% of the haploid genome, with at least 800 coding for proteins. Genes located on the X chromosome play a crucial role in the development of gender in both males and females. The X chromosome has two pseudoautosomal regions (PARs) located at the distal end of each arm, which are similar to the Y chromosome. These regions, along with a few adjacent chromosomes, escape X inactivation and remain active in both sexes [4]. As recombination is absent between the X and Y chromosomes, mutations and deletions accumulate on the Y chromosome due to genetic drift and weak selection. The regions where the X and Y chromosomes share homology are extremely limited, with only two small areas known as pseudoautosomal regions 1 and 2 (PAR1 and PAR2) remaining. These regions are located at the ends of the X and Y chromosomes. Men are more prone to X-linked mutations than women because they have two copies of each gene on the X chromosome, resulting in quasi-hemizygous status[5].

It is commonly believed that recombination between the X and Y chromosomes occurs solely in the pseudoautosomal regions (PARs) situated at the ends of each sex chromosome, excluding the autosomes. A human-specific X-transposed region has been duplicated from the X chromosome to the Y chromosome in PAR1 and PAR2 [6]. It is believed that this pseudo-autosomal boundary, which divides the Y chromosome's non-PAR (non-PAR) segment from the PAR1 region, suppresses X-Y recombination [7]. The PAR recombines and functions like an autosome during meiosis. Because of this, the genes in this area are inherited via autosomal inheritance rather than being completely sex-linked [8]. The main sex-determining gene in humans, Sex determination region (SRY), is found on the Y chromosome. The human sex chromosomes are also exposed to unusual evolutionary processes because there is no recombination and one of the X chromosomes is inactivated in females.

Here are some of the structural characteristics of the human sex chromosomes, some of which are shared and some of which are specific to each [9, 10].

1.2. Population and Forensic Genetics:

The X chromosome plays a crucial role in population and forensic genetics, providing valuable information about human genetic variation and aiding in the identification of individuals. Here are some advances and applications of X chromosome markers in these fields:

A. Population Genetics

1. Population Structure and Migration Patterns: X chromosome markers are used to study population structure and migration patterns, providing insights into historical demographic events.
2. Haplotype Diversity: Analysis of X chromosome haplotypes helps in understanding the genetic diversity within populations, which is important for studying population history and evolution.
3. Sex-Biased Migration and Gene Flow: The X chromosome is useful for investigating sex-biased migration and gene flow, discerning differences in male and female migration patterns.
4. Demographic History Inference: X chromosome markers are employed to infer demographic history, such as population expansions, contractions, and bottlenecks, by examining the distribution of genetic variation.

B. Forensic Genetics

1. Identification of Individuals: X chromosome markers contribute to the development of more accurate forensic DNA profiling, aiding in the identification of individuals in criminal investigations.
2. Parental Testing: X chromosome markers are used in cases of disputed parentage, providing

additional genetic information for paternity and maternity testing.
3. Population-Specific Databases: X chromosome data contribute to the development of population-specific databases that enhance the accuracy of forensic analyses by considering population-specific genetic variations.
4. Phenotypic Prediction: Some X-linked markers are associated with specific phenotypic traits, allowing for predictions related to certain physical characteristics of an individual.
5. Familial Relationships: X chromosome markers are useful in establishing familial relationships, including sibling relationships, which can be crucial in missing persons cases or when trying to identify unknown remains.
6. Admixture Analysis: X chromosome markers contribute to admixture analysis, helping forensic investigators understand the genetic makeup of individuals with mixed ancestry.
7. Forensic Genealogy: X chromosome markers, along with other genetic information, aid in forensic genealogy, helping in the identification of unknown individuals through the reconstruction of family trees.

As technology continues to advance, the resolution and efficiency of X chromosome marker analysis in both population and forensic genetics are likely to improve, providing even more valuable insights into human genetic variation and aiding in criminal investigations [11].

