

Contribution of ABO-Rhesus/Electrophoresis of hemoglobin methods and Short Tandem Repeats analysis in the determination of paternity in Burkina Faso, West Africa

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Abstract

Background: the establishment of filiation by the current ABO, HLA, MNS, Kells and serum tests, pose a real reliability problem. It is then necessary to combine these methods with or to use high-performance methods such as microsatellite genetic analysis or short tandem repeats. This study aimed to compare the short tandem repeat technique with ABO/Rhesus system in combination with electrophoresis of hemoglobin.

Methods: Fourteen (14) contested paternity trios were investigated. Blood samples were collected to determine blood groups using the Beth-Vincent method and the type of hemoglobin by electrophoresis. Blood spots on FTA paper were used for the analysis of 16 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, Amel) by capillary electrophoresis on the ABI 31310 Genetic Analyzer. The generated sequences were analyzed with GeneMapper® software version 3.2.1. The data were analyzed to determine the paternity index and the probability of paternity.

Results: Of the fourteen (14) trios studied, ten (10) cases were probable inclusion, three (03) cases were exclusion and one (01) case was an undetermined paternity outcome with the ABO-Rhesus/ electrophoresis of hemoglobin system. While the analysis of genetic polymorphisms in DNA gave five (05) inclusions versus nine (09) exclusions of paternity. Of the 10 probable inclusion cases given by the ABO-Rhesus/Electrophoresis of hemoglobin system, only 05 cases (50%) were confirmed for inclusion by Short tandem repeat analysis.

Conclusion: The analysis of short tandem repeat with sixteen genetic markers is more reliable in determining paternity than ABO-Rhesus/hemoglobin electrophoresis techniques.

1. Background

The biological determination of filiations is an old problem. The study is based on the genetic polymorphisms of individuals and the Mendelian transmission of these polymorphisms [1, 2]. Referring to techniques based on blood group determination, Mosinger and Rochette stated that it is never possible to demonstrate the certain filiation between a child and an alleged father, but that it can be demonstrated that an alleged father having known blood group cannot have produced a child whose blood group is also known [3]. In other words, in some cases, the exclusion of paternity may occur. Conversely, the difficulties of using these methods to include paternity of "alleged fathers" are related to random transmission of alleles in the general population. The high frequency of phenotype of these alleles carries the risk of randomly finding a person with a phenotype compatible with paternity. The late maturation of antigens is a barrier to the determination of filiation through the use of the ABO system. Thus, the establishment of parentage, by the current tests such as ABO, HLA, MNS, Kell, is a real problem, with low exclusion probabilities in the order of 0.17 [4]. Taking these limitations into account, in the context of paternity research, it is necessary to combine several systems [ABO, rhesus, HLA, MNS, Kell, serum

systems...] or to use other more efficient systems such as microsatellite genetic analysis, or "short tandem repeats" (STR) [5, 6].

STR is a polymorphic locus present in all eukaryotic genomes. They are generally composed of tandem matrices of short repeated sequences of 2 to 6 base pairs and polymorphism occurs when the number of copies of the repeated sequence present at a given STR locus varies between individual chromosomes [7–9]. Hundreds of microsatellites have been studied and some are used as markers for the determination of genetic fingerprints to discriminate or genetically link individuals (families, immigrants, etc.) [10]. They have a wide diversity and can be used in the identification of paternity testing cases [6, 11]. The application of STRs to the search for parentage in 877 paternity cases had in the past ruled out 35.2% of cases and found a probability of paternity of 99.9999% [12]. In Africa, the demand for paternity testing is less frequent and samples are sent to Europe or the United States when the need is felt due to a lack of skills and advanced equipment. There are no studies yet in this area from West Africa. In Burkina Faso, a West African country, the demand for paternity tests is increasing. To fill the gap, we conducted a study to compare the effectiveness of STR analysis with that of the ABO/Rhesus system in combination with electrophoresis of hemoglobin used in the determination of paternity on proven cases of a claim made by the court.

2. Methods

2.1. Sample collection

This study involved 14 trios (mother-child-alleged father) of paternity test applications, i.e. 42 samples received at the Biomolecular Research Centre Pietro Annigoni (CERBA) in Ouagadougou at the request of the Tribunal de Grande Instance de Ouagadougou on cases of contested paternity. The persons concerned were contacted and blood samples were taken on EDTA tube for blood grouping and electrophoresis of hemoglobin and then on FTA paper (NucleiCard, Brescia, Italy) for the determination of the genetic polymorphism of each individual's DNA.

