Features and Prospects of the Use of X-chromosomal Tandem Repeats in Forensic Molecular Genetic Examination

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When conducting molecular genetic examinations, biological specialists in Ukraine usually use kits for autosomal STR repeats and Y-chromosome repeats and do not use kits for X-chromosomal STR repeats at all. The research paper's purpose is to analyze the literature data to determine the characteristics of the X chromosome, the possibilities of using X chromosome typing kits, and their implementation in the forensic practice of experts in molecular genetic examinations. The criteria for searching and STR loci are defined, the characteristics of commercial kits (in particular, Argus X-8 PCR Amplification Kit from Biotype AG and Investigator Argus X-12 QS Kit (Germany), X19 X-STRs amplification kit from AGCU ScienTech Inc. and 19X Direct ID system (China)) the directions of their use are considered. The data of scientific and methodological literature on the frequency of mutations, the prevalence of alleles in populations, and the prospects for creating a common database of X-STR repeats are studied. To achieve this goal, general and special methods were used, among which the main one is the structural genetic analysis and synthesis of X-chromosome research data from the standpoint of forensic genetics. The methods of formalizing the characteristics of commercial X-STR repeat kits and their comparative analysis, systematizing possible areas of STR repeat use taking into account

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the peculiarities of X chromosome inheritance, and generalizing population studies conducted in European countries were also used.

Keywords: *molecular genetic examination; X-chromosome; STR-repeat; tandem repeat; linkage disequilibrium.*

Research Problem Formulation

In the conditions of a full-scale Russian invasion of the territory of independent Ukraine, the issue of the fastest possible identification of persons, in particular, through molecular genetic research, is becoming especially urgent.

When conducting molecular genetic examinations to identify individuals and establish the fact of kinship, biological experts actively use short tandem repeat, (*STR*s). Experts in international and domestic practice use kits based on autosomal STR markers. As additional tools, kits of STR repeats of the Y chromosome and mitochondrial DNA are used, but in domestic practice, kits for DNA identification of STR repeats of the X chromosome are not used at all, which can be an auxiliary method for comprehensively establishing the fact of kinship between direct and indirect relatives.

Analysis of Recent Researches and Publications

The possibilities of using X-chromosome began to be studied in the late 1990s, directing these studies to the search for STR and SNP markers, nucleotide insertion/deletion markers (*INDEL*), and generalizing them into multiplex sets. The main criteria for searching for STR loci on the X chromosome have been formed:

1) high polymorphism of loci;
2) possibility of automating DNA analysis by using *Polymerase Chain Reaction*, (hereinafter referred to as *PCR)*, in names of foreign-made — *PCR*) capillary electrophoresis;
3) short length of amplicons in *STR*-kits for working with degraded DNA.

The scientists Iva Homesh, Nadia Pinto, Sofiia Antao-Suza, Veronika Homesh, Leonor Husmao and Antoniu Amorim conducted a meta-analysis of 236 publications with results from existing population-based X-STR studies using commercial kits available in different countries. The scientists noted the disparity in the study of the population of some regions and the small total number of loci analyzed which does not allow for a full study of the imbalance (disequilibrium) of allele links on one chromosome [1].

Article Purpose

Analysis of literature data to determine the peculiarities of using X-chromosome typing kits and the possibilities of introducing these kits for molecular genetic research in the Expert Centers of the Ministry of Internal Affairs.

1 Gomes I., Pinto N., Antão-Sousa S., Gomes V., Gusmão L., Amorim A. Twenty Years Later: A Comprehensive Review of the X Chromosome Use in Forensic Genetics. *Frontiers in genetics*. 2020. Vol. 11. Pp. 1—17. DOI: 10.3389/fgene.2020.00926 (date accessed: 10.08.2023).

Objectives: to consider the biological basis and peculiarities of inheritance of human sex chromosomes; to highlight possible aspects of the use of X chromosome typing kits; to compare commercial kits for DNA identification on the market; to analyze population studies of the frequency of alleles and the rate of their mutagenesis.

Research Methods

The methodological basis of the study is general and special methods, among which the main one is structural genetic analysis and synthesis of X-chromosome research data from the standpoint of forensic genetics. The methods of formalizing the characteristics of commercial X-STR repeat kits and their comparative analysis, systematizing possible areas of STR repeat use, taking into account the peculiarities of X-chromosome inheritance, and generalizing population studies conducted in European countries were also used.

Main Content Presentation

X-chromosome is 156,040,895 nucleotide pairs that form 857 protein-coding genes, 692 non-protein-coding genes, and 888 pseudogenes [2]. The X-chromosome is the sex chromosome shared by men and women. A feature of sex chromosomes is their inheritance by subsequent generations: females receive one X-chromosome from each parent, while males inherit only one maternal X-chromosome. Two X-chromosomes in females increase the level of expression of sex-linked genes, which could lead to abnormalities in the development of organisms. Therefore, there is an evolutionarily developed mechanism for the equalization of dosage compensation of genes in female mammals, which occurs due to the inactivation of one of the X-chromosomes, which remains inactive in all somatic cells of the body [3].

X-chromosome inheritance also has evolutionary differences: in males, the X-chromosome is almost entirely transmitted to daughters as an invariant block (except for the pseudoautosomal *PAR* regions, which retain homology and conjugate to the *X*-chromosome region during male meiosis), then as women have two X-chromosomes that recombine with each other, creating genetic variability and increasing haplotype diversity in offspring [4].

A feature of working with repeats on the *X*-chromosome is the formation of linkage groups due to the close location of loci on the chromosome. Linkage is a consequence of genetic recombination and causes markers to be more likely to be inherited together than physically separated repeats [5]. Linkage calculation is carried out according to the speed or frequency of chromosomal recombination, and if the markers are

2 Chromosome X: 1-155,270,560 / e!Ensembl. July 2023. URL: http://www.ensembl.org/Homo_sapiens/Location/Chromosome?r=X:1-155270560 (date accessed: 10.08.2023).

