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An analysis of the results of genetic paternity tests performed for judicial purposes in the years 2018–2023 at the Chair and Department of Forensic Medicine of the Medical University of Warsaw

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ABSTRACT

Introduction: In legal paternity cases, genetic testing holds crucial evidential value. The standard practice involves the participation of the mother, child, and alleged father in these tests. There is an approach that considers the child's mother's profile unnecessary for such tests. While testing fewer participants may be cost-effective, it is essential to obtain clear and conclusive results. Therefore, it is worth examining whether the absence of the mother's profile in the tests negatively impacts the results of kinship analysis.

Materials and methods: The results of disputed paternity genetic tests that included the mother, child, and alleged father were evaluated. These tests were conducted between 2018–2023 at the Department of Forensic Medicine of the Medical University of Warsaw. Genetic profiles were obtained using the PowerPlex® Fusion 6C System reagent kit (Promega, USA).

Results: Analysis of 557 paternity tests revealed that 148 cases resulted in the exclusion of paternity, 406 confirmed paternity, and 3 cases remained unsolved. Further analysis of cases with paternity exclusions showed inconsistencies in an average of 14 *loci*. The most frequently excluding marker was SE33, while TPOX had the weakest excluding strength. No false positive results were obtained in tests that included the mother's profile, and the percentage of unresolved cases was 0.54%. When simulating the results without the mother's profile, 5.21% of cases remained unresolved, and it was determined that a false positive result could occur.

Conclusions: The necessity of the mother's participation in genetic paternity testing has been confirmed. Omitting the mother's profile may lead to an inconclusive result or even an erroneous genetic opinion.

Keywords: paternity testing; STR *loci*; PowerPlex® Fusion 6C; excluding *loci*; false positive/negative paternity.

INTRODUCTION

When paternity is disputed, genetic testing serves as crucial evidence in establishing the biological truth necessary for legal confirmation of paternity. The participation of the mother, child, and alleged father is the standard practice in such testing. However, some laboratories offer testing without the participation of the child's mother, claiming that the results remain reliable and sufficient for effectively confirming or excluding paternity. While analyzing fewer genetic profiles may have financial advantages, the key to resolving questions of paternity lies in obtaining unequivocal test results that allow for categorical confirmation or exclusion.

Even in the first decade of the 21st century, the reagent kits used – although more advanced than earlier methods [1, 2] – did not always enable the determination of relationships between tested individuals with near-certainty. Moreover, the percentage of unresolved cases remained too high [3]. In subsequent years, technological advancements led to the development of new reagent kits with expanded genetic markers, reducing the percentage of unresolved cases.

Considering these advancements, it is necessary to assess whether, despite using the most up-to-date reagent kits, the absence of the mother's genetic profile negatively impacts the statistical analysis of kinship.

MATERIALS AND METHODS

We obtained our results from 557 disputed paternity cases between 2018–2023, in which genetic testing was performed on DNA samples collected from buccal swabs of trios consisting of the mother, child, and alleged father. Two swabs were collected from each individual, and tests were performed in duplicates. DNA isolation was conducted using the SwabSolution™ Kit (Promega, USA). The isolated DNA was then amplified by polymerase chain reaction (PCR) using the PowerPlex® Fusion 6C System (Promega, USA) reagent kit, allowing for the analysis of 23 autosomal STR *loci*: D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, and FGA, as well as Amelogenin and 3 Y-STR *loci*: DYS391, DYS576, and DYS570. Capillary electrophoresis was performed on the amplification products using an ABI Prism 3500xl (Applied Biosystems, USA) with POP-4® polymer (Applied Biosystems, USA), and the results were analyzed using GeneMapper® ID-X v1.4 software. All steps were carried out according to the manufacturers' protocols, with positive and negative controls included, both of which yielded correct results.

Statistical analysis of kinship was performed using the Familias software (ver. 3.3.1, Norway) [4, 5, 6], based on allele

frequencies found in the Polish population, available on the website: https://strider.online/. The likelihood ratio (LR) calculations used in genetic testing measure the relative credibility of 2 alternative hypotheses: H1 – the alleged father is the biological father of the child and the son/daughter of the mother; H2 – another random man from the population, unrelated to the alleged father, is the biological father of the child and the son/daughter of the mother.