2.2. Carrying out blood grouping and electrophoresis of hemoglobin

The determination of blood and Rhesus groups was performed using the Beth-Vincent technique with Anti-A, Anti-B, Anti-AB, and Anti-D sera. The determination of the hemoglobin type was made on the HELENA electrophoresis chain (Helena Biosciences Europe, Queensway South, Gateshead Tyne and Wea) according to the manufacturer's instructions. Hemolysate was prepared by mixing 1 volume of whole blood with 3 volumes of Helena hemolysis reagent (0.005 M EDTA and 0.01% potassium cyanide). Electrophoresis was performed at 350 V for 25 minutes in a boric acid/Tris-EDTA buffer (pH 8.4, ionic strength = 0.035).

2.3. Amplification by polymerase chain reaction (PCR)

PCR amplification was performed using 1.2 mm of bloodstained disc obtained by a punch on FTA paper previously soaked in blood and containing 5 to 20 ng of DNA. A multiplex PCR amplification of 16 loci of tandem repeat strap (polymorphic STR loci) was performed using the AmpFISTR® identifier® Direct kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Among the 16 STRs, the Amelogenin marker was included to allow genetic identification of the sex of each individual. The characteristics of the 16 STRs are shown in Table 1. The PCR was performed in 25 µL of reaction volume containing 5–20 ng DNA, 12.5 µL primers and 12.5 µL Master Mix on the Gene Amp PCR System 9700 thermocycler (Applied Biosystems, USA) according to the following program: an initial denaturation at 94 °C for 11 minutes, 28 cycles of 94 °C for 20 seconds, 59 °C for 3 minutes and 72 °C for 1 minute, and a final extension at 60 °C for 25 minutes.

Table 1
16 STR loci and alleles with their characteristics

Locus	Location on the chromosome	Included alleles	Fluorochrome
D8S1179	8	8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	6-FAM
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38	
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	VIC
D3S1358	3p	12, 13, 14, 15, 16, 17, 18, 19	
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	
D13S317	13q22-31	8, 9, 10, 11, 12, 13, 14, 15	
D16S539	16q24-qter	5, 8, 9, 10, 11, 12, 13, 14, 15	
D2S1338	2q35-37.1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	
D19S433	19q12-13.1	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	
vWA	12p12-pter	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	
TPOX	2p23-2per	6, 7, 8, 9, 10, 11, 12, 13	
D18S51	18q21.3	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27	
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y	PET
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16	PET
FGA	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	

2.4. Capillary electrophoresis

The amplification fragments obtained were then analyzed on the ABI 3130 Genetic Analyzer (Applied Biosystem, USA) on a 96-well plate containing 1 µL of PCR product, 8.7 µL of Hi-Di Formamide and 0.3 µL of GeneScan 500 LIZ Size Standard followed by denaturation at 95 °C for 3 min and immediate cooling on ice for 3 minutes. The electrophoresis was performed with Performance-Optimized Polymer 4 (POP4) with a capillary of 36 cm. After electrophoresis, GeneMapper® ID version v3.2.1 software was used to

assemble the obtained sequences and compare them to the allele scale to determine the allele types present in each analyzed sample.

2.5. Statistical analyses

The paternity index (PI), which measures the weight of scientific evidence obtained from the paternity test, was calculated for each STR locus using the method described by Eisenberg, 2003. Then the combined paternity index (CPI) was estimated by multiplying the individual paternity index with the others. The probability of paternity (POP), a conditional probability of knowing whether an alleged father is the biological father of a child, was calculated using the following equation: $CPI \times 0.5 / [CPI \times 0.5 + (1 - 0.5)]$, the CPI being the combined paternity index and 0.5 is the prior probability [13].

3. Results

3.1. Inclusion and exclusion by the ABO/Rhesus system and electrophoresis of hemoglobin

Of the 14 trios, the ABO/Rhesus system showed only one case of exclusion while electrophoresis of hemoglobin reported two cases of exclusion. The trio affected by the exclusion revealed by the ABO/rhesus system is different from the other two found by electrophoresis of hemoglobin. Two cases were considered inconclusive because of fetal hemoglobin (Hb F) immaturity in children (Table 2).