3 Ross M. T., Grafham D. V., Coffey A. J., Scherer S., McLay K., et al. The DNA sequence of the human X chromosome. *Nature*. 2005. Vol. 434. Pp. 325—337. DOI: 10.1038/nature03440 (date accessed: 10.08.2023).

4 Gomes I., Pinto N., Antão-Sousa S., Gomes V., Gusmão L., Amorim A. Op. cit. DOI: 10.3389/fgene.2020.00926 (date accessed: 10.08.2023).

5 Tillmar A. O., Kling D., Butler J. M., Parson W., Prinz M., Schneider P. M., Egeland T., Gusmão L. DNA Commission of the International Society for Forensic Genetics (ISFG): Guidelines on the use of X-STRs in kinship analysis. *Forensic Science International: Genetics*. 2017. Vol. 29. Pp. 269—275. DOI: 10.1016/j.fsigen.2017.05.005 (date accessed: 10.08.2023).

not linked, then in each meiosis there will be recombination of chromosome sections and 50% of the gametes will be recombined. In the case of change in this indicator, we can talk about *linkage disequilibrium* (*LD*). Given the length of the X-chromosome, a maximum of four unrelated X-STRs can be analyzed simultaneously. Each linkage group forms a haplotype, which is inherited in the pattern of allelic inheritance. Inheritance frequencies of haplotypes must be taken into account to calculate relatedness, but they are not as accurate as alleles, and much larger databases are needed to study their inheritance [6].

In addition, when studying consanguinity, it is necessary to have information about the frequency of mutations, but because of their overall low frequency, the study of a large number of meioses is necessary to obtain statistically significant results [7].

Thus, the main characteristics for searching for loci on the *X-chromosome* were revealed:

- location on the entire *X-chromosome*;
- the smallest physical distance between two loci must exceed 5 Mb;
- loci are located in different linkage groups;
- high heterozygosity (≥ 0.4);
- suitable for creating primers for multiplex *PCR* [8].

Taking into account these characteristics, manufacturers have created commercial kits for use in research laboratories. The most commonly used kits:

1) *Argus X-8 PCR Amplification Kit* by *Mentype* (*Biotype AG*, Germany) [9]. It contains 8 markers on the *X*-chromosome and a sex marker. The kit contains the loci *Amelogenin*, *DXS7132*, *DXS7423*, *DXS8378*, *DXS10074*, *DXS10101*, *DXS10134*, *DXS10135* and *HPRTB*. The markers form pairs that are inherited as haplotypes and are present in each of the four linkage groups on *X-chromosome*. The sensitivity limit of the *Mentype Argus X-8 PCR Amplification Kit* is approximately 0.1 ng of DNA, but the manufacturers recommend using concentrations of 0.1-1.0 ng of DNA in the sample. The amplification process takes 177 minutes for 30 cycles and 191 minutes for 34 cycles, the latest being recommended for objects with a low amount of DNA. The kit can be used on *GeneAmp® 9700* amplifier, *ABI PRISM 310,* and *ABI PRISM 3100/3130* genetic analyzers (all — *Applied Biosystems*, the USA) [10].
2) *Investigator Argus X-12 QS Kit* by *Qiagen* (Germany). It contains

6 Gomes I., Pinto N., Antão-Sousa S., Gomes V., Gusmão L., Amorim A. Op. cit. DOI: 10.3389/fgene.2020.00926 (date accessed: 10.08.2023).

7 García M. G., Catanesi C. I., Penacino G. A., Gusmão L., Pinto N. X-chromosome data for 12 STRs: Towards an Argentinian database of forensic haplotype frequencies. *Forensic Science International: Genetics.* 2019. Vol. 41. Pp. e8—e13. DOI: 10.1016/j.fsigen.2019.04.005 (date accessed: 10.08.2023).

8 Jia J., Liu X., Fan Q., Wang M., Zhang J., Li W., Shi L., Zhang X., et al. Development and validation of a multiplex 19 X-chromosomal short tandem repeats typing system for forensic purposes. *Scientific Reports*. 2021. Vol. 11. Art. 609. DOI: 10.1038/s41598-020-80414-x (date accessed: 10.08.2023).

9 Тут і далі у дужках зазначено виробника.

10 Mentype® Argus X-8 PCR Amplification Kit Manual. Germany : Biotype AG, 2009. 36 p. URL: https://www.biotype.de/fileadmin/user/MANUALS/Mentype_Argus_X-8_eng.pdf (date accessed: 10.08.2023).

12 *X*-chromosome markers, a sex marker (*Amelogenin*) and one autosomal marker (*D21S11*). The autosomal marker is included in the kit to allow comparison with any *STR* loci kits to reduce the risk of confounding. *The set contains Amelogenin, DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148, HPRTB* loci grouped into four linkage groups. The optimal amount of DNA to obtain a high-quality DNA profile under standard conditions is 0.2—0.5 ng, during internal testing, the manufacturer of the kit obtained reliable results from concentrations < 0.1 ng of DNA in the object. Internal control was added to the reaction mixture to determine the presence of DNA in the sample and verify the success of the PCR. The amplification process takes 80 minutes thanks to *Qiagen*'s rapid cycle technology, and the kit can also be used for direct amplification reactions. The kit can be used on the *GeneAmp PCR System 9700* amplifier and *3500 Genetic Analyzer* (both from *Applied Biosystems*, the USA) [11];