1.3. Pseudoautosomal Regions

Each human sex chromosome has two corresponding pseudoautosomal regions (PARs), as depicted in. These regions are situated at the ends of the chromosomes; PAR1 is located on the short arm (Xp/Yp) of the sex chromosomes, while PAR2 is situated on the long arm (Xq/Yq). PAR1 spans a length of 2.7 Mb, while PAR2 covers a length of 320 kb. The areas' end locations on the chromosomes are represented by a distinct pseudoautosomal boundary (PAB) [12, 13]. The PAR1 region undergoes at least one recombination every male meiosis, which is a high recombination rate relative to the autosomes for a region that is less than 3 Mb in size. Although it is lower in females, the average autosomal recombination rate is lower than the PARs recombination rate [14].

1.4. X-transposed Region

Around three to four million years ago, a segment of the X chromosome measuring 3.4 Mb in length was transferred to the Y chromosome, giving rise to the X-transposed region (XTR), which presently contains only two genes [15]. Previous studies suggest that recombination between the X and Y chromosomes is probable in this region [16]. Genotyping (both sequencing and chip genotyping) may encounter difficulties in this region due to the shared homology between the X and Y chromosomes. Identifying the origin of SNPs or regions is challenging because two identical sections exist on both chromosomes [17].

1.5. X-degenerate Region

The term "X degenerate region" refers to certain sections of the Y chromosome that are highly conserved among primates. It has 16 genes that are single copies and have X chromosome homologues [15]. Areas with the same color are homologous between two sex chromosomes, as seen in (figure 1). Pink and green, respectively, depict the PARs and X-STR. The chromosome's centromeres are depicted as tightening [10].

1.6. X-chromosome Inheritance Pattern

The forensic significance of the X chromosome stems primarily from its unique inheritance pattern in females. Unlike males, who are hemizygous for most regions of their X and Y chromosomes, females can recombine during meiosis due to their possession of two homologous copies of the X chromosome. As a result, a female receives one non-recombinant copy and one recombinant copy of the X chromosome from each of her parents [1]. In contrast, a male inherits only one recombinant copy of the X chromosome from his mother, which he will pass on to his female offspring without alteration (Figure 2).