Table 2

Inclusion and exclusion results according to the ABO-Rhesus/Electrophoresis of hemoglobin

Trio	ABO/rhesus (Electrophoresis of hemoglobin)			Inclusion and exclusion			Conclusion
	Mother	Child	Alleged father	ABO	Rhesus	Electrophoresis of hemoglobin	
1	B+ (AC)	O+ (AA)	A+ (AA)	Inclusion	Inclusion	Inclusion	Probable inclusion
2	A+ (AA)	AB+ (AA)	B+ (AA)	Inclusion	Inclusion	Inclusion	Probable inclusion
3	B+ (AC)	B+ (AC)	B- (AA)	Inclusion	Inclusion	Inclusion	Probable inclusion
4	O+ (AA)	B+ (AF)	A+ (AA)	Exclusion	Inclusion	unconclusive	Exclusion
5	O+ (AA)	B+ (AF)	B+ (AS)	Inclusion	Inclusion	unconclusive	unconclusive
6	B+ (AC)	B+ (AA)	B+ (AS)	Inclusion	Inclusion	Inclusion	Probable inclusion
7	B+ (AC)	B+ (AA)	B+ (AA)	Inclusion	Inclusion	Inclusion	Probable inclusion
8	O+ (AA)	B+ (AA)	AB+ (AA)	Inclusion	Inclusion	Inclusion	Probable inclusion
9	O+ (AA)	B+ (AC)	AB+ (AA)	Inclusion	Inclusion	Exclusion	Exclusion
10	O+ (AA)	A+ (AC)	AB+ (AA)	Inclusion	Inclusion	Exclusion	Exclusion
11	B+ (AC)	AB+ (AA)	A+ (AS)	Inclusion	Inclusion	Inclusion	Probable inclusion
12	A+ (AA)	O+ (AA)	A+ (AS)	Inclusion	Inclusion	Inclusion	Probable inclusion
13	A+ (AA)	A+ (AA)	AB+ (AC)	Inclusion	Inclusion	Inclusion	Probable inclusion
14	A+ (AA)	A+ (AA)	A+ (AA)	Inclusion	Inclusion	Inclusion	Probable inclusion

3.2. Inclusion and exclusion according to the STR analysis

The analysis of the 16 STRs identified the DNA profile of each trio (mother-child-alleged father). These results reveal cases of inclusion and exclusion by comparing the child's alleles with those of both parents

and by calculating the PI and POP. Of the 14 trios, 5 alleged fathers (trios 1, 3, 7, 8 and 13) were included in paternity while 9 (trios 2, 4, 5, 6, 9, 10, 11, 12 and 14) were excluded from paternity. The paternity index ranged from 0 to 37,072,170,900 and the highest POP was 99.9999999997% found in trio 3 (Table 3). Figures 1 and 2 show examples of inclusion and exclusion of paternity.

Table 3
Results of the combined paternity index (CPI) and the probability of paternity (POP) in trio cases

N°	Cases	IPC	POP	Conclusion of paternity
1	trio	3 263 198 110	0,99999999968	Inclusion
2	trio	0	0,00	Exclusion
3	trio	37 072 170 900	0,99999999997	Inclusion
4	trio	0	0,00	Exclusion
5	trio	0	0,00	Exclusion
6	trio	0	0,00	Exclusion
7	trio	196 349 727	0,99999999490	Inclusion
8	trio	12 695 452 599	0,99999999992	Inclusion
9	trio	0	0,00	Exclusion
10	trio	0	0,00	Exclusion
11	trio	0	0,00	Exclusion
12	trio	0	0,00	Exclusion
13	trio	2 526 793	0,999999996	Inclusion
14	trio	0	0,00	Exclusion

3.3. Evaluation of the number of inclusion and exclusion of paternity according to the test used

Comparison of the results of the two methods used shows that 5 alleged fathers (35.71%) were included of paternity with the analysis of STR as opposed to 10 inclusions (76.92%) of paternity found with the ABO-rhesus/Electrophoresis of hemoglobin method. But all 5 inclusions reported by analysis of genetic polymorphism of DNA were also found by the ABO-rhesus/Electrophoresis of hemoglobin method.

