CHIMERISM AS A CHALLENGE IN FORENSIC DNA ANALYSIS: LESSONS FROM A PATERNITY CASE

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Abstract

DNA forensic investigations can sometimes produce unexpected results, even in seemingly straightforward cases. One such case involved a paternity test where an alleged father sought confirmation of his biological relationship with a young girl. After two prior examinations produced contradictory results, the test was repeated at the DNA Laboratory of the Institute of Scientific Police in Tirana. Samples included three buccal swabs from the alleged father, one from the mother, and one from the child. Sixteen autosomal STR loci and AMELO were analyzed using PCR, followed by capillary electrophoresis on a Genetic Analyzer 3500 HID. The alleged father's samples revealed a mixed DNA profile: three loci with three alleles, three loci with four alleles, and ten loci with two alleles. His medical history disclosed a prior bone marrow transplant, which explained the chimeric DNA pattern. This represents the first documented case of chimerism affecting DNA-based paternity testing in Albania. The case highlights how chimerism can lead to false exclusions in parentage testing and complicate genetic investigations. Awareness of medical history and expert consultation are essential to reduce misinterpretation and ensure reliable conclusions in such cases.

Key words: *forensic genetics, chimera, DNA paternity testing, STR profile.*

Përmbledhje

Hetimet forenzike të ADN-së ndonjëherë mund të japin rezultate të papritura, edhe në raste që duken të thjeshta. Një rast i tillë përfshinte një test atësie, ku një baba i prezumuar kërkonte të konfirmonte lidhjen e tij biologjike me një

vajzë të mitur. Pas dy ekzaminimeve të mëparshme që kishin dhënë rezultate kontradiktore, testi u përsërit në Laboratorin e ADN-së pranë Institutit të Policisë Shkencore në Tiranë. Mostrat përfshinin tre tamponë oralë, nga babai i prezumuar, një nga nëna dhe një nga fëmija.

U analizuan gjashtëmbëdhjetë lokuse STR autosomale dhe AMELO duke përdorur metodën PCR, e ndjekur nga elektroforeza kapilare në Genetic Analyzer 3500 HID. Mostrat e babait rezultuan në profil miks të ADN-së: tre lokuse me tre alele, tre lokuse me katër alele dhe dhjetë lokuse me dy alele. Historia e tij mjekësore tregoi një transplant palce kockore të kryer disa vite më parë, gjë që shpjegoi modelin kimermik të ADN-së. Ky përbën rastin e pare të dokumentuar të kimerizmit që ndikon në testimin e atësisë me bazë ADN-je në Shqipëri. Rasti thekson se kimerizmi mund të çojë në përjashtime të rreme në testimin e prindërve dhe të komplikojë hetimet gjenetike. Njohja e histories mjekësore dhe konsultimi me ekspertë janë thelbësore për të shmangur keqinterpretimet dhe për të siguruar përfundime të besueshme në raste të tilla.

Fjalë kyçe: *gjenetikë forenzike, kimerë, testim i atësisë me ADN, profil STR.*

Introduction

The majority of organisms have a single set of DNA that is present and identical in each and every cell in their body as we inherit 50% of DNA from the father and the other 50% from the mother. Organisms known as chimeras possess two distinct sets of DNA or more. A chimerahuman is an individual who hastwo or more genetically distinct cell lines in their body that makes them genetically a mix of two individuals, although they appear as one person. Chimerism may be either acquired by transfusion or transplantation of donor cells which in some articles is referred also as "artificial chimerism", or congenital arising from embryo fusion or dizygotic twin-twin transfusion referred also as "natural chimerism" (Grazen, 2014& Wenk, 2018).

The "Fairchild case" is one of the well-knowncases that represents congenital chimerism. Prior to 2002, Lydia Fairchild had no indication that she might be a chimera, and she did the DNA test for some legal procedures that required both paternity and maternity tests. She was told by tests (cheek swabs) she was not the mother of her children. The DNA results were surprising but later tests on cervical tissue matched. After further medical investigations the doctors reached the conclusion that she was a chimera (ABC News, 2006).

Another type of chimerism known and studied is "transplacental chimerism". A pregnant woman carrying a male fetus represents a form of transplacental

chimerism, as fetal cells can cross the placenta and persist in the mother's blood for decades. This phenomenon, known as microchimerism, involves a small population of genetically distinct cells or DNA within an individual and may arise through maternal–fetal cell exchange, twin chimerism, or blood chimerism between twin fetuses (George et al., 2013).

This study examines acquired chimerism and its potential implications in paternity testing. Such testing typically relies on the comparison of specific genetic markers between the mother, the alleged father, and the child to determine biological relationships. Standard approaches, such as short tandem repeat (STR) analysis, are designed to identify alleles at defined loci that are inherited from each parent. However, in individuals exhibiting chimerism, the coexistence of two distinct genetic profiles may complicate this process, particularly when analyses are conducted on blood samples.