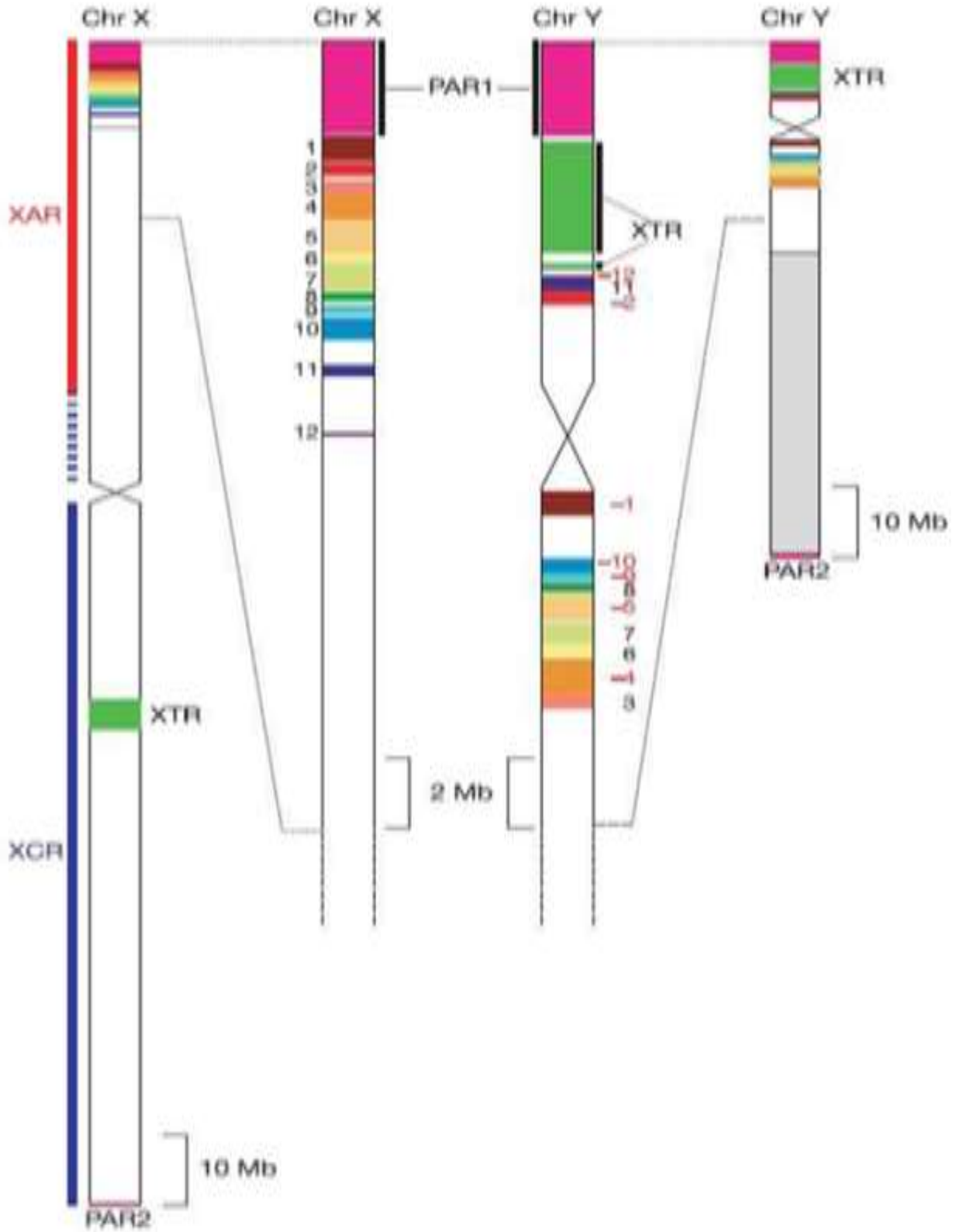

Figure 1. An overview of different chromosomal sections of X and Y chromosomes [10].

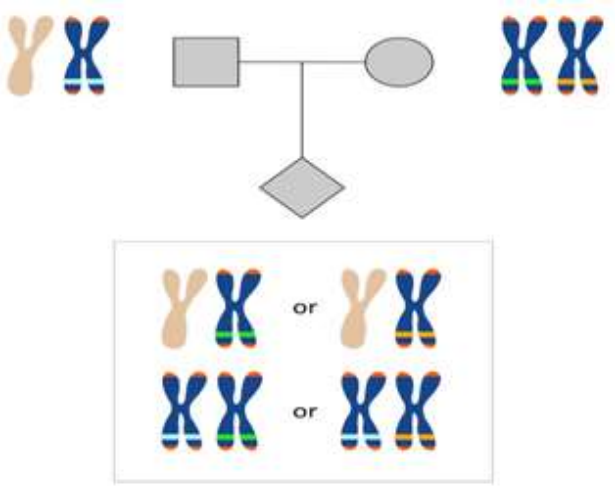

Figure 2. The Pattern of Inheritance in X-chromosome in Males (XY) and Females (XX) [10].

1.7. X-Chromosome Markers Analysis

Due to its distinct pattern of inheritance, X-STR research has recently attracted a lot of interest in the field of forensic genetics. Men inherit one X chromosome from their mothers, while women inherit two X chromosomes one of each parent. The examination of the X-chromosomal loci may be helpful [16]. The son carries the mother's X-Chr, whereas the daughters carry the entire paternal X chromosome, which allows for the use of the X-Chr as an additional tool for autosomal STR and Y-chromosomal STR (Y-STR) DNA typing as in (figure 3)[17, 18]. Here are some examples of possible genotypes: These examples illustrate the variety of genotypes possible when considering X chromosome markers with three alleles (A, B, and C). The actual frequencies of these genotypes in a population would depend on factors such as allele frequencies, recombination rates, and population history.

i. *Individual with Two Different Alleles:* XAXb: This individual has inherited allele A from one parent and allele B from the other parent.
ii. *Individual with Two Identical Alleles:* XbXb; This individual has inherited allele B from both parents.
iii. *Individual with All Three Alleles:* XAXBXc: This individual has inherited allele A from one parent, allele B from the other parent, and allele C from both parents.
iv. *Individual with Two of the Three Alleles:* XAXc: This individual has inherited allele A from one parent and allele C from the other parent.
v. *Individual with a Different Combination:* XCXB: This individual has inherited allele C from one parent and allele B from the other parent.

