PATERNITY TEST: USE OF MARKERS IN GENOMIC ANALYSIS TO INVESTIGATE BIOLOGICAL BOND

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Abstract

This study aimed to investigate new techniques used for DNA testing in paternity analysis, presenting novel methods that ensure quality and reliability in their results. It highlights the most commonly used approaches, such as STR markers, while also addressing their limitations and the need for additional strategies, including SNP identification and emerging perspectives in the investigation of biological relationships. Furthermore, it discusses how genetic markers have a broad applicability, even in situations where only a limited amount of sample is available, enabling effective analysis. This is due to the use of key molecular markers, with STRs being the most commonly employed in such cases. Paternity testing has become increasingly detailed, allowing, in cases where the alleged father is deceased or missing, the confirmation of biological relationships through samples from close relatives. Additionally, even when samples are degraded or available in small quantities, analysis remains feasible. This level of accuracy is achieved through techniques such as STRs, SNPs, Y-chromosome STRs, and autosomal STRs. The results become even more reliable when two or more of these markers are analyzed together, increasing the robustness of the conclusions.

Keywords: Polymorphism, DNA test, Forensic genetics, Genetic test.

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INTRODUCTION

With scientific advances, studies continue to improve and new techniques are developed, especially in the field of genomics involving DNA (deoxyribonucleic acid). Likewise, in paternity testing—particularly in cases involving victims of sexual abuse, when an investigation must be carried out during pregnancy—new techniques are chosen so that there is no risk to either the fetus or the pregnant woman, as is the case, for example, with amniocentesis, since it is considered an invasive examination (Damour, 2023).

Thus, mention should be made of the non-invasive prenatal test (NIPT), which initially began using the STR genetic marker (Short Tandem Repeats), conventionally employed in these analyses; however, its detection proved insufficient to obtain a precise result, since its reach extended to only a limited number of alleles (Shen, 2021). Other markers have also been used in recent years, such as SNPs (single nucleotide polymorphisms), DNA methylation, and microhaplotypes; however, for database purposes, their public reference still lacks evaluation (Shen, 2021).

Additionally, forensic genetic genealogy (FGG), whose genomic databases contain hundreds of thousands of single nucleotide polymorphisms (SNPs), provides broad scope for identifying both close and distant maternal and paternal relatives, due to this extensive allele coverage (de Vries, 2021).

It is known that autosomal DNA has been used over the years, although, regarding the reconstruction of a genetic profile of individuals who lived in the last century, it is still considered a recent line of research. As in the case of Joseph Smith Jr., in which DNA testing was employed to study his ancestry, the investigation began with the use of autosomal STR markers; however, they would not have been sufficiently informative, and thus SNP genetic information was used, since these enabled the typing of second- to fifth-degree relatives separated by two centuries (Perego, 2019).

In view of the foregoing, this study aimed to investigate new techniques used in DNA paternity testing, presenting new methods that ensure quality and confidence in their results, portraying the most commonly used methods, such as STR markers, while also addressing their limitations, the need to use

additional approaches such as SNP identification, and the perspectives in the investigation of biological relationships. It also considers new DNA tests and cases involving kinship through family members, even when samples are obtained from relatives up to the fifth degree.

METHODOLOGY

In this scientific article, an integrative review of the scientific literature was carried out for discussion of the theme guided by the following research question: DNA testing for the investigation of paternity, and its new methods, and its importance for the reliability of results? – identifying the principal techniques used, their advantages and disadvantages. The scientific databases used were: National Institutes of Health (PubMed), Scientific Electronic Library Online (SciELO), Latin American and Caribbean Health Sciences Literature (LILACS), and ScienceDirect.

For the inclusion criteria, studies published between 2014 and 2024 in Portuguese, English, and Spanish were selected, using the descriptors contained in Health Sciences Descriptors (DeCS): paternity, DNA, genetic markers, SNP, STR, forensic genetics, genetic testing, and polymorphism.

Studies that did not encompass the established theme and terms, were outside the publication period, or were duplicates in the databases were excluded.