According to Polish jurisprudence and the recommendations of the Polish Society of Forensic Medicine and Criminology (Polskie Towarzystwo Medycyny Sądowej i Kryminologii – PTMSiK), an LR score of ≥1,000,000 is considered sufficient to conclude in favor of paternity. Due to the possibility of mutations in microsatellite *loci*, paternity is excluded when at least 4 differences in gene *loci* are found [7]. Thus, in our review, any LR score below 1,000,000 was classified as inconclusive.

For each analyzed family, 2 statistical calculations were performed: one including all tested individuals (mother, child, and alleged father) and another excluding the child's mother's profile from the statistical analysis. In each case, the obtained LR value was recorded as the result of the analysis. Additionally, in cases where paternity was excluded, the specific markers responsible for the exclusion were noted. In cases where 3 or fewer differences in gene *loci* were identified, the possibility of mutations was considered. In such instances, statistical kinship calculations were performed using the Stepwise (Stationary) mutation model, with the mutation rate obtained from the Association for the Advancement of Blood & Biotherapies (AABB) report [8] and a mutation range of 0.1.

To assess the possibility of obtaining false positive results, a family search of all available profiles was conducted. First, we examined whether a random match to the child was possible in cases where the mother's profile was available, followed by the same search excluding the mother's profile. Additionally, all available profiles were compared to determine whether parent-child relationships could be identified among them.

RESULTS

After analyzing 557 cases of disputed paternity, we found:

- 148 cases excluding the paternity of the alleged father, constituting 26.6% of all cases. This result was identical for calculations performed with the full mother-child-alleged father trio and those without the child's mother's profile. However, after removing the child's mother's profile from the calculations, a decrease in the number of excluding markers was observed. In trios, the number of differences ranged of 6–21 (average: 14), while in duos, it ranged of 4–17 (average: 10). The frequency of individual markers as excluding *loci* is presented in Figure 1. In both calculations, the most frequent excluding marker was SE33, while the least frequent was TPOX;
- for calculations performed with the full trio, 406 cases confirmed paternity (72.9% of all cases). The average LR value was 1.29 x 10^{16}, with a maximum of 2.31 x 10^{18}. Three cases remained unresolved (0 < LR < 1,000,000), accounting for 0.54% of all cases;
- for calculations that did not consider the profile of the child's mother, there was a decrease in the number of confirmed paternity cases (380 cases, 68.2% of all) and an increase in unresolved cases (29 cases, 5.21% of all). The average LR value was 1.85 x 10^{12}, with a maximum of 6.64 x 10^{14}.

No false positive matches were found during family searches of all profiles that included the child and mother. However, after removing the mother's profile and conducting the search again, 7 possible matches were identified, representing 1.26% of all cases. In each of these matches, 1 or 2 mismatches were observed between the profiles of the analyzed individuals, with calculated LR values ranging from 1.67 x 10^{-11} to 3 x 10^{-2}, supporting the hypothesis of no kinship.

After comparing all available profiles, 15 results were found that could indicate kinship (number of exclusions below 4). The results were categorized based on LR values following the recommendations of the European Network of Forensic Science Institutes (ENFSI Guideline for Evaluative Reporting in Forensic Science, 2015):

- 10 cases with an LR value below 1, indicating greater support for the hypothesis of no relationship;
- 3 cases with an LR value between 2–10;
- 1 case with an LR value between 10–100;
- 1 case with an LR value between 1,000–10,000 (Fig. 1).

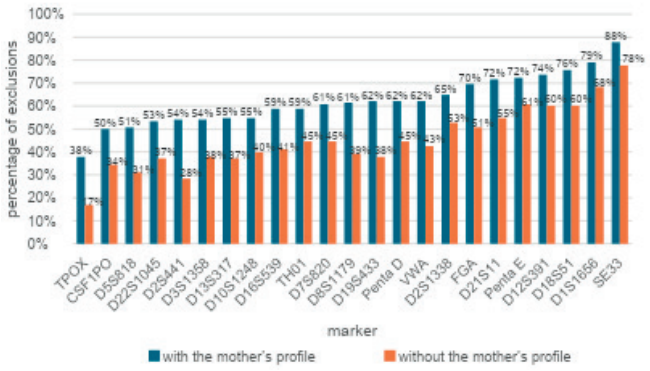

FIGURE 1. Efficiency of markers in excluding paternity

CONCLUSIONS

The obtained percentage of cases that excluded paternity (26.6%) was comparable to the results observed at the Department of Forensic Medicine of the Medical University of Białystok [9] and at the University of Bloemfontein in the Republic of South Africa [10]. Considering data from other publications, this percentage oscillated around ±6%. Higher results were observed in laboratories specializing in forensic paternity testing [11, 12], while laboratories performing testing for

forensic and private purposes recorded a lower result [8]. It could therefore be concluded that the obtained result depends largely on the specifics of the laboratory and the type of cases performed there.