In practical applications, genotyping techniques, such as polymerase chain reaction (PCR) and DNA sequencing, would be used to determine the specific alleles present in an individual's X chromosome markers. Analyzing the distribution of these alleles in populations can provide insights into genetic diversity, population structure, and other aspects of population genetics. In forensic applications, such as DNA profiling, the presence or absence of specific alleles at X-chromosome markers can be used for individual identification and relationship testing.

1.8. X-Chromosome (X-STR)

X-chromosome short tandem repeats (X-STRs) are of particular importance in forensic investigations due to their distinctive genetic inheritance pattern. X-STRs complement other DNA markers such as autosomal STRs, Y-chromosomal STRs, and mitochondrial DNA (mtDNA) markers. This makes them a valuable tool in cases involving complex biological relationships. The benefits of using X-STRs in forensic analysis are summarized in Table (1). [20]. Incomplete mother-son and father-daughter testing as well as paternity deficiency testing have both been linked to X- STRs. Because of this, females who were fathered by the same male share the parental X chromosome [24].

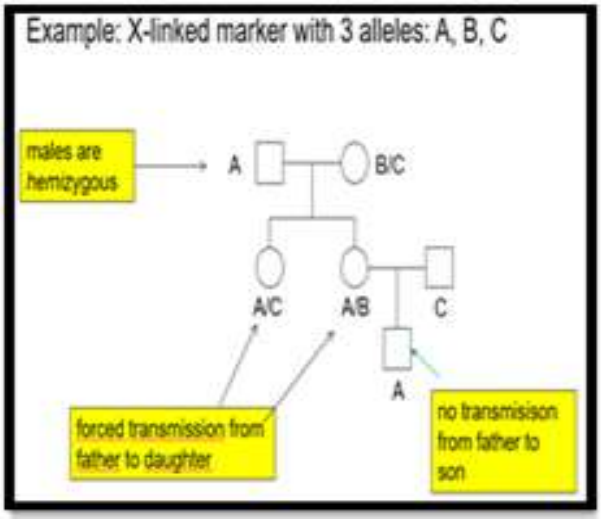

Figure 3. X-Chromosome markers analysis with three Alleles: A, B, C (XAXb , XbXb and XAXBXc) [19].

Table 1. Region of uses X-STR DNA typing adapted from [21, 22 and 23].

Uses	Advantages
Forensic case works	Effectively for testing mother-son kinship, father-daughter relationships, and deficient paternity cases.
Variation	Given the short length of amplicons, they are simple to genotype, even from damaged DNA samples.
Genetic applications	Hemizygous Males have direct access to the haplotypes that make up the genetic population.

They are currently employed to analyze parentage and relationships, including first cousin and avuncular relationships. Stable haplotypes of closely related X-chromosome markers have shown to be useful for kinship analysis with respect to X-STRs, particularly when examining father-daughter relationships. In cases of paternity involving close blood relatives as alternative presumed fathers and in cases of paternity deficiency, where the DNA sample of the presumed father was not available and the DNA of the paternal relative should instead be analyzed, they also have an advantage over autosomal STRs [26]. The ability of autosomal STRs to rule out alternative putative dads who are close blood relations is greatly diminished in certain paternity scenarios, and X STRs may even be more powerful than autosomal (AS) markers. As an illustration, if the two putative dads were Since a father and son do not inherit any identical X-chromosomal alleles, ChrX markers are more precise than Autosomal (AS) markers (ibd) for determining relatedness between them[25]. A more efficient method of X-STR analysis for extremely degraded DNA has been discussed and confirmed by several earlier investigations [23].

1.9. Applications of Forensic Haplotyping and Haplotyping

A particular set of alleles at connected loci that are found on one of the two homologous chromosomes is referred to as a "haplotype" in biology. When a batch of data contains data from loci in the same linkage group, a haplotype must be produced. During population studies of a linkage group, the frequencies of haplotypes, not alleles, are obtained. [27, 28].