3) *17 X-Plex System* (*SWGDAM*) represented by loci *DXS8378, DXS9898, DXS7133, GATA31E08, GATA172D05, DXS6801, DXS7423, DXS6809, DXS6799, DXS7132, DXS9902, DXS6800, DXS6789, DXS10075, DXS10079, DXS6807, DXS6803*. The sizes of the amplicons of the selected repeats are less than 294 bp. Amplification lasts 95 minutes, it was carried out on *GeneAmpR 9800 PCR System*, electrophoresis on ABI PRISM *Genetic Analyzer* using *POP-7* and *LIZ-500TM Size Standard* (all - *ThermoFisher Scientific*, the USA). The final incubation can be extended for 30 minutes in the presence of adenine fragments. The optimal concentration of DNA in the reaction is 100 pg. Complete DNA profiles were obtained from 250 ng/ml humic acid and 150-300 mM hematin. The height of the staters varies from 4.0 % for the *DXS6800* locus to 15.2 % for the *DXS6809* locus. The average heterozygous balance is 82.6 % (total range from 71.8 to 87.9 %) [12];

4) *X19 X-STRs amplification kit* (*AGCU ScienTech Inc., Wuxi, Jiangsu,* China) contains 19 markers on *X*-chromosome and a sex marker: *DXS7423, DXS10148, DXS10159, DXS6809, DXS7424, DXS8378, DXS10164, DXS10162, DXS7132, DXS10079, DXS6789, DXS101, DXS10103, DXS10101, HPTRB, DXS10075, DXS10074, DXS10135* i *DXS10134*. The amplification cycle lasts 79 minutes, the kit can also be used for direct amplification. The kit is tested on *GeneAmp® 9700* amplifier and 3130, 3500, and 3700 *Genetic Analyzer* (both from *Applied Biosystems*, the USA). Complete profiles were obtained from concentrations of 0.5-0.125 ng. Concentrations of inhibitors were calculated at which at least one locus in the DNA profile was lost (*DXS10135* locus was the first to be inhibited): humic acid — 60 μg/ml, calcium ions — 3.0 mmol/l, hemoglobin — 500 mmol/l. The kit is used to extract traces of blood and buccal epithelium from FTA cards,

11 Investigator® Argus X-12 QS Handbook. Germany : Qiagen, 2022. 90 p. URL: https://qiagen.com (date accessed: 10.08.2023).

12 Prieto-Fernández E., Baeta M., Núñez C., Zarrabeitia M. T., Herrera R. J., Builes J. J., de Pancorbo M. M. Development of a new highly efficient 17 X-STR multiplex for forensic purposes. *Electrophoresis*. 2016. Vol. 37. Is. 12. Pp. 1651—1658. DOI: 10.1002/elps.201500546 (date accessed: 10.08.2023).

cigarette butts, nail fragments, hair follicles, and saliva [13];

5) *19X Direct ID system* (*Microread Genetics Co, Suzhou*, Китай) містить 20 маркерів, серед яких *19 X-STR*-локусів: *DXS6795, DXS6803, DXS6807, DXS9907, DXS7423, ATA172D05, DXS101, DXS9902, DXS7133, DXS6810, GATA31E08, DXS6800, DXS981, DXS10162, DXS6809, GATA165B12, DXS10079, DXS10135, HPRTB* та *Amelogenin*. Amplification can be carried out on a *Veriti™ 96-Well Thermal Cycler* device (*Thermo Fisher Scientific*, the USA) with 29 cycles set and a total execution time of 36 minutes, electrophoresis on a 3500xl *Genetic Analyzer device (Applied Biosystems*, the USA). Complete profiles were obtained from concentrations of 250 pg of DNA and more. The maximum concentrations of inhibitors at which a complete DNA profile can be obtained were found: humic acid — 0.5 ng/ml, hemoglobin — 500 mmol/l, EDTA — 0.8 mmol; with the addition of higher concentrations of inhibitors, loss of *HPRTB* and *DXS10135* loci occurred [14].

In addition, further searches are being conducted for new *X-STR* repeats to expand existing sets. For example, the study compared 9 new loci in three linkage groups in 198 unrelated samples from Shanghai residents and 168 samples from 43 families in duos and trios. Amplification lasts 30 cycles and 50 minutes, analysis was performed on an ABI 3130xl genetic analyzer with барвником *GeneScan 500 LIZ Size Standard* dye (both from *Applied Biosystems*, the USA). The size of the loci was from 146 bp. (X033) to 477 BP. (X028). The minimum number of alleles is 5 (locus *X008*), the maximum is 12 (locus *X006*). Two single-step mutations were identified during the study of families [15]. Therefore, the further development and search for new loci on the *X*-chromosome is a promising direction of research.

The above *X-STR*-kits can be used to:

- establish paternity with a daughter — in the absence of the father, but with the possibility of taking samples from his mother or another daughter (sister or half-sister), in this X-chromosome study of grandmothers and daughters completely coincides with the parental one and gives a more effective result than autosomal STR-repeats [16];
- establishment of paternity with a person-son — in the presence of

13 Yang X.-Y., Wu W.-W., Chen L., Liu Ch.-H., Zhang X.-F., Chen L., Feng X.-L., Wang H.-J., Liu Ch. Development of the 19 X-STR loci multiplex system and genetic analysis of a Zhejiang Han population in China. *Electrophoresis.* 2016. Vol. 37. Is. 15—16. Pp. 2260—2272. DOI: 10.1002/elps.201500540 (date accessed: 10.08.2023).

14 Yang Q., Qian J., Shao Ch., Yao Y., Zhou Zh., Xu H., Tang Q., Qian X., Xie J. Identification and Characterization of Nine Novel X-Chromosomal Short Tandem Repeats on Xp21.1, Xq21.31, and Xq23 Regions. *Frontiers in Genetics.* 2021. Vol. 2. Pp. 1—9. DOI: 10.3389/fgene.2021.784605 (date accessed: 10.08.2023).

15 Xiao Ch., Yang X., Liu H., Liu Ch., Yu Zh., Chen L., Liu Ch. Validation and forensic application of a new 19 X-STR loci multiplex system. *Legal medicine*. 2021. Vol. 53. Pp. 1—4. DOI: 10.1016/j.legalmed.2021.101957 (date accessed: 10.08.2023).

