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Investigating Biological Relationships in Burkina Faso Using DNA Testing

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Abstract

DNA testing for biological relationships is becoming increasingly common in Burkina Faso. STR analysis remains the most reliable technique for resolving disputes or claims in court regarding biological relationships. This study aimed to establish the links of biological relationships between subjects by analyzing 21 STR loci. The participants were 14 individuals referred to CERBA by the Justice in 2022. Oral or blood samples were taken for each subject. DNA was extracted, and the analysis of DNA polymorphism by PowerPlex® 21 Kit (Part No. DC8902) was performed by capillary electrophoresis on the ABI PRISM 3130 sequencer. DNA profiles were compiled using the GeneMapper IDX software version 1.2. Of the fourteen subjects examined, twelve of these samples had complete genetic profiles, while the other two had partial and absent profiles. The results confirmed the inclusion of three cases of Paternity, one case of maternity, one case of a relationship of brotherhood, and the exclusion of one case of maternity and one case of a relationship of brotherhood. DNA tests improve the resolution of filiations, but they require ethical and cultural awareness and a strengthened legal framework to prevent and protect society.

Keywords

DNA, STR, Identification, Genetic Fingerprint, Burkina Faso

1. Introduction

Genetic testing is a technique used to establish a medical diagnosis. They also find their place in criminal investigations. The term "genetic fingerprint" or "DNA fingerprinting" describes a technique that allows for the identification of individuals with certainty by DNA analysis, unlike conventional techniques. By definition, DNA profiling is a filiation test on the maternal or paternal line using autosomal, mitochondrial, or chromosomal DNA markers [1]-[3]. It was first described by Jeffreys et al., who discovered that certain regions of DNA contained repeated DNA sequences. These repeated DNA sequences exist in all types of sizes and are collectively referred to as variable number of tandem repeats (VNTR). They are usually designated by the length of the central repeater unit and the number of repeater units [4]. DNA regions with repeated units of 2 to 6 bp in length are called microsatellites or short tandem repetitions (STR). The number of repetitions in STR markers varies greatly from individual to individual, making these markers effective for probing filiations [5] [6]. The small size of STR alleles compared to the minisatellites used by Jeffreys et al. makes STR markers better candidates for use in forensic applications [7]-[9]. Furthermore, PCR makes it possible to characterize a particular microsatellite easily. The band profiles thus obtained are unique for each human being, except for true twin boys [9]. Thus, the probability that two people have the same genetic fingerprint is, depending on the region of the genetic heritage observed, 1 per 100,000 to 1 per 1,000,000 [10]-[12]. The genetic fingerprint is also used to verify paternity links. The comparison of the mother's DNA with that of the child and that of the presumed father allows us to clarify the paternity relationship in a certain way. It is now considered that one child in thirty (2% to 5% of children) is not fathered by the man considered as his father, but considerable differences are observed between populations [13]. Moreover, the people concerned are often in difficult psychological situations. They often require specialized support and legal mediation as appropriate. All this shows the importance and necessity of carrying out tests of filiations. In this context, we have found it necessary to contribute to establishing biological kinship links through reliable methods in Burkina Faso. This work investigated biological relationships by analyzing loci STRs in Burkina Faso.

2. Material and Methods

2.1. Study Population

This descriptive study occurred in Ouagadougou (Burkina Faso) at the Pietro Annigoni Biomolecular Research Center (CERBA). The study participants were 14 individuals referred to CERBA in 2022 by the Justice. They were divided into files according to the nature of the DNA test request: Case 1 was the paternity link search involving 06 individuals, file 2 was the maternity with 04 individuals, and the third file was the fraternity with 04 individuals.

2.2. Samples Collection

The biological sampling techniques used were oral swabs and FTA filter paper

blood sampling. Oral samples (07) were taken using double sterile swabs and coded for molecular analysis in biological parents. For subjects seeking a link of filiation, the paper FTA NUCLEIcardTM copan Flock Technologies SARL was used to make their blood collection [14]. All samples were kept at −20˚C pending molecular biology analysis. Study participants gave their free and informed consent. The samples were also taken with the authorization of the judicial authorities according to the regulations in force. The various analyses of the samples taken were carried out in accordance with good laboratory practice and ethical considerations.