Table 4
Inclusion and exclusion with the ABO-Rhesus/Electrophoresis of hemoglobin and analysis of STR.

Paternity	ABO-Rhesus/ Electrophoresis of hemoglobin	Analysis of STR
Inclusion	10 (76,92%)	5 (35,71%)
Exclusion	3 (13,04%)	9 (64,29%)

4. Discussion

The technique of paternity determination based on blood grouping, the rhesus factor combining electrophoresis of hemoglobin, has identified some limitations related to the profile of hemoglobin in young infants in trios 4 and 5. This may be explained by the fact that there is still a significant proportion of fetal hemoglobin (Hb F) in these infants due to their very young age [14]. Taking into account the blood grouping of parents and children, a match discrepancy was observed (trio4). Electrophoresis of hemoglobin also showed match discrepancies in trios 9 and 10. These match discrepancies would make it difficult for the ABO-rhesus and electrophoresis of hemoglobin association to determine paternity. In general, the high frequencies of the alleles of the ABO system would make it difficult to include the alleged father in a paternity case; but could rather exclude him if, however, given his blood type, he did not present the possibility of being the father. From the above, the ABO-Rhesus technique associated with electrophoresis of hemoglobin used to determine paternity has limitations. The analysis of STRs consisted of determining the genetic markers of each trio to compare the alleles of the alleged fathers with those of the children. The Identifiler Direct Kit made it possible to compare 15 alleles between the individuals in each trio. The genetic analysis of the fourteen paternity search trios comprising mother, child, and alleged father revealed 64.29% of cases of exclusion compared to 13.04% with ABO/Rhesus associated with electrophoresis of hemoglobin. This trend is consistent with the studies conducted by Souiden *et al.*, 2007, [14]. The determination of STRs would correct inclusion and exclusion errors induced by the ABO/Rhesus technique associated with electrophoresis of hemoglobin. The ABO/rhesus system is easily suited to the search for the exclusion of paternity. For example, in trio 4, the mother has group O+, the child has group B + and the alleged father has group A+. In this case, paternity is excluded with certainty and without recourse to other systems to confirm the result. Similarly, the rhesus system alone may reveal an exclusion, as is the case for example for an O- mother, O + child and O- father [14]. But in some particular cases, such as mother O+, child A1, father O + and if the ABO system is the only exclusion system, paternity is excluded only if it can be shown that one of the parents does not have the Bombay phenotype [15]. On the other hand, the immaturity of antigens in all newborns should be considered; for example, in the following example: mother O, child A2, father A1B, the child's blood type should be checked a few months later before reporting an exclusion, as this may be a delay in the development of the A1 antigen. For example, in the case of trios 4 and 5, the determination of paternity by electrophoresis by hemoglobin is uncertain because children have fetal hemoglobin. Taking all these

limitations into account, in the context of a paternity search, it is necessary to combine several systems (ABO, rhesus, HLA, MNS, Kell, serum systems...) [4, 16] or to use other systems such as STR genetic analysis [5]. In this study, STRs solved all the cases studied, as it is based on DNA polymorphism analysis for the identification of an individual. Based on the Bayesian probability law, we determined the PI and the CPI. These PIs make it possible to determine the probability of paternity of an alleged father "to be" or "not to be" the biological father of a child. The results of this study showed a PCI of more than 100 million, unlike a study conducted in Egypt which found CPI of more than 1 million [17]. The CPI can be high depending on whether the PI calculated from the allele frequencies is high or low. In the ABO system, three alleles are possible and therefore six possible genotypes are present in the human population. In contrast, STR multiplex markers produce a greater number of possible genotypes, as a large number of alleles are present for each STR locus. Thus, although the ABO-Rhesus/Electrophoresis system of Hb is useful for excluding a person from paternity, this technique cannot be used to declare a truth inclusion of paternity. The conclusion of paternity from this technique is "Exclusion" or "probable inclusion". The term "probable inclusion" means that there is a possibility of inclusion of paternity but this situation needs reliable techniques to confirm. In paternity tests, the results of the probability of filiation would be either 0% to exclude someone in situations of paternity, siblings, etc. as the biological parent of a child or the same filiation or at least 99% to confirm someone as the biological parent. Legally, a 99% or greater probability of a biological relationship is considered proof of paternity [17, 18]. The inclusion of paternity comes from the fact that one of the child's alleles is identical to one of the alleles of the alleged father for all the markers studied. While the exclusion is explained by the fact that the child did not receive any allele from the alleged father for one or more STR markers. In this study, genetic amplification of STRs excluded 9 presumed paternity fathers and identified 5 others as the biological fathers of the children. The biological father is the one who transmitted his genes to his child and this can be verified by DNA tests [19]. STR markers provide sufficient discriminatory power to exclude or include an alleged father in contested paternity cases. Indeed, PCR amplification based on STRs remains the preferred method for conducting paternity tests [20, 21]. In this study, the allele frequencies of the African American population provided in the kit's user manual were used to calculate the paternity index because there are no great genetics variations between this population and West Africans [22]. Nevertheless, it would be more appropriate to conduct a study to determine the allelic frequencies of the 16 STRs specific to the population of Burkina Faso.