16 Zhang Y., Yu Zh., Mo X., Zhao X., Li W., Liu H., Liu Ch., Wu R., Sun H. Comparative evaluation of autosomal STRs and X-chromosome STRs as a complement of autosomal STRs in kinship testing in Southern Han Chinese. *Annals of human biology*. 2021. Vol. 48. Is. 1. Pp. 66—69. DOI: 10.1080/03014460.2020.1856926 (date accessed: 10.08.2023).

the mother [17]. In these cases, the study of loci on the X-chromosome gives a 100 % probability of consanguinity, whereas autosomal STR repeats give at most only a 25 % probability of sister consanguinity;

- confirmation or exclusion of paternity or kinship in a pair with at least one female person in the presence of inconclusive results of autosomal *STR* analysis, namely the presence of a mutation in two loci [18];
- establishing the fact of father-daughter incest and female offspring. If the father of the child is also the father of the mother and in the absence of a mutation, the child is either homozygous (for one allele present in the mother) or heterozygous for the same alleles of the mother, in this case, information about incest can be obtained even without examining the father's sample [19];
- establishing the fact of inbreeding — those belonging to the same autosomal class. In case of connection of one of the parents with the grandfather / grandmother or uncle / aunt, or half-brother / sister, the born child will belong to the inbred line [20];
- investigation of mixed traces from sexual assault crime scenes to identify both female and male fractions and to complement traditionally used autosomal *STR* repeat methods [21]. It is also advisable to use *X*-markers when studying mixtures of two males: studies show higher resolution due to fewer targets involved in the mixture and less stochastic *PCR* effects (such as unbalanced heterozygotes) [22];
- carrying out an additional method of analysis of the identification of bone remains in the case of armed conflicts, mass disasters, disappearance of missing persons, in the presence of at least one woman in a pair for research.

It is important that the use of X-repeats in practice has the ability to examine DNA mixtures, especially for the extraction of the female part of the mixture (with

17 Pinto N., Gusmão L., Amorim A. X-chromosome markers in kinship testing: a generalisation of the IBD approach identifying situations where their contribution is crucial. *Forensic Science International: Genetics*. 2011. Vol. 5 Is. 1. Pp. 27—32. DOI: 10.1016/j.fsigen.2010.01.011 (date accessed: 10.08.2023).

18 Jusic B., Pilav A., Dzehverovic M., Čakar J. H. DNA Paternity Testing with Two Mismatches: Our Experience. *Jordan Journal of Biological Sciences*. 2022. Vol. 15. Is. 3. Pp. 387—394. DOI: 10.54319/jjbs/150307 (date accessed: 10.08.2023).

19 Gomes I., Pinto N., Antão-Sousa S., Gomes V., Gusmão L., Amorim A. Op. cit. DOI: 10.3389/fgene.2020.00926 (date accessed: 10.08.2023).

20 Pinto N., Gusmão L., Amorim A. Op. cit. DOI: 10.1016/j.fsigen.2010.01.011 (date accessed: 10.08.2023).

21 Diegoli T. M., Coble M. D. Characterization of X Chromosomal Short Tandem Repeat Markers for Forensic Use. U. S. Department of Justice, 2015. 136 p. URL: https://www.ojp.gov/pdffiles1/nij/grants/249510.pdf (date accessed: 10.08.2023).

22 Kakkar S., Sarma Ph., Panigrahi I., Mandal S. P., Shrivastava P., Kumawat R. K. Development of a new screening method for faster kinship analyses in mass disasters: a proof of concept study. *Scientific reports*. 2022. Vol. 12. Is. 1. DOI: 10.1038/s41598-022-22805-w (date accessed: 10.08.2023).

significant excesses of male DNA). Such traces are scratches or other wounds on the parts of the man's body involved in the attack (such as areas of contact with epithelial cells and saliva). In such cases, the result of the analysis is a mixed profile, where the main contributor is a man, but it is not able to completely mask the female profile, if it is not homozygous for male alleles [23]. Several studies have been conducted for various commercial kits to investigate this issue. Mixtures of human DNA *F312* and *M308* were used to test *Microreader™ 19X Direct ID System* kit. Complete sample profiles were obtained for concentrations of 1:1 and 1:4, and 18 out of 19 loci of the minor contributor were detected for the ratio of 1:9. In the 1:19 mixture, a higher rate of dropouts of the secondary participant was found, namely 63.16%, which makes it impossible to qualitatively identify the DNA profile [24].

Another set of nineteen *X-STR* repeats was tested on control DNAs *9948* (male) *9947A* (female) at ratios of 1:0, 1:1, 1:3, 1:5, 1:8, and 0:1, maintaining the total amount of DNA at the level of 1 ng. Dropouts of alleles were not detected in any ratio, but it was noted that the height of the peaks is proportional to the amount of introduced DNA. Therefore, the new genotyping kit being developed is suitable for testing mixtures of DNA samples from two individuals [25].

Similar results were also obtained for *TYPER-X19 multiplex assay* kit. When examining male-female mixtures reduced to 1 ng of DNA, 100%, 70.3%, and 14.8% of loci were identified in three replicates of 1:1, 1:5, and 1:10, respectively. The presence of male loci could not be detected in a ratio of 1:20, however, increasing the amount of genomic DNA, according to the authors, can increase the sensitivity of detecting DNA mixtures [26].