2.3. DNA Extraction

The DNA extraction from swabs was performed with the DNA Swap solution extraction kit DC8271. The cottony tips of the stem were cut and placed in an Eppendorf tube of 1.5 mL, and then 1 mL of Swap solution was added. The tubes were incubated at 70˚C for 20 minutes in a heated block, and the extracted DNA was kept at −80˚C for further analysis. DNA extraction from blood samples on the FTA paper was performed with the DNA IQ System Kit (ref: DC6701) according to the protocol provided by the manufacturer

2.4. DNA Amplification

DNA amplification is performed using the PowerPlex® 21 Kit (Part No. DC8902) in a total reaction volume of 12.5 µL. The amplification mixture comprised 2.5 µL master mix, 2.5 µL primer, and 5.5 µL molecular water. Add two µL of extracted DNA to 10.5 µL of PCR mixture for amplification. Amplification was performed using an Applied Biosystems PCR Thermocycler 9700. The amplification program included an initial step of denaturation at 96˚C for 1 min followed by 24 cycles of denaturation at 94˚C for 10 s, hybridization of primers at 59˚C for 1 min, and extension to 72˚C for 30 s, a final step of extension to 60˚C for 20 min would end the program. The amplified products were kept at −20˚C under protection from light until the capillary electrophoresis stage.

2.5. Capillary Electrophoresis of DNA

Capillary electrophoresis was performed using the DNA analyzer 3130 AB prism. The length of the capillaries used was 36 cm with polymer type 4 (POP4). The reaction medium consisted of 9.5 µl Hi Di formamide and 0.5 µl of a size standard (WEN ILS 500). A 96-well plate was used for electrophoresis in the DNA analyzer. Per well, 1 µL of PCR product, allelic marker, or control (Positive/negative) was added to 10 µL of the reaction medium. The ABI 3130 DNA analyzer had four capillaries; in each set, only 3 samples could be simultaneously analyzed with the allelic marker of the fourth wall. A denaturation step of the DNA was performed just before the beginning of capillary electrophoresis. The electrophoresis conditions were 5 seconds for injection time and 15 volts for voltage. The electrophoresis sequence files were collected using Data Collection v3.0. Software in the format “fsa”.

2.6. Data Analysis

The sequence files "fsa." generated are processed with GeneMappers IDX software version 1.2 to determine the marker alleles in each sample analyzed. A total of 20 markers were analyzed per subject, and two alleles per marker were read. Indeed, on the two alleles of subjects in search of kinship links, we identify the mandatory paternal/maternal/allele. In the absence of this allele, the genetic profile of this sample is excluded from identification because the index of paternity/maternity is zero. A combined probability must be estimated for each region examined if this allele is present. No regional allele frequency database in West Africa accurately represents Burkina Faso. The African population, including that of Burkina Faso, exhibits significant genetic diversity due to a complex evolutionary history and migration patterns. Research has demonstrated that utilizing Afro-American allele frequencies is as close as possible to the African population and can aid in establishing genetic relationships and variations within the Burkinabe population [15]. Based on the Bayesian probability law, we used allelic frequencies of markers in the African-American population to determine combined indices of motherhood, Paternity, and combined brotherhood relationship [16]-[18]. The establishment of biological relationships was done on the basis of the formulas described by Eisenberg in 2003.

3. Results

3.1. DNA Profile Relative to Paternity Test Samples

Table 1 and Table 2 present record 1, which consisted of the genetic profiles of individuals (OZ1, OZ2, and OZ3) looking for potential paternity affiliations with individuals (OY1, OY2, and OY3). Out of the six samples analyzed, six complete profiles were obtained. For these biological samples, the study population consisted of 03 samples of supposed fathers and 03 samples of subjects seeking paternity links.

Table 1. Genetic profiles of alleged parents for paternity relationship research.