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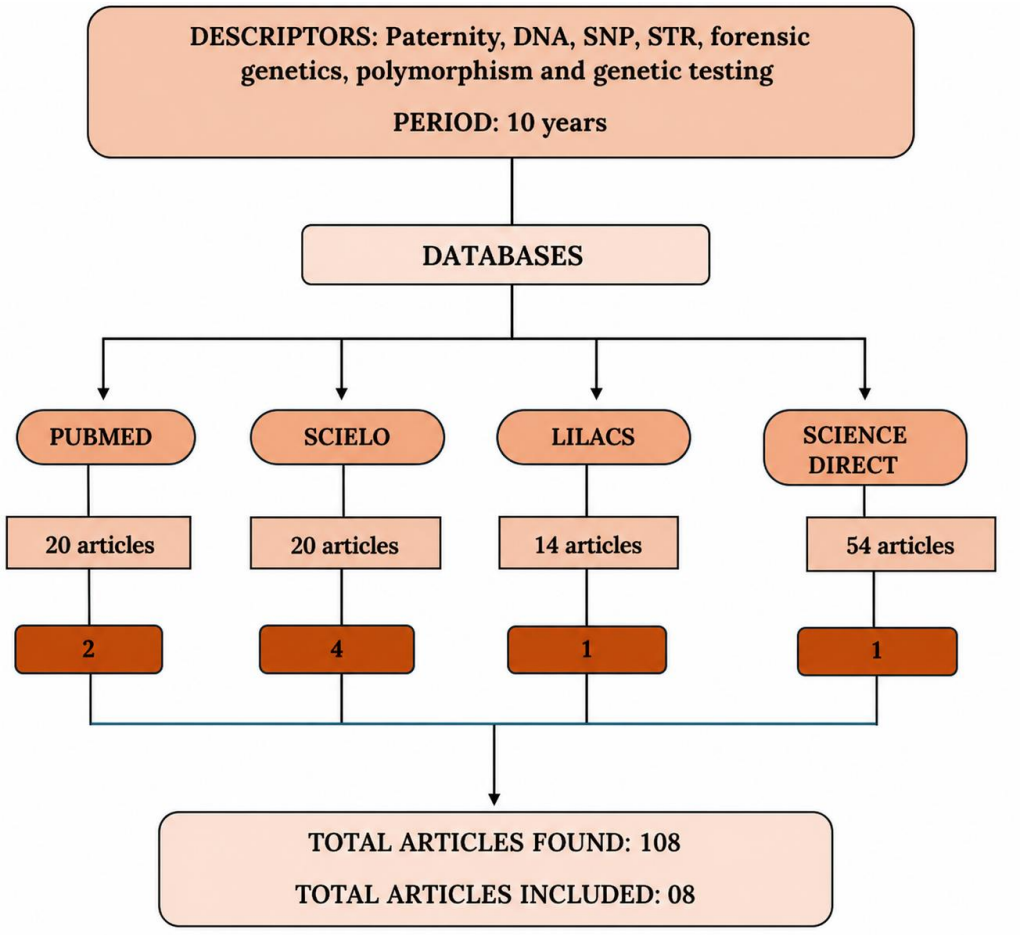
RESULTS AND DISCUSSION

This research was conducted through a systematized search in the selected databases using the established descriptors. After the initial search, the studies were filtered according to the inclusion and exclusion criteria. Figure 1 demonstrates the methodology established for this process, as well as the total number of studies found in this search and the articles included to compose this integrative review of the scientific literature.

Figure 1

Search for studies to carry out the integrative review, descriptors, databases, established period, total number of articles found and included.



Source: Authors

The following table (Table 1) was prepared to provide a better understanding of the selected articles and presents the distribution of each study included in the review. The data are presented according to author and year, article title, study design, study objective, and main results.

Table 1

Description of studies included in the integrative review.