The main disadvantage of using *X-STR* in forensics is the hybrid nature of *X*-chromosome inheritance in primates. On the one hand, the presence of one copy of the *X*-chromosome in men, which is directly transmitted to daughters, makes it possible to specifically detect its inheritance, on the other hand, female *X*-chromosomes, like autosomes, undergo recombination, which increases the variety of possible options among descendants. Pseudoautosomal *PAR* regions also remain on the sex chromosomes, which have homologous regions between the *X*-chromosome and the *Y*-chromosome and retain the ability to recombine between them, which reduces the limits of searching for possible markers and building specific primers for the *PCR* reaction. In addition, *X*-chromosomal repeats are uninformative for establishing paternity with sons and have the same statistical power as autosomal loci for mother-daughter dyads. X-chromosome

23 Diegoli T. M. Forensic typing of short tandem repeat markers on the X and Y chromosomes. *Forensic Science International: Genetics*. 2015. Vol. 18. Pp. 140—151. DOI: 10.1016/j.fsigen.2015.03.013 (date accessed: 10.08.2023).

24 Xiao Ch., Yang X., Liu H., Liu Ch., Yu Zh., Chen L., Liu Ch. Op. cit. DOI: 10.1016/j.legalmed.2021.101957 (date accessed: 10.08.2023).

25 Jia J., Liu X., Fan Q., Wang M., Zhang J., Li W., Shi L., Zhang X., et al. Op. cit. DOI: 10.1038/s41598-020-80414-x (date accessed: 10.08.2023).

26 Zhang Y., Yu Zh., Mo X., Zhao X., Li W., Liu H., Liu Ch., Wu R., Sun H. Development and validation of a new 18 X-STR typing assay for forensic applications. *Electrophoresis*. 2021. Vol. 42. Is. 6. Pp. 766—773. DOI: 10.1002/elps.202000168 (date accessed: 10.08.2023).

marker testing may also be ineffective in ruling out close relatives of the true father: a full brother of a child's true father and a niece will share 50 % of the same X-chromosome alleles as a true father and his daughter [27].

At the same time, in case of mass disasters, it is advisable to use *X-STR* loci in all pairs of relatives to identify a significant number of people in a short period of time. On the basis of *X*-chromosomal repeats, methods are being tested to further narrow down the search for individuals for whom it is necessary to conduct a comparative study of kinship by other methods. One of these methods of narrowing the search circle is the method of cutting off the correspondence of alleles, which occurs due to the determination of the minimum number of matches in different loci, taking into account the degree of kinship of the compared individuals, without taking into account the frequencies of the selected alleles in the populations. This method can be potentially effective if it is used in practice after conducting studies of a significant number of individuals [28].

Another disadvantage when using *X-STR* typing kits is the presence of linkage groups, which complicate the calculation of the probability and reduce the efficiency of the detection system [29]. It is possible to obtain reliable values of the frequency of mutations during the study of a significant number of meioses. However, few studies have been conducted to determine the frequency of mutations on the *X*-chromosome [30].

Mutation studies carried out to date demonstrate different frequencies of mutability of loci. For example, during the study of 38 *X-STR*-repeats, which contained sets of *AGCU X19 multiplex system (AGCU ScienTech Incorporation*, China) and *ID X19 multiplex system* (China), 31 cases of mutations in 16 loci were found on a sample of 619 meioses in several generations: all but one is a shift of 1 repeat. The average frequency of mutations for 38 loci is 1.32× 10-3 per meiosis; 20 of the 31 mutations were passed down from the grandfather to the mother, 8 of which were passed down to the next generation. Of the total number of mutations, 11 were transmitted from mother to child, of which 4 were not received from grandparent. Loci *DXS10135* and *DXS8377* showed the highest level of mutations, which calls into question their use in forensic identification of a person [31].

The Spanish-Portuguese Group of the International Society of Forensic Genetics (*GHEP-ISFG*) also conducted population studies in triplets (father-mother-child) and identified mutations and the presence of "silent" alleles. During the study,

27 Gomes I., Pinto N., Antão-Sousa S., Gomes V., Gusmão L., Amorim A. Op. cit. DOI: 10.3389/fgene.2020.00926 (date accessed: 10.08.2023).

28 He H.-J., Zha L., Cai J.-H., Huang J. The forensic value of X-linked markers in mixed-male DNA analysis. *International Journal of Legal Medicine.* 2018. Vol. 132. Is. 5. Pp. 1281—1285. DOI: 10.1007/s00414-018-1841-5 (date accessed: 10.08.2023).

29 Gomes I., Pinto N., Antão-Sousa S., Gomes V., Gusmão L., Amorim A. Op. cit. DOI: 10.3389/fgene.2020.00926 (date accessed: 10.08.2023).

30 García M. G., Catanesi C. I., Penacino G. A., Gusmão L., Pinto N. Op. cit. DOI: 10.1016/j.fsigen.2019.04.005 (date accessed: 10.08.2023).

31 Song F., Wei X., Zhou C., Wang S., Deng C., Liao M., Luo H. Resolving the recombination pattern of 38 X-STRs from Chinese Han three-generation pedigrees. *Legal Medicine (Tokyo, Japan)*. 2022. Vol. 59. DOI: 10.1016/j.legalmed.2022.102135 (date accessed: 10.08.2023).

mutations associated with the appearance or loss of one repeat (174 / 181 mutations) with a change in the sequence by 2 repeats (5 / 181 mutations) and an increase in the length of the chain by 1 pair of bases (2 / 181 mutations) were detected. At the same time, 134 / 181 detected mutations were of paternal origin, 26 / 181 were of maternal origin, and 21 / 181 were of possible origin from both parents. Consequently, a significantly higher rate of mutations in the male *X*-chromosome was found, which increases with the age of the father; no such correlation was found for mothers. The increased level of mutations in men must be taken into account when calculating probabilities in the event of establishing kinship. In addition, *DXS10135* locus was the most unstable to mutations (frequency of mutations 1.1E-02) [32].

Analytical and statistical problems are one of the main reasons for the reduced interest in the identification of individuals based on *X-STR*-repeats, however, software applications have already been developed to estimate *X*-chromosomal relatedness without limiting the use of *X*-repeats, among them *MINX (MERLIN in X)*, *Familias, FamLinkX*. As an example, consider *FamLinkX* software, the basis of which is the use of Markov chains for estimating linkage disequilibrium and genetic inconsistency in the form of mutations and the ability to model recombinations within a cluster (the latter is an advantage compared to other programs for kinship estimation) [33].