Duo1	Requestor		Alleged parent		Duo2	Requestor		Alleged parent	
Type of relation	OZ1		OY1		Type of relation	OZ2		OY2	
	Alleles obtained					Alleles obtained			
STR Locus	Allele 1	Allele 2	Allele 1	Allele 2	STR Locus	Allele 1	Allele 2	Allele 1	Allele 2
AMEL	X	X	X	Y	AMEL	X	X	X	Y
CSF1PO	10	10	10	12	CSF1PO	10	12	10	12
D12S391	15	16	15	17	D12S391	20	20	19	20
D13S317	11	11	11	11	D13S317	12	12	12	12
D16S539	11	13	11	11	D16S539	11	13	11	11

Continued

D18S51	16	20	15	20	D18S51	12	18	18	19
D19S433	14	14	14	20	D19S433	12	12.2	12	13
D1S1656	14	19.3	14	14	D1S1656	14	15	14	16
D21S11	27	32.5	27	28	D21S11	32.2	36	28	36
D2S1338	21	25	16	21	D2S1338	24	24	21	24
D3S1358	16	18	16	16	D3S1358	15	16	15	16
D5S818	12	13	12	12	D5S818	12	12	12	13
D6S1043	12	18	12	20	D6S1043	15	16	12	16
D7S820	8	11	8	11	D7S820	9	10	10	10
D8S1179	15	16	13	16	D8S1179	15	15	13	15
FGA	22	23	22	26	FGA	19	22	15	22
Penta D	2.2	8	2.2	5	Penta D	8	11	9	11
Penta E	9	16	8	9	Penta E	12	15	15	17
TH01	6	7	6	7	TH01	7	9	7	9
TPOX	8	9	8	10	TPOX	8	12	11	12
vWA	16	16	16	18	vWA	17	17	15	17

Legend: Duo1 (comparison of profiles of the presumed father OY1 and OZ1) and Duo2 (comparison of profiles of the presumed father OY2 and OZ2).

Table 2. Genetic profiles of biological parents (paternity Duo 3).

Duo3	Requestor		Alleged parent	
Type of relation	OZ3		OY3	
STR Locus	Allele 1	Allele 2	Allele 1	Allele 2
AMEL	X	Y	X	Y
CSF1PO	10	11	10	11
D12S391	19	20	17	19
D13S317	11	11	11	12
D16S539	12	13	11	13
D18S51	17	18	15	17
D19S433	13	13	13	15.2
D1S1656	14	14	12	14
D21S11	28	32.2	29	32.2
D2S1338	18	19	19	21

Continued

D3S1358	15	15	15	17
D5S818	10	11	11	14
D6S1043	12	13	12	20
D7S820	8	8	8	10
D8S1179	13	14	12	14
FGA	19	21	21	27
Penta D	2.2	10	10	12
Penta E	8	8	5	8
TH01	7	7	7	7
TPOX	6	9	9	11
vWA	17	18	18	

3.2. DNA Profile of Maternity Test Samples

Case 2 concerned four subjects seeking a maternity link. Of the 04 samples analyzed, two complete genetic profiles were obtained, and the rest yielded partial and null genetic profiles. (Table 3)

Table 3. DNA profile of maternity test samples (Duo 4 and 5).

Duo6	Requestor		Alleged parent		Duo7	Requestor		Alleged parent	
Type of relation	OZ6		OY6		Type of relation	OZ7		OY7	
STR Locus	Allele 1	Allele 2	Allele 1	Allele 2	STR Locus	Allele 1	Allele 2	Allele 1	Allele 2
AMEL	X	X	X	X	AMEL	X	Y	X	X
CSF1PO	10	10	-	-	CSF1PO	7	10	10	10
D12S391	17	17	-	-	D12S391	18	18	18	18
D13S317	11	11	-	-	D13S317	11	12	11	12
D16S539	9	9	-	-	D16S539	11	12	9	12
D18S51	16	19	-	-	D18S51	16	19	14	19
D19S433	12	13	-	-	D19S433	15	16	13	16
D1S1656	14	17	-	-	D1S1656	16.3	19.3	16	19.3
D21S11	29	30	-	-	D21S11	28	30	27	30
D2S1338	22	23	-	-	D2S1338	17	19	17	20
D3S1358	14	16	-	-	D3S1358	14	16	16	16
D5S818	12	13	-	-	D5S818	12	13	12	12
D6S1043	12	19	-	-	D6S1043	13	13	13	12