One of the most known reasons for acquired chimerism is BMT (bone marrow transplant). Bone marrow transplantation, which is often performed for hematologic disorders such as leukemia or lymphoma, is a life-saving procedure that can result in long-term survival for many patients. However, it comes with a series of potential complications, including graft-versus-host disease (GVHD), rejection, and chimerism. The development of chimerism is a well-known consequence of BMT, with most recipients becoming "mixed chimeras," wherein both recipient and donor DNA co-exist within the patient. In some cases, chimerism can be nearly complete, with little to no recipient DNA remaining in the blood or marrow, while in others, it can be partial, creating a genetic mosaic (Verfaillie et al., 2001).In the case of a bone marrow transplant (BMT), a patient receives hematopoietic stem cells from a donor, which repopulate the patient's bone marrow and give rise to a new set of blood cells with the donor's genetic material.

Over time, the donor's DNA can be found in various blood components (leukocytes, red blood cells, and platelets) as well as in tissues derived from the hematopoietic system (Santos et al., 2012).In most cases, the donor-derived DNA remains restricted to the blood and marrow, while the rest of the individual's tissues retain the original genetic makeup. However, chimerism can extend beyond the blood, potentially affecting other tissues such as skin, liver, and even the brain in some rare instances (Bertoni et al., 2005). The degree of chimerism can vary, with some individuals exhibiting nearly complete donor DNA in their blood, while others may have a more mosaic pattern, where certain cells are derived from the donor, but others retain the

recipient's original DNA (Cai et al., 2006).In bone marrow transplant recipients, the blood-derived DNA may not match the DNA in other tissues, leading to conflicting results when paternity tests are conducted using different tissue samples (Donnelly et al., 2003).

Material and methods

Sample Collection

The case initially appeared straightforward, involving a paternity test requested by an alleged father seeking to confirm whether a young girl was his biological child. The analysis was subsequently conducted for a third time at the DNA laboratory of the Institute of Scientific Policein Tirana, after two contradictory conclusions. Three separated buccal swabs were collected from the alleged father, one from the mother and one from the child. The study was conducted in accordance and approved by the Institutional Review Board of Institute of Forensic (protocol code 5582, and date 26.12.2024 of approval).

DNA Extraction and Amplification

DNA from buccal swabs was manually isolated using QIAamp DNA Investigator Kit (Qiagen, Germany) for extraction. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes between 20 and 100 µl, in our case the final volume of extracted DNA is 50 µl, DNA is eluted in buffer ATE.The commercial STR kit used for this case is AmpFLSTR™ NGM SElect™ kit (Applied Biosystem)a five dyes kit which simultaneously amplifies 16 separate STRs: D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, THO1, FGA, D10S1248, D22S1045, D2S441, D1S1656, D12S391 and SE33 and the sex determining marker Amelogenin. Several PCR reactions were performed to see the reproducibility of data.

Capillary Electrophoresis and Data Analysis

The amplified products were analyzed using capillary electrophoresis on the Genetic Analyzer ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems) using POP-4 polymer and the collection software Data Collection, version 2.0. Data was sized using GeneMapper™ID-X Software version 1.5 (Applied Biosystems). Allelic profiles were subsequently compared across all loci to assess genetic relationships

Results and discussion

All samples - comprising three independent DNA extractions and nine PCR amplifications (three PCRs with different dilutions prepared from each extraction) - analyzed from the alleged father produced a mixed DNA profile, showing three alleles at three loci (D16S539, D22S1045, D19S433), three loci with four alleles (D21S11, D12S391, SEE33) and ten loci with two alleles. The reproducibility of data and comparing the alleles per each loci in different PCR reactions, generated the DNA profiles of the alleged father, child and the mother and are presented in Table 1.

Table 1. Allelic profiles generated from buccal swab samples collected from the alleged father, the child and the mother

STR Genetic loci	Allelesfrom the alleged father	Alleles from the child	Alleles from the mother
D10S1248	14-15	14-15	14-15
HumvWA31	17-18	17-17	17-17
D16S539	9-11-12	11-12	12-12
D2S1338	17-20	17-20	17-23
AMELO	X-Y	X-X	X-X
D8S1179	12-14	12-13	13-14
D21S11	29-30-31-32	29-30	30-33.2
D18S51	12-13	12-16	16-17
D22S1045	11-15-16	15-16	15-16
D19S433	13-14-15	13-13	13-13
HumTH01	6-9.3	6-6	6-6
HumFGA	22-23	22-23	19-22
D2S441	10-11	11-11	11-14
D3S1358	15-17	17-17	17-18
D1S1656	11-18.3	16-18.3	15-16
D12S391	17-19-23-25	19-22	18.3-22
SE33	16-19-24.2-27.2	18-19	18-21

According to Mendelian principles of inheritance, an offspring receives one allele from the biological father and the other from the biological mother at each locus. By comparing the allelic profiles across all studied loci, it is possible to determine the genetic relationship between the individuals. As is presented in this table for D10S1248 locus the alleged father, child and the mother has the combination of alleles 14,15; for D16S539 locus the baby has inherited the allele 12 from the mother and the allele 11 from the father.