To introduce *X-STR* repeats into the routine conduct of forensic molecular genetic examination, first of all, it is necessary to create a database of genetic diversity of alleles to establish the suitability of using loci among a certain population, the possible genetic relatedness of populations and the spread of alleles among the population for further calculation of the probability of coincidence of genetic characteristics of individuals. Over the past 10 years, China has made significant progress in the study of population diversity, and similar studies are also being conducted in Switzerland, Italy, India, Eritrea, Argentina, Mexico, the UAE, the United States, and Japan, but not as large-scale [34].

The closest geographic location to Ukraine is a study of the Croatian population with a sample of 397 people from 5 historical and cultural regions of the country and a comparison with previous studies that revealed similarities between all regions of Croatia (except for small isolated populations: for example, islands and remote villages). The researchers found out that *DXS10135* locus is the most informative (35 alleles are present in the population), and *DXS8378* locus (7 alleles) is the least informative. The *DXS10079* locus demonstrated a high

32 Kling D., Dell'Amico B., Tillmar A. O. FamLinkX — implementation of a general model for likelihood computations for X-chromosomal marker data. *Forensic Science International: Genetics*. 2015. Vol. 17. Pp. 1—7. DOI: 10.1016/j.fsigen.2015.02.007 (date accessed: 10.08.2023).

33 Garcia F. M., Bessa B. G. O., dos Santos E. V. W., Pereira J. D. P., Alves L. N. R., Vianna L. A., Casotti M. C., Trabach R. S. R., Stange V. S., Meira D. D., Louro I. D. Forensic Applications of Markers Present on the X Chromosome. *Genes*. 2022. Vol. 13. Is. 9. Pp. 1—12. DOI: 10.3390/genes13091597 (date accessed: 10.08.2023).

34 Mršić G., Ozretić P., Crnjac J., Merkaš S., Sukser V., Račić I., Rožić S., Barbarić L., Popović M., Korolija M. Expanded Croatian 12 X-STR loci database with an overview of aberrant profiles. *Forensic Science International: Genetics*. 2018. Vol. 34. Pp. 249—256. URL: https://core.ac.uk/reader/245883170 (date accessed: 10.08.2023).

level of instability, which was expressed in obtaining a biallelic locus on the graphs in males and a triallelic locus in females. This indicates the potential mutability of this locus. 7 out of 12 studied markers are in unbalanced linkage, but no dependence was found between different groups of linkage of markers. During the study using the *Investigator Argus X-12 kit (Qiagen,* Germany) it was also established that the population of Europe is homogeneous, Croatia overlaps with Belarus and other Slavic peoples in terms of allele frequencies and at the same time forms a cluster with seven countries of Central and Eastern Europe. Among the European population, only regions with specific population processes (genetic drift, inbreeding) stand out: Ibiza, Greenland, Sardinia, Southern Italy, and Albania [35].

It is urgent to assess the genetic substructure in the Western Mediterranean (eastern Spain, southern Italy and Morocco), because there are geographical and/or cultural isolates. Study of 716 individuals from 11 populations using two X-chromosome genotyping kits: 9 X-chromosome *Alu*-inserts and *Investigator Argus X-12 kit (Qiagen,* Germany). In the presented populations, *DXS10135* locus was the most discriminating (32 alleles), while *DXS10103* was the least diverse and represented only in 7 allelic variants. Linkage disequilibrium was found in the loci *DXS10101-DXS10146* (for Moroccans), *DXS10103-DXS10101* (for the Sicilian population), and *DXS10148-DXS10135* (for the Ibizan population), the last two loci being located in the same linkage group. All populations showed high average genetic diversity for this *X-STR* kit, with values ranging from 0.791 in the Catanzaro population to 0.831 in the Moroccan Sahara population. The most polymorphic linkage group is *LG1,* and the smallest is *LG3*. Populations from Ibiza and Sicily showed the lowest haplotype diversity with a low percentage of unique haplotypes compared to the other populations studied, while those from the Moroccan Sahara and Valencia had the most diversity. Overall, the Moroccan population showed higher proportions of unique haplotypes than the Italian and Spanish populations. In addition, populations of North Africa are genetically more similar to Europeans than other populations located south of the Sahara. For both kits of markers, the Ibiza population shows a distant position relative to the populations that surround it geographically (possibly as a result of genetic drift). In general, studies have shown the presence of migration around the Mediterranean Sea, the level of which was higher for women than for men [36].

The first population studies of *X*-chromosome were also conducted in Switzerland. 1198 people (592 women, 606 men) were studied using *Investigator Argus X-12 kit (Qiagen,* Germany). The most diverse loci in terms of the number of alleles are *DXS10146* (38 variants) and *DXS10135* (37 variants), the least diverse — *DXS7423* (7 variants). The researchers discovered 12 new alleles, 6 of which are represented in *DXS10146* locus, which indicates the need for the expansion of the allelic ladder by the reagent manufacturer. 9 multiallelic

35 Ferragut J. F., Bentayebi K., Barbaro A., Ramírez M., Saguillo A. Y., Ramon C., Picornell A. Exploring the Western Mediterranean through X-chromosome. *International Journal of Legal Medicine*. 2021. Vol. 135. Is. 3. Pp. 787—790. DOI: 10.1007/s00414-020-02498-4 (date accessed: 10.08.2023).

36 Bottinelli M., Gouy A., Utz S., Zieger M. Population genetic analysis of 12 X-chromosomal STRs in a Swiss sample. *International Journal of Legal Medicine*. 2022. Vol. 136. Is. 2. Pp. 561—563. DOI: 10.1007/s00414-021-02684-y (date accessed: 10.08.2023).

repeat variants were identified, 6 of the 9 were found at *DXS10079* locus, whose duplications were observed in the studies mentioned above. Also, a dropout at *DXS10101* locus and a potential dropout at *DXS10146* locus were detected due to a decrease in peak heights. In three of the four repetition groups, clutch imbalance was detected [37].