Continued

D7S820	11	12	-	-	D7S820	8	12	8	11
D8S1179	13	14	-	-	D8S1179	13	16	13	15
FGA	22	25	-	-	FGA	24	24	24	15
Penta D	2.2	7	-	-	Penta D	8	8	8	10
Penta E	5	8	-	-	Penta E	7	11	11	11
TH01	9	9	-	-	TH01	6	7	5	7
TPOX	9	10	-	-	TPOX	8	11	11	11
vWA	16	16	-	-	vWA	15	16	15	17

3.3. DNA Profile of the Brotherhood Test Samples

Case 3 also concerned four subjects seeking a bond of brotherhood. Two of the four samples tested resulted in a complete DNA profile, and the remaining two samples showed a partial and null genetic profile (Table 4).

Table 4. DNA profile of the brotherhood test samples.

Duo4	Requestor		Alleged parent		Duo5	Requestor		Alleged parent	
Type of relation	OZ4		OY4		Type of relation	OZ5		OY5	
STR Locus	Allele 1	Allele 2	Allele 1	Allele 2	STR Locus	Allele 1	Allele 2	Allele 1	Allele 2
AMEL	X	Y	X	Y	AMEL	X	Y	X	X
CSF1PO	8	8	8	9	CSF1PO	12	13	12	13
D12S391	18	18	18	20	D12S391	18	18	-	-
D13S317	11	12	12	12	D13S317	11	12	11	12
D16S539	8.3	9	9	12	D16S539	12	14	12	14
D18S51	17	21	17	15	D18S51	14	17	14	17
D19S433	13	14	13	14	D19S433	12	13	OL	OL
D1S1656	13	15	15	15	D1S1656	10	16	-	-
D21S11	28	29	27	29	D21S11	28	30	-	-
D2S1338	17	23	17	23	D2S1338	23	23	-	-
D3S1358	15	17	15	16	D3S1358	15	16	-	-
D5S818	8	12	9	12	D5S818	11	12	-	-
D6S1043	15	20	12	19	D6S1043	17	20	-	-
D7S820	8	9	9	10	D7S820	11	11	-	-
D8S1179	14	14	13	14	D8S1179	15	15	-	-
FGA	22	23	17.2	22	FGA	22	25	-	-

Continued

Penta D	8	8	6	7	Penta D	10	10	-	-
Penta E	5	15	5	12	Penta E	8	16	-	-
TH01	5	6	5	8	TH01	5	5	-	-
TPOX	9	11	8	9	TPOX	9	11	-	-
vWA	15	16	15	16	vWA	19	19	-	-

3.4. Analysis of Genetic Profiles

In a paternity or maternity test, the analysis looks for numerical value matches of alleles between the biological parent and the subject looking for kinship. The subject must receive a STR allele from their close relative for the markers studied. The Paternity/Maternity index is a way to measure the strength of a particular match based in part on the game's uniqueness. Moreover, it is a draw if there is no match between the two profiles in this marker. The creation of database with the genetic profile of close relatives and those of subjects seeking a kinship link would allow us to identify its mandatory alleles in order to be able to perform paternity or maternity index calculations.

Inclusion or Exclusion

Analysis of the 21 STRs established the DNA profile for each sample. These results, by comparing the alleles of biological parents with those subjects seeking kinship and by calculating the paternity index and the fraternity coefficient, revealed inclusion cases. Of the 07 pairs, 03 paternity cases (OZ1 and OY1 Pair, OZ2 and OY2, OZ3, and OY3), 01 maternity test case (OZ7 and OY7), and 01 fraternity test (OZ4 and OY4) were included for Paternity. The paternity/maternity/fraternity index was 99.99% for inclusion cases (Table 5).

Table 5. Result of the combined Paternity/maternity/fraternity and probability index.