According to the data included in these publications [9, 11, 12, 13, 14], it can be noted that when there are a greater number of markers, more exclusion *loci* are observed. For the PowerPlex® Fusion 6C System kit (Promega, USA), the average number of exclusion *loci* was 14. The most frequently excluding marker was SE33, where incompatibility was observed in 87.84% of all exclusions. This marker has the most possible variants and is also one of the most frequently mutating markers [8]. In the Zimbabwean population, it also turned out to be the most frequent excluding marker [12]. In other analyzed publications, it was not included in the scope of the reagent sets used. The most frequently mentioned excluding markers were D2S1338, D18S51, and Penta E, which were also markers with a high exclusion rate in our study. The least frequent excluding marker was TPOX, which was observed in only 37.84% of exclusions, corresponding with earlier reports for Thailand [13] and Białystok [9]. It is noteworthy that the D12S391 marker, which was the least frequent excluding marker in the Zimbabwean population [12], was ranked the fourth most frequent in our study (73.65%).

For calculations made using the profiles of the mother, child, and alleged father, 0.54% of cases were unresolved. In the AABB publication, unresolved cases were calculated to be between 0.41–0.45% [8], which can be considered a value similar to that observed in our study. In other publications, the cut-off value for results considered decisive ranged between 1,000 [13] and 1,000,000 [9], meaning that a result deemed inconclusive in 1 publication could be considered decisive under another publication's criteria. However, all cases with an inconclusive result involved at least 1 discrepancy between the profile of the alleged father and the child.

The inclusion of the mother's profile in testing had a clear influence on the final test results. In cases excluding paternity, the absence of the mother's profile reduced the number of differences. This trend has also been confirmed in other publications, such as studies conducted in: Korea [15], China [16], Brazil [17], and South Africa [10]. To obtain comparable results in statistical analysis without the mother's profile, a reagent set with a larger number of genetic markers should be used [16, 18]. In our study, after removing the mother's profile from cases excluding paternity, no false positive results were obtained. However, a report presented by the Chair of Forensic Medicine in Wrocław documented 3 cases of false positive results. During the analysis of disputed paternity for the full trio (mother, child, and alleged father), exclusions were found in 4 systems in 1 case and in 5 systems in another. After removing the mother's profile, no discrepancy was found in the first case between the profiles of the alleged father and the child, while in the second case, only 1 exclusion remained [19]. In Brazil, 7 mismatches were identified for the full trio, while only 1 difference remained when the mother's profile was excluded [17]. In these examples, it was determined that the analyzed individuals were related, which influenced the obtained analysis results. A study from South Africa reported 42 false positive matches after removing the mother's profile from the calculations in 1,673 cases [10].

In our study, omitting the mother's profile from cases with confirmed paternity resulted in lower LR scores, which in turn contributed to a higher proportion of inconclusive cases, e.g., 1 in 19 duos vs. 1 in 185 trios.

Furthermore, in the analysis of profiles for the possibility of obtaining a false positive result – taking into account the profiles of both the mother and the child – no matches were found. However, when comparing all 1,602 profiles with each other, 15 possible matches were identified that could indicate a parent–child relationship. In ten cases, the obtained LR value provided stronger support for the hypothesis of no kinship. In 4 cases, the result provided moderate or weak support for the hypothesis confirming kinship. One result had an LR value above 1,000. This value is high enough that, according to some guidelines [13], it could be considered sufficient to confirm kinship. Given that the simulation was based on unrelated individuals, such a result can be classified as a false positive.

Based on our analysis of genetic test results, the need for the mother's participation in genetic testing for disputed paternity cases has been confirmed. Omitting the mother's profile may lead to an inconclusive result or even an erroneous genetic opinion. We recommend performing genetic tests for the purpose of establishing paternity with the full participation of the mother, child, and alleged father.