Author and year	Article title	Study design	Study objective	Main results
Fonseca, 2019	DNA e o deslocamento de certezas no direito de família brasileiro [DNA and the displacement of certainties in Brazilian family law]	Original article	Shows the impacts of DNA paternity testing on decisions and policies in Brazilian family law	This study reports cases in which alleged children did not come forward for years before the emergence of DNA paternity testing, since it was normally not possible to obtain sufficient witnesses to report the case, and with the advent of the test it became possible to be certain of the biological father. In Brazil, even with several advanced technologies for genetic identification, the area did not attract as much attention from the media and society as paternity testing.
Machado; Leite; Barcelos, 2017.	Aplicabilidade do cromossomo X no DNA forense [Applicability of the X chromosome in forensic DNA]	Review article	Assists in solving cases by making use of genetic polymorphisms of molecular markers located on the X chromosome	This article investigated molecular polymorphisms and, because complete genetic material was unavailable or degraded, nuclear molecular markers and mitochondrial DNA were also used. The greatest use of X-chromosome polymorphism was in complex cases of human identification that could not be solved due to the lack of genetic material from relatives.
Caulfield; Stern, 2017.	Sombras da dúvida: a difícil incorporação da ciência de identificação na determinação legal da paternidade no Brasil [Shadows of doubt: the difficult incorporation of identification science in the legal determination of paternity in Brazil]	Original article	Even the most precise technology for human identification becomes irrelevant, and therefore such technologies were incorporated as supplementary methods in legal, social, and cultural decisions surrounding families	It reports the history of paternity testing, including in the colonial period, when illegitimacy rates were high, especially among the poor enslaved and free population. When Brazil's first Civil Code was approved, children could sue for paternal recognition, and when this code was implemented it instantly became one of the most common proceedings in family court. As the examination became more popular, the rights of families in favor of single mothers were also revolutionized, particularly by putting an end to humiliation in trials. However, in the search for truth in identification and legal science, it was not found in DNA, but rather in relation to the biology and culture of individual and collective identity.
Finamori, 2015.	Cuidado e consanguinidade na	Original article	The trajectory of DNA testing intertwined with the determination of	This article reports family cases involving famous individuals, in which the first denied being the biological

	atribuição de responsabilidade intergeracionais [Care and consanguinity in the attribution of intergenerational responsibility]		laws and with some cases involving famous individuals was highlighted, in which the test result assists in resolving cases	father of his alleged daughter, even though the examination result affirmed positive consanguinity, claiming that because he had not had affective contact with her, it projected a negative image. In the other case, even after years of assuming he was the biological father, the DNA examination proved negative; nevertheless, even with the result, he did not sever affective ties with his son, despite not being biologically related. These were two cases with different endings and corresponding results, also generating different repercussions before the public.
Fonseca, 2016.	Deslocando o gene: o DNA entre ouras tecnologias de identificação familiar [Displacing the gene: DNA among other technologies of family identification]	Original article	Shows the reach of the technology and its advances, especially concerning DNA testing, and highlights cases in which the entire outcome was followed, potentially involving feelings of affection and abandonment	This article presents an analysis of the hypothesis that the use of DNA testing in judicial paternity investigations may have caused a “geneticization” of social life. It contextualizes its effects on the evolution of scientific paternity evidence, on improving governance practices, and on the affective and material framework of some families.
Ambrosio *et al.,* 2020	Dados mutacionais e perfil populacional de 23 Y-STRs em três populações brasileiras [Mutational data and population profile of 23 Y-STRs in three Brazilian populations]	Original article	In order to achieve broader scope in forensic identification, Y-STRs are applied mainly in more complex cases, serving as a complement to autosomal STRs; however, for accurate interpretation, estimates of Y-STR haplotype frequencies must be reliable	This study made it possible to improve estimates of the frequency of the PowerPlex Y23 haplotype, increasingly expanding the number of profiles in the Brazilian database. Markers with GAAA or GATA repeats showed an increase in mutation rate with allele size, and there was also an increase in mutation rate with the father’s age.

Basgalupp *et al.,* 2014	Investigação de paternidade com suposto pai falecido ou desaparecido análise do êxito ao final do relatório [Paternity investigation with alleged father deceased or missing: analysis of success at the end of the report]	Original article	A study in which the alleged father is deceased or missing, and, through DNA testing and its results in these cases, the genetic profile was reconstructed using DNA profiles from close relatives	The results showed that the greater the number of close relatives, the higher the success rate of conclusive reports. In these cases, without the presence of the alleged father, the investigation becomes more complex and ultimately generates inconclusive results, resulting in the loss of all effort and investment.
Rêgo; Carvalho; Gusmão, 2019	Linhagens masculinas em populações brasileiras e desempenho de ferramentas de predição de haplogrupos [Male lineages in Brazilian populations and the performance of haplogroup prediction tools]	Original article	Reports on the use of the Y chromosome in forensic investigations, contributing to Brazilian databases, showing the development of software in its results, and in other tests with their result percentages	These studies are aimed at increasingly supporting the growing list of genetic profiles in Brazilian databases, with results showing our high diversity of haplotypes across all samples.

DNA – deoxyribonucleic acid; Y-STRs – Short Tandem Repeats genetic marker of the Y chromosome; GAAA – nitrogenous bases Adenine and Guanine; GATA – nitrogenous bases Guanine, Adenine, and Thymine. Source: Authors, 2024.