The alleged father in this locus has presence of three alleles. Even loci with two alleles had a different ratio from a normal case in which the heterozygote ratio between the peaks would be > 60%. There were three loci which had the presence of four alleles. The alleles for D16S539, TH01 and SE33 are shown in Figure 1.

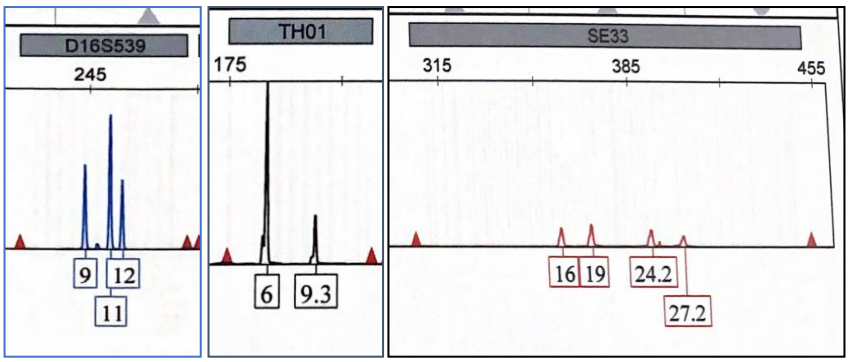

Figure 1. Electropherogram of three loci with discrepant alleles identified.

A-three alleles in D16S539, B-two alleles in THO1, C-four alleles in SE33

The positive and negative control clearly indicated that technical processes were optimal, and we were in front of the facts that this individual had more than one DNA profile in his epithelial cells.The alleged father underwent through a bone marrow transplant some years ago and the donor was his brother - these facts explained the surprising results we had and identified this case as chimera.Chimerism has important implications for forensic genetics

and paternity testing, as it can produce mismatches in DNA profiles, which may not necessarily indicate non-paternity (Fletcher et al., 2007).

Chimerism could be a major cause of false negative conclusions in parentage testing as it often produces curious results of medical and genetic investigations. In cases involving chimerism, a comprehensive approach is necessary. DNA testing from multiple tissue types - such as buccal swabs, hair follicles, or skin biopsies - may be required to accurately assess paternity. Studies suggest that a deeper understanding of the patient's chimeric status, including the percentage of donor versus recipient DNA present in different tissues, is crucial for correctly interpreting paternity results (Santos et al., 2012).

In recent years, several studies have reported the identification of human chimeras, often discovered incidentally during genetic or medical investigations. For example, germline chimerism was identified in monochorionic dizygotic twins through advanced molecular analysis (Rodríguez-Buritica et al., 2021). Similarly, a congenital case of chimerism was revealed during routine paternity testing using short tandem repeat genotyping, which detected more than two alleles at multiple loci (Wu et al., 2025). In prenatal settings, chimerism has also been mistaken for specimen confusion, as shown in a 46,XY/46,XY case that was later confirmed through genetic testing (Lee et al., 2024). Another case involved a fetus with both trisomy 21 and normal male karyotype (47,XX,+21/46,XY), further illustrating the complexity of detecting such conditions (Su et al., 2012).

In the context of assisted reproduction, "chimerism" can occur when cells or genetic material from more than one individual are present within the same person. This may result from events such as the transfer of multiple embryos during in vitro fertilization (IVF), where one embryo can incorporate cells from another. Such cases of chimerism can complicate the interpretation of genetic tests. For example, it has even led to paternity confusion, raising ethical and procedural questions for assisted reproductive technology (ART) practitioners (Souter et al., 2007).

This should come as no surprise, given that genetic counseling and testing are increasingly used by the scientific and medical communities-for instance, to predict the development of diseases such as cancer, to prepare for organ transplantation, or to establish biological paternity. The issue becomes particularly relevant when the father has undergone a bone marrow transplant, since the procedure replaces some or all of his blood-forming (hematopoietic)

cells with the donor's DNA. As a result, DNA tests performed on blood or saliva may show the donor's genetic profile rather than the individual's own, leading to misleading or conflicting results (Pope et al., 2006).

Conclusion

This case represents the first documented instance of chimerism identified in the context of DNA paternity testing in Albania, highlighting the critical importance of obtaining comprehensive medical histories. In this case, it was not possible to analyze alternative biological samples such as blood, sperm, or hair; however, in suspected chimerism cases, it is recommended to analyze multiple types of biological material to increase the reliability of results.

In situations involving bone marrow transplantation, access to DNA from both the donor and the recipient can help clarify mixed DNA profiles observed in the chimeric individual. These findings underscore the broader implications for forensic and clinical practice: as genetic testing becomes increasingly widespread, cases of chimerism may be detected more frequently than previously anticipated. Our findings suggest that clinicians and forensic experts should carefully consider an individual's medical history, including prior transplants, when interpreting DNA evidence to avoid erroneous conclusions, particularly in parentage testing.

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