REFERENCES

1. Karpiewska A, Kowalczyk E, Dobosz T. Paternity testing at the Department of Forensic Medicine of Wroclaw Medical University (Poland). Leg Med (Tokyo) 2017;26:18-24.
2. Gręda A. Dowód z badań DNA w sprawach o ustalenie lub zaprzeczenie pochodzenia dziecka. Palestra 2015;3-4:69-79.
3. Babol-Pokora K, Jacewicz R, Pepinski W, Szram S. Is SGM Plus™ the sufficient system for paternity testing? Int Congr Ser 2006;1288:450-2.
4. Drábek J. Validation of software for calculating the likelihood ratio for parentage and kinship. Forensic Sci Int Genet 2009;3(2):112-8.
5. Egeland T, Kling D, Mostad P. Relationship inference with Familias and R. Statistical method in forensic genetics. Amsterdam: Elsevier; 2016.
6. Kling D, Mostad PF, Egeland T. Manual for Familias 3. 2017. https://familias.name/Files/manualFamilias.pdf (21.11.2024).
7. Zasady atestacji laboratoriów genetycznych przy Polskim Towarzystwie Medycyny Sądowej i Kryminologii na rok 2016. Polskie Towarzystwo Medycyny Sądowej i Kryminologii. http://www.ptmsik.pl/komisja-genetyki-sadowej/zasady-atestacji-na-rok-2016 (21.11.2024).
8. Relationship Testing. Technical report for testing in 2022. As compared to reported data from 2020 and 2021. Association for the Advancement of Blood & Biotherapies. https://www.aabb.org/docs/default-source/default-document-library/accreditation/2022-technical-report-summary.pdf?sfvrsn=4ef9c537_1 (21.11.2024).
9. Wójcik MA, Skawrońska M, Niemcunowicz-Janica A, Pepiński W. Analiza wykluczeń ojcostwa w materiale Zakładu Medycyny Sądowej Uniwersytetu Medycznego w Białymstoku w latach 2008–2017. Arch Med Sadowej Kryminol 2018;68(4):281-9.
10. De Kock AA, Kloppers JJ. The impact of motherless paternity testing in a South African population. Afr Health Sci 2021;21(1):379-84.
11. García-Aceves ME, Jasso-Razo DG, Díaz-Navarro XX, Rangel-Villalobos H. A posteriori parameters from paternity tests of a Mexican laboratory with the powerplex fusion system. Leg Med (Tokyo) 2023;64:102296.

12. Thelingwani RS, Jonhera CA, Masimirembwa C. Analysis of data and common mutations encountered during routine parentage testing in Zimbabwe. Sci Rep 2024;14:1385.
13. Plodthong N, Chareonsirisuthigul T, Lumjiaktase P, Rerkamnuaychoke B. Analysis of paternity testing results by Identifiler™ system in Thailand. Thai J Genet 2014;7(2):133-8.
14. Huang YM, Wang J, Jiao Z, Yang L, Zhang X, Tang H, et al. Assessment of application value of 19 autosomal short tandem repeat loci of GoldenEye™ 20A kit in forensic paternity testing. Int J Legal Med 2013;127(3):587-90.
15. Lee HS, Lee JW, Han GR, Hwang JJ. Motherless case in paternity testing. Forensic Sci Int 2000;114(2):57-65.
16. Zhang MX, Gao HM, Han SY, Liu Y, Tian YL, Sun SH, et al. Risk analysis of duo parentage testing with limited STR loci. Genet Mol Res 2014;13(1):1179-86.
17. Aguiar VRC, de Castro AM, Pinto LM, Ferreira ACS, Dos Santos EVW, Louro ID. Assessing false paternity risk in simulated motherless cases from more than 20 000 real exclusion trios. Transfusion 2021;61(3):678-81.
18. Dogan M, Canturk KM, Emre R, Kara U, Filoglu G. Demonstration of false inclusion risks of duo parentage analyses in the Turkish population in light of parentage acceptance criteria. Aust J Forensic Sci 2017;49(3):326-31.
19. Bonar M, Czosnykowska M, Kowalczyk E, Dobosz T. Pomijanie matki w testach DNA ustalających ojcostwo może prowadzić do błędu. Przypadki Medyczne 2012;18:65-70.