Brazil was the first country in Latin America to develop DNA testing, which is not surprising given its history of advanced use in genetic research and testing, both in clinical medicine and in the forensic field, since the 1980s (Caulfield; Stern, 2017). Innovation in DNA technology, particularly aimed at Brazilian family rights (Fonseca, 2019), also made it possible to elucidate criminal investigations, enabling individuals to be accused or exonerated even in cases with minimal quantities of biological samples (Fonseca, 2019). This gave rise to new scientific technologies aimed at supporting science in the lives of the population (Fonseca, 2016).

Judicially, this test became the gold standard for paternity examinations, being widely requested between the 1990s and 2000s as its use became more widespread. Television media helped popularize it, leading many individuals to seek the courts to confirm or contest paternity (Finamori, 2015). Although it remains a widely debated topic, especially due to the defendant's refusal to undergo testing or the argument that DNA alone would be sufficient to attest compatibility, case law maintained that this alone was not enough to eliminate paternity in unlawful cases (Caulfield; Stern, 2017).

This issue also opens space for another discussion: the true family bond between father and child is not limited to biological relationships but also involves the so-called "socio-affective truth." This conflict arises especially when fathers who raised and registered children as their own discover, through DNA testing, that there is no biological compatibility, alleging that they were deceived by the mother (Fonseca, 2019).

In cases where there is paternal absence or absence of ascendants, difficulty arises in elucidating autosomal molecular markers, Y-chromosome markers, and those located in mitochondrial DNA, making the use of DNA testing indispensable (Machado; Leite; Barcelos, 2017).

According to Machado, Leite, and Barcelos (2017), in the field of forensic genetics, the main molecular markers are STRs and SNPs, with autosomal STR markers being the most widely used due to their high polymorphic level and low mutation rates. This allows effective analysis even in degraded or highly fragmented samples, as they require only small quantities of base pairs.

For Ambrosio et al. (2020), obtaining results in more complex cases in which paternal relatives are distant, or understanding paternal ancestry, became possible through Y-chromosome STRs, which, when combined with autosomal STRs, have high discriminatory power between individuals.

According to Basgalupp et al. (2014), in investigations to reconstruct the genetic profile of an alleged missing or deceased father, approximately 81.0% of genetic markers are analyzed in first-degree relatives. This enables obtaining a result, although accuracy increases proportionally to the number of close relatives' samples available for analysis.

Likewise, Jannuzzi et al. (2020) state that Y-chromosome genetic markers form databases and characterize male lineages, mainly because they do not undergo recombination, as most of their length—the non-recombining region of the Y chromosome (NRY)—does not recombine with the X chromosome. Thus, they are passed from generation to generation without mutation, making them valuable allies in investigations where autosomal DNA analysis alone is insufficient.

As cited by Ambrosio et al. (2020), to study Y-STR mutation rates, only father–son pairs were compared to avoid diverse mutations. As a result, many studies were unable to compile data to estimate allele mutation rates because they did not provide information on the distribution of allele frequencies in paternal samples.

However, Machado et al. (2017) state that X-chromosome markers complement and increase reliability together with autosomal markers, as they present high Mean Exclusion Chance (MEC) values, thus providing greater discriminatory power. In paternity investigations in which genetic material from the alleged father is unavailable, autosomal tests are normally used; however, their analysis may be limited or incomplete. In such cases, X-chromosome analysis has greater applicability. Nevertheless, in situations involving direct analysis between father and male child, the use of the X chromosome becomes redundant, since men do not inherit the X chromosome from their father, but from their mother.

CONCLUSION

This study analyzed various aspects related to paternity testing, considering that it has become one of the most frequently requested examinations in judicial proceedings. It represents hope for those seeking to legally prove biological paternity or for individuals who, through the examination, wish to demonstrate genetic incompatibility with a child whom the mother alleged to be their biological offspring.

Given the increase in such cases, the judiciary has sought to adapt in order to judge and conclude these demands in the best possible manner. However, many still argue that paternity testing does not constitute definitive proof, questioning the reliability of its results. Despite this, in many cases the

examination is essential, as it provides conclusive biological evidence regarding the existence or absence of a paternity relationship.

Paternity test analysis has become increasingly detailed, allowing, in cases where the alleged father is deceased or missing, the confirmation of ancestry using samples from close relatives. In addition, even in situations where samples are degraded or available in small quantities, analysis is possible.

This precision is achieved through techniques such as STR, SNP, Y-chromosome STR, and autosomal STR. The results become even more reliable when two or more of these markers are analyzed together, increasing the robustness of the conclusions.

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