The inheritance pattern of X chromosome markers is specific: females have two copies, while males have only one, inherited from their mother and father, respectively. As a result, daughters inherit haplotypes from both parents. Analysis of X-chromosomal loci can be valuable in cases of paternity testing when investigating half-sisters and/or grandmothers. In the joint typing of a father and his daughter, the ChrX haplotypes of both daughters can be fully determined because they always inherit the full paternal X chromosome (ChrX) [29]. Because males possess only one X chromosome and fathers pass their entire X chromosome to their daughters, X-STR markers are valuable supplements to Y-chromosomal STR markers (Y-STRs) and autosomal short tandem repeat markers (STRs) in cases where genetic relationships need to be established [30]. Additionally, some X-STR loci exhibit strong linkage disequilibrium (LD) and group as haplotypes [31]. If a male displays multiple traits that are linked to the X chromosome, it indicates that all relevant loci alleles are combined into a single haplotype [25]. This explains the simplicity of ChrX-linkage analysis. Understanding such basic circumstances is important for kinship testing as well as clinical genetics. Establishing methods that can span wide lineage gaps is one of the difficulties in kinship testing. Clinical genetics findings have shown that individuals who have a relatively unusual genetic trait can be grouped in a shared pedigree [32].

1.10. X-chromosome Short Tandem Repeats in Forensic Genetics

Forensic genetics has recently shown a lot of interest in studying short tandem repeats (STRs) on the X chromosome (X-STRs) [33]. These markers have proven useful in solving challenging cases that cannot be resolved using other forensic markers [34]. X-STRs are particularly helpful in identifying victims of war, where the majority of profiles analyzed belong to second-or third-degree relatives, such as grandparents' grandchildren, maternal uncles and nephews, among others [35]. X-STRs are also valuable in natural or man-made disasters for establishing the relationship between the

deceased and their relatives, who may be any degree of family members [36].

1.11. X-STR loci

In forensic research, roughly 33 X-STR loci are used. Similar to autosomal STR loci used in forensic research, tetranucleotide repeats are more typically chosen because they produce fewer stutter products than dinucleotide or trinucleotide repeats. Based on a linkage/recombination map created at Rutgers University, each locus' chromosomal position, geographic location, and genetic distance are described [14].

A chromosome's loci are said to be connected if they are physically adjacent to one another and are not passed down independently. A characteristic of the physical space between the sequences of the DNA within a chromosome is genetic linkage [36].

1.12. The Usefulness of X-Chromosome Markers

i. Kinship Testing using Chr-X Markers

Gonosomal markers are highly effective in cases of kinship insufficiency, especially in situations such as war and international migration where only distant relatives are available for testing. X-chromosome markers, in particular, have proven to be useful in identifying war and mass disaster victims, where sophisticated techniques are required for skeleton and corpse identification. In some cases, X-chromosome markers may even be more effective than autosomal markers [37].

ii. Chr-X Markers in Kinship Testing (trios and duos)

Although STRs are typically sufficient to resolve paternity cases involving the common triple constellation of mother, child, and putative father, additional or alternative markers do not appear to be necessary. ChrX markers are completely useless for evaluating father-son connections. While X-chromosomal testing may be beneficial for paternity tests involving daughters or more complicated family relationships [38]. ChrX markers may be useful for checking in case of father or daughter parentage in issue. This is particularly true if challenging-to-analyze template materials, such as DNA from excavated skeletons, etc, are involved. The ChrX markers are comparable to the AS markers in terms of mother-daughter relationships and offer no distinct advantages. ChrX markers, however, are more effectively used when testing (mother-son kinship). ChrX marker typing utilizing short amplicons should be considered if skeletal human remains or other difficult samples need to be evaluated. Because ChrX markers need fewer low-size STRs to provide enough statistical power than autosomal markers do, they may be more successful in certain situations [25].