For Ukraine, similar databases of population studies are not presented in the scientific literature, which complicates the practical use of *X-STR* markers, but is a promising direction for future research.

Conclusions

X-STR-repeats are a full-fledged tool for conducting molecular genetic examinations, which can be proposed for implementation in the Expert Service of the Ministry of Internal Affairs of Ukraine. Such tools are not only useful for demonstrating significant genetic variation between populations: they can also be used to identify individuals, especially in the context of russian aggression against Ukraine. Developed commercial kits make it possible to standardize the results obtained in different laboratories, and to conduct searches and comparative studies of individuals during molecular genetic examinations. Population studies and the creation of an international database of DNA profiles of *X-STR* repeats are promising.

Особливості та перспективи використання *X*-хромосомних тандемних повторів у судовій молекулярно-генетичній експертизі

Софія Зюбрій

Під час проведення молекулярно-генетичних експертиз фахівці-біологи в Україні зазвичай використовують набори аутосомних STR-повторів і повтори на Y-хромосоми і взагалі не послуговуються наборами для дослідження X-хромосомних STR-повторів. Метою цього дослідження є аналіз літературних даних для визначення характеристик X-хромосоми, можливостей використання наборів для типування X-хромосоми та впровадження їх у судову практику фахівців молекулярно-генетичних експертиз. Визначено критерії пошуку й аналізу STR-локусів, розглянуто характеристики комерційних наборів (зокрема, Argus X-8 PCR Amplification Kit від Biotype AG та Investigator Argus X-12 QS Kit (Німеччина), X19 X-STRs amplification kit від AGCU ScienTech Inc. та 19X Direct ID system (Китай)) і напрями їх використання. Досліджено дані науково-методичної літератури щодо частоти мутацій, поширеності алелів у популяціях й перспективи створення загальної бази даних X-STR-повторів. Для досягнення поставленої мети застосовано загальні та спеціальні методи, з-поміж яких основним є структурно-генетичний аналіз і синтез даних досліджень X-хромосоми з позицій судової генетики. Використано також методи формалізації характеристик комерційних наборів X-STR-повторів і їхній порівняльний аналіз, систематизації можливих напрямів використання STR-повторів із урахуванням особливостей наслідування X-хромосоми й узагальнення популяційних досліджень, проведених у європейських державах.

***Ключові слова**: молекулярно-генетична експертиза; X-хромосома; STR-повтор; тандемний повтор; порушення рівноваги зчеплення.*

37 Pinto N., Pereira V., Tomas C., Loiola S., Carvalho E. F., Modesti N., et all. Paternal and maternal mutations in X-STRs: A GHEP-ISFG collaborative study. *Forensic Science International: Genetics*. 2020. Vol. 46. Pp. 1—7. DOI: 10.1016/j.fsigen.2020.102258 (date accessed: 10.08.2023).

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References

Bottinelli, M., Gouy, A., Utz, S., Zieger, M. (2022). Population genetic analysis of 12 X-chromosomal STRs in a Swiss sample. *International Journal of Legal Medicine*. Vol. 136. Is. 2. DOI: 10.1007/s00414-021-02684-y.

Chromosome X: 1-155,270,560 (2023) / e!Ensembl. URL: http://www.ensembl.org/Homo_sapiens/Location/Chromosome?r=X:1-155270560.

Diegoli, T. M. (2015). Forensic typing of short tandem repeat markers on the X and Y chromosomes. *Forensic Science International: Genetics*. Vol. 18. DOI: 10.1016/j.fsigen.2015.03.013.

Diegoli, T. M., Coble, M. D. (2015). Characterization of X Chromosomal Short Tandem Repeat Markers for Forensic Use. U. S. Department of Justice. URL: https://www.ojp.gov/pdffiles1/nij/grants/249510.pdf.

Ferragut, J. F., Bentayebi, K., Barbaro, A., Ramírez, M., Saguillo, A. Y., Ramon, C., Picornell, A. (2021). Exploring the Western Mediterranean through X-chromosome. *International Journal of Legal Medicine*. Vol. 135. Is. 3. DOI: 10.1007/s00414-020-02498-4.

Garcia, F. M., Bessa, B. G. O., dos Santos, E. V. W., Pereira, J. D. P., Alves, L. N. R., Vianna, L. A., Casotti, M. C., Trabach, R. S. R., Stange, V. S., Meira, D. D., Louro, I. D. (2022). Forensic Applications of Markers Present on the X Chromosome. *Genes*. Vol. 13. Is. 9. DOI: 10.3390/genes13091597.

García, M. G., Catanesi, C. I., Penacino, G. A., Gusmão, L., Pinto N. (2019). X-chromosome data for 12 STRs: Towards an Argentinian database of forensic haplotype frequencies. *Forensic Science International: Genetics*. Vol. 41. DOI: 10.1016/j.fsigen.2019.04.005.

Gomes, I., Pinto, N., Antão-Sousa, S., Gomes, V., Gusmão, L., Amorim, A. (2020). Twenty Years Later: A Comprehensive Review of the X Chromosome Use in Forensic Genetics. *Frontiers in genetics*. Vol. 11. DOI: 10.3389/fgene.2020.00926.

He, H.-J., Zha, L., Cai, J.-H., Huang, J. (2018). The forensic value of X-linked markers in mixed-male DNA analysis. *International Journal of Legal Medicine*. Vol. 132. Is. 5. DOI: 10.1007/s00414-018-1841-5.

Investigator® Argus X-12 QS Handbook. Germany : Qiagen (2022). URL: https://qiagen.com.