Nature of the link	Index combines Paternity or Index combines Maternity or Brotherhood Coefficient	Index combines Paternity% or Index combines Maternity% or Brotherhood Coefficient	Conclusion
Paternity test (0Z1 and 0Y1)	24423.00897	99.9959056681	INCLUSION
Paternity test (0Z2 and Y02)	6161104.81	99.99998376915	INCLUSION
Paternity test (0Z3 and 0Y3)	1034980930	99.999199307	INCLUSION
Maternity test (0Z7 and 0Y7)	1987577649	99.99999994969	INCLUSION
Brotherhood Test (0Z4 and 0Y4)	336895000000	99.9999681	INCLUSION

4. Discussion

The study aimed to establish the biological relationship by analyzing STR in Burkina Faso. Genetic profiles are used to differentiate individuals or link them

through polymorphism [19]-[22]. Subjects sharing biological links together share a number of polymorphisms received independently according to the Mendel laws [19]-[22]. This variability is explained by the fact that about 0.3% of DNA is heterogeneous and contributes to the characterization and distinction of each individual [7] [14] [23]. Indeed, the analysis of the 20 STR loci of the subjects in Case 1 presented a complete profile. In contrast, the samples of subjects seeking a link of descent and the complete genetic profile obtained were five (05) profiles out of seven (07) samples. Both (02) samples had partial and null DNA profiles. This partial or zero profile situation is observed in the work of Lello et al. (2021), who worked on allelic frequencies for 20 microsatellite autosomal loci in the Kenyan population [24]. The partial or null profile would be due to low DNA concentration or total absence of DNA [25]-[27]. In our results, we observed the presence of "off ladder (OL)" or off-scale alleles that are not recognized by the allelic marker we used. The existence of these OLs could be due to a known variant or micro variant that does not fit with the allelic scale used; these OLs would provide important information on the specific genetic diversity and variability of the population [3] [28] [29]. Internal evaluation of analytical methods could be a key solution for addressing partial or null DNA profiles. Multiple authors have documented specific instances of "off-ladder (OL)" alleles at various STR loci, including D12S391, PentaE, FGA, and D19S433, using the KIT PowerPlex Fusion and GlobalFiler systems. This phenomenon affects both short and long alleles [30]. Regarding the calculation of filiation indices, a genetic database is necessary for determining the filiation link through tests that may include or exclude their profile in a paternity and/or maternity fraternity relationship. Currently, Burkina Faso does not have a legal framework and a formal database of genetic data with the frequencies of alleles of the markers studied. Only the judiciary has the authority to issue requisitions or orders for DNA profiling, as there are currently no regulations governing forensic DNA databases. To address this issue, it is essential to establish a legislative and legal framework for this area. Additionally, determining the allelic frequencies of the Burkinabe population would be beneficial. This data could serve not only as a genetic database for neighboring countries but also as a reference for those countries when establishing their own databases. In Burkina Faso, the CERBA laboratory has an HID-certified SeqStudio and NGS to address these challenges. Indeed, for the purposes of the study, we propose that a database project based on the Excel table of markers studied could be implemented. The proposed database could refer to recommendations made by Interpol on the development of the legislative framework governing the operation and management of the genetic database [23] [30] [31]. This is the case in Canada, where the DNA Identification Act, enacted on June 30, 2000, creates a national data bank [11]. Similarly, in the USA, the "DNA Identification Act" of 1994 authorized the creation of a DNA profile data bank (system CODIS). This database was used to search for paternal and maternal mandatory alleles in order to determine the indices of paternity/maternity [32]. This calculation of filiation determination is done using

the 20 loci, and the inclusion probability is around 99.99% with a probability of error in the order of 1 in one hundred million [19] [32]. In addition, based on the Bayesian probability law, we used the allelic frequencies of the markers of the African-American population that would be closest to our population. The results of our work have resulted in a combined index of Paternity, maternity, and a combined relationship of brotherhood of 99.99%. This gives us an inclusion of 3 cases of Paternity, a case of maternity, and a case of brotherhood relationship, and an exclusion of a case of maternity and a case of brotherhood relationship.

5. Conclusion

The application of DNA tests to investigate kinship links in Burkina Faso represents a significant step forward in resolving filiation and family disputes. This innovative technology, which enables accurate and reliable verification of biological relationships, plays a crucial role in various legal and social contexts. However, while genetics offer promising prospects for identifying biological links, its application must be carefully framed.

Authors' Contribution

BBVEJT, ZAA, and SM developed the study protocol and initiated the manuscript plan in collaboration with all co-authors under the Scientific Direction of JS. BBVEJT and OM coordinated the manuscript writing activities. SS, BP, KI, and SA contributed to the molecular analyses. SST, DF, TL, SM, ZL, ZTM and YA contributed to the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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