iii. Deficiency Paternity Cases

The primary applications of Chr-X markers are in complex kinship testing and deficient paternity testing. X-chromosomal tracks can be used to assign pedigree members over great distances using Chr-X markers. However, they fall short when father-son relationships disrupt X-chromosomal lines. The same paternal Chr-X is always shared by females who share the same father. Thus, paternity can be ruled out based on the Chr-X markers of two sisters or stepsisters, even if the parents' DNA is not accessible. AS markers are unable to offer such qualitative data. Although less trustworthy, a positive paternity test is nonetheless feasible without knowledge of the mother's genotype. Sisters typically receive partially matched haplotypes from their mothers, which accounts for this. The co-inheritance of two identical maternal Chr-X without recombination is feasible, although unusual. If Chr-X markers are checked in a deficient case, the most important person to pay attention is the mother of an unavailable putative father (sometimes referred to as the putative grandmother) [39]. In a strict sense, the moments she is available to type are not deficit cases. Investigating her will reveal the putative father's whole set of Chr-X alleles. The putative grandmother's Chr-X marker genotype can also be partially inferred from her offspring. If she has numerous daughters, it is possible to ascertain the grand maternal genotype and the parental origin of the majority of their Chr-X alleles. It is considerably more instructive if the putative father's brothers are accessible [25]. The grand maternal genotype must then be heterozygous for each Chr-X locus where the putative father's brothers have different alleles. If they share the same alleles, the constellation is only partially predictive since the mother could be homozygous or heterozygous at the relevant location. If closely connected loci have already been unambiguously defined as homozygous or heterozygous, haplotyping can be used to evaluate the chance of homozygosity at the originating locus [25].

iv. The use of Chr-X markers in cases of paternity involving blood relatives.

The STRs exclusion strength is significantly diminished in paternity instances where close blood relatives are suspects, and Chr-X STRs may be preferable to AS markers. Since two putative dads would not share any X-chromosomal alleles that are similar by descent, Chr-X markers would be more accurate than AS markers in this case (ibd). Conversely, when it comes to having a specific mother Chr-X allele, brothers have a 0.5 chance of sharing exactly one allele through descent at an AS location [40].

v. Use of Abortion Material in Paternity Testing with Chr-X Markers in Incest and Rape Cases

Suction abortion is a method of post-incest or post-criminal sexual assault pregnancy termination. Small amounts of unidentifiable fetal organs, along with maternal blood and other tissues, can be found in an aborted six–eight weeks product of conception. In these situations, it is typically unsuccessful in detecting embryonic organs or chorionic villi under the microscope, and samples will have a blend of maternal and fetal DNA. The emergence of a Chr-Y signal and the straightforward amelogenin dimorphism can reveal the sex. Additional Chr-Y testing can simply be used to determine the paternity of male fetuses. When incest must be explored and the clean fetal material may be acquired through a biopsy of the chorion, the scenario is considerably different. When all of the fetal alleles match those of the pregnant woman, Chr-X testing for a female fetus would demonstrate that the father was a father-daughter incest [41].

vi. Chr-X Markers in Maternity Testing

Mother-child testing may be required in certain circumstances. For instance, official agencies in charge of aliens frequently permit family reunions only after kinship has been established. Sequencing mitochondrial DNA (mtDNA) can also be used to prove maternity; however, the accuracy of this method is not always guaranteed. For instance, Sequences of mtDNA are identical to individuals of their maternal line's nieces and nephews as well as to those of their children. Due to the significant prevalence of illegitimate paternity in contemporary society, mother-child testing is also more reliable than determining father-child ties when identifying skeletons or corpses. ChrX STR typing may therefore be a reasonable alternate method for determining pregnancy. ChrX markers are equal to AS markers for examining mother-daughter connections and offer no distinct advantages. ChrX markers, however, are more effective for determining mother-son connections [23].

vii. Chr-X Testing May Be Particularly Beneficial for Certain Lost People or Catastrophe Victims

When direct reference samples are unavailable for particular identification scenarios, Individuals who are second or third-degree biological relatives, including but not limited to grandchildren, grandparents, maternal uncles, and nephews, are tested instead. [42].