Jia, J., Liu, X., Fan, Q., Wang, M., Zhang, J., Li, W., Shi, L., Zhang, X., et al. (2021). Development and validation of a multiplex 19 X-chromosomal short tandem repeats typing system for forensic purposes. *Scientific Reports*. Vol. 11. Art. 609. DOI: 10.1038/s41598-020-80414-x.

Jusic, B., Pilav, A., Dzehverovic, M., Čakar, J. H. (2022). DNA Paternity Testing with Two Mismatches: Our Experience. *Jordan Journal of Biological Sciences*. Vol. 15. Is. 3. DOI: 10.54319/jjbs/150307.

Kakkar, S., Sarma, Ph., Panigrahi, I., Mandal, S. P., Shrivastava, P., Kumawat, R. K. (2022). Development of a new screening method for faster kinship analyses in mass disasters: a proof of concept study. *Scientific reports*. Vol. 12. Is. 1. DOI: 10.1038/s41598-022-22805-w.

Kling, D., Dell'Amico, B., Tillmar, A. O. (2015). FamLinkX — implementation of a general model for likelihood computations for X-chromosomal marker data. *Forensic Science International: Genetics*. Vol. 17. DOI: 10.1016/j.fsigen.2015.02.007.

Mentype® Argus X-8 PCR Amplification Kit Manual (2009). Germany : Biotype AG. URL: https://www.biotype.de/fileadmin/user/MANUALS/Mentype_Argus_X-8_eng.pdf.

Mršić, G., Ozretić, P., Crnjac, J., Merkaš, S., Sukser, V., Račić, I., Rožić, S., Barbarić, L., Popović, M., Korolija, M. (2018). Expanded Croatian 12 X-STR loci database with an overview of aberrant profiles. Forensic Science International: Genetics. Vol. 34. URL: https://core.ac.uk/reader/245883170.

Pinto, N., Gusmão, L., Amorim, A. (2011). X-chromosome markers in kinship testing: a generalisation of the IBD approach identifying situations where their contribution is crucial. *Forensic Science International: Genetics*. Vol. 5 Is. 1. DOI: 10.1016/j.fsigen.2010.01.011.

Pinto, N., Pereira, V., Tomas, C., Loiola, S., Carvalho, E. F., Modesti, N., et all. (2020). Paternal and maternal mutations in X-STRs: A GHEP-ISFG collaborative study. *Forensic Science International: Genetics*. Vol. 46. DOI: 10.1016/j.fsigen.2020.102258.

Prieto-Fernández, E., Baeta, M., Núñez, C., Zarrabeitia, M. T., Herrera, R. J., Builes, J. J., de Pancorbo, M. M. (2016). Development of a new highly efficient 17 X-STR multiplex for forensic purposes. *Electrophoresis*. Vol. 37. Is. 12. DOI: 10.1002/elps.201500546.

Ross, M. T., Grafham, D. V., Coffey, A. J., Scherer, S., McLay, K., et al. (2005). The DNA sequence of the human X chromosome. *Nature*. Vol. 434. DOI: 10.1038/nature03440.

Song, F., Wei, X., Zhou, C., Wang, S., Deng, C., Liao, M., Luo, H. (2022). Resolving the recombination pattern of 38 X-STRs from Chinese Han three-generation pedigrees. *Legal Medicine (Tokyo, Japan)*. Vol. 59. DOI: 10.1016/j.legalmed.2022.102135.

Tillmar, A. O., Kling, D., Butler, J. M., Parson, W., Prinz, M., Schneider, P. M., Egeland, T., Gusmão, L. (2017). DNA Commission of the International Society for Forensic Genetics (ISFG): Guidelines on the use of X-STRs in kinship analysis. *Forensic Science International: Genetics*. Vol. 29. DOI: 10.1016/j.fsigen.2017.05.005.

Xiao, Ch., Yang, X., Liu, H., Liu, Ch., Yu, Zh., Chen, L., Liu, Ch. (2021). Validation and forensic application of a new 19 X-STR loci multiplex system. *Legal medicine*. Vol. 53. DOI: 10.1016/j.legalmed.2021.101957.

Yang, Q., Qian, J., Shao, Ch., Yao, Y., Zhou, Zh., Xu, H., Tang, Q., Qian, X., Xie, J. (2021). Identification and Characterization of Nine Novel X-Chromosomal Short Tandem Repeats on Xp21.1, Xq21.31, and Xq23 Regions. *Frontiers in Genetics*. Vol. 2. DOI: 10.3389/fgene.2021.784605.

Yang, X.-Y., Wu, W.-W., Chen, L., Liu, Ch.-H., Zhang, X.-F., Chen, L., Feng, X.-L., Wang, H.-J., Liu, Ch. (2016). Development of the 19 X-STR loci multiplex system and genetic analysis of a Zhejiang Han population in China. *Electrophoresis*. Vol. 37. Is. 15—16. DOI: 10.1002/elps.201500540.

Zhang, Y., Yu, Zh., Mo, X., Zhao, X., Li, W., Liu, H., Liu, Ch., Wu, R., Sun, H. (2021). Comparative evaluation of autosomal STRs and X-chromosome STRs as a complement of autosomal STRs in kinship testing in Southern Han Chinese. *Annals of human biology*. Vol. 48. Is. 1. DOI: 10.1080/03014460.2020.1856926.

Zhang, Y., Yu, Zh., Mo, X., Zhao, X., Li, W., Liu, H., Liu, Ch., Wu, R., Sun, H. (2021). Development and validation of a new 18 X-STR typing assay for forensic applications. *Electrophoresis*. Vol. 42. Is. 6. DOI: 10.1002/elps.202000168.

Ziubrii, S. (2023). Features and Prospects of the Use of X-chromosomal Tandem Repeats in Forensic Molecular Genetic Examination. *Theory and Practice of Forensic Science and Criminalistics*. Issue 3 (32). Pp. 148—161. DOI: 10.32353/khrife.3.2023.09.