viii. X-chromosome and population genetic

For population genetic investigations, the X-chromosome offers aspects that make it a reliable source of information. Males have only one copy of the X chromosome, which makes it possible to distinguish between different X chromosomal haplotypes in men. In comparison to autosomes, the X-chromosome has lower rates of recombination, mutation, and effective population size, which causes genetic drift to occur more quickly [43]. As a result, it is anticipated that linkage disequilibrium (LD) and population structure in the X chromosome will be stronger than those in the autosomes. The X chromosome has been studied in females for two-thirds of its history. X-chromosome polymorphisms thus primarily represent the history of women. Recombination has caused the X chromosome markers in females to give a multilocus system, whereas the Y chromosome and mtDNA are linked haplotypes [44]. As a result, X-chromosome markers are crucial for population genetic research. Additionally, if other scientific fields like evolutionary anthropology are going to pay attention to ChrX markers as well, they'll require accurate ChrX marker data collected from a variety of ethnic groups throughout the world. ChrX typing in this field will never have the same significance that ChrY marker research has because of the very different manner of inheritance [37]. The ChrX marker manifests in a hemizygous condition in males. As a result, haplotypes are automatically provided by ChrX typing of marker clusters. The frequencies of haplotypes cannot be computed by multiplying the frequencies of the single alleles of the

implicated haplotypes; rather, they must be inferred from the study of population samples since extremely tightly related markers frequently exhibit linkage disequilibrium. The number of haplotypes can reach several hundred or even more than a thousand if two or more STR loci are employed [45].

Therefore, rather than only including allele frequencies, an X-STR database must also include haplotype frequencies. The forensic community can visit a website called www.chrx-str.org which offers a reference database of both ChrX STRs and ChrX STR haplotypes with published demographic data for populations from many different nations [46].

1.13. Population Genetics

Population genetics examines how inherited variety changes through time and place as a result of processes like mutation, selection, migration, and genetic drift. By assessing the various DNA alleles and their frequency within and between populations, information on variables like population composition, development, size, and age may be found [47].
A population group's or a particular population group's variation in allele and genotype levels is measured using population genetics. Within the population, there is considerable genetic variability at the level of individual nucleotides. For instance, there are around 10 million nucleotides that might differ across individuals in humans. More genetic variation exists within the human racial groups than there is on average between them [48]. Each one of the autosomal gene or DNA marker in the diploid individuals has two copies: one from the paternal ancestor (sperm) and one from the maternal ancestor (egg). For that locus, an individual is said to be heterozygous if alleles they acquired from sperm and egg are different, but they are called homozygous if they inherited two identical alleles from egg and sperm. The variability of a locus must be stable enough to reliably transfer an allele to the following generation (i.e., have a low mutation rate), but not too stable as this would lead to a small number of alleles over time and lessen the locus' utility (i.e., useful in human identity testing applications). The frequency distribution of genotypes is the simplest way to describe variance. The population's heterozygote population size provides a gauge of this variance [49]. Allele frequency in a population is influenced by population genetic processes such as mutation, migration (gene flow), random genetic drift and natural selection. Isolated populations gradually diverge from one another as a result of inbreeding, which causes each to lose heterozygosity. There is less gene switching because the gene selection pool is smaller in isolated groups. The definition of a population must be established upfront. A population in the context of forensic genetics is a collection of individuals with a common ancestor [50].
In forensic nomenclature, several subgroups are combined and classified as Caucasian, Sub-Saharan African, and East Asian, for example, even though these groups may differ in language, culture, and religion.

1.14. Population database

To accurately estimate the prevalence of alleles in a population, it is essential to identify and analyze each of the commonly occurring alleles multiple times when constructing a population database. The process of building and testing such a database can be used to determine the frequency of a DNA profile, as shown in Figure 5, within a given population [51]. Studies on the appropriate population should be carried out to establish allele frequencies and look into potential substructure before novel DNA markers are introduced into forensic casework [52]. Another aspect to take into account is the DNA markers' forensic effectiveness in casework including criminal investigations and relationship testing. These computations distinguish between case-specific values and the statistical significance of using the same markers under different forensic genetic settings [53]. In a perfect world, DNA databases would have STR genotypes from every member of a specific group, enabling incredibly precise assessments of DNA profile frequencies. However, a smaller database is necessary due to time and money constraints. Thankfully, similar to how a phone survey of several hundred individuals is used to try and predict the outcome of an election, it is possible to properly estimate allele and genotype frequencies in a small subset of the population. The difficulty is to collect information from a sufficient number of individuals to precisely estimate the frequency of the principal alleles at a genetic locus [54].

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