5. Conclusion

The ABO/Rhesus system associated with electrophoresis of hemoglobin is not very effective in the search for paternity and filiation, but its interest lies in the fact that it allows a certain exclusion. This classic analysis can only give a simple approach to the result. Besides, the analysis of STRs has increased the performance of the results. This latter method is more reliable and is the reference method for paternity tests and even forensics in criminology (rape, murder, etc.) because it compares the genetic fingerprints of two individuals.

Abbreviations

CPI

combined paternity index; DNA:deoxyribonucleic; HbF:foetal hemoglobin; PCR:polymerase chain reaction; PI:paternity index; POP:probability of paternity; POP4:Performance-Optimized Polymer 4; STR:short tandem repeat

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. The court and institutional ethics committee of CERBA/LABIOGENE gave their approbation to carry out the study. All parents gave their written consent before sample collecting. Moreover, anonymous and confidentiality of data were respected.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MM and STS have done data analysis and interpretation and drafted the article, BVJTB and TMZ participated in data collection and interpretation and revised for intellectual content,

ATY participated in the revision of the manuscript, JS designed the study. All authors have read and approved the manuscript.

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Figures

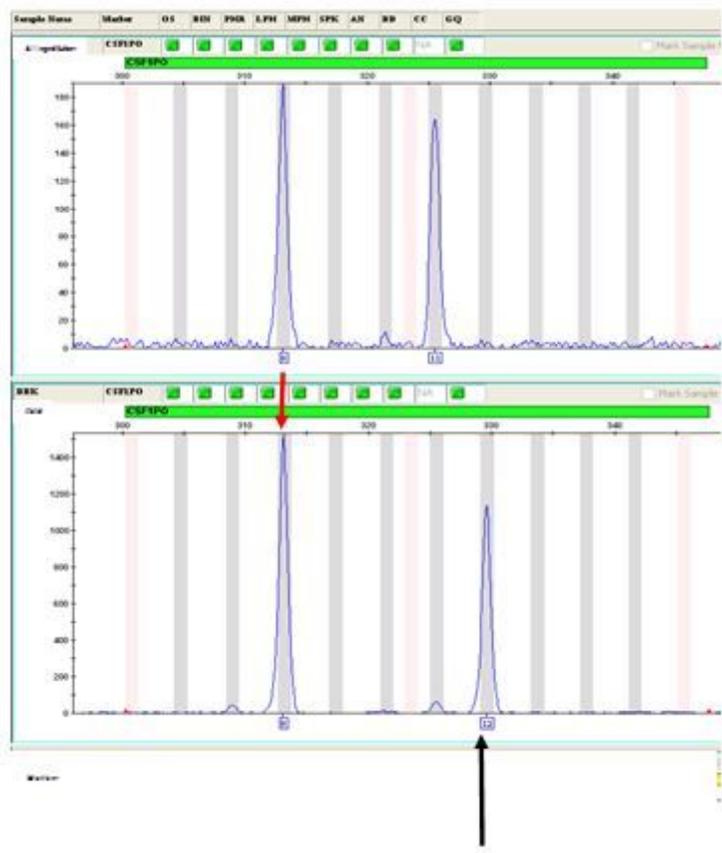


Figure 1

Inclusion of paternity for the trio 13: example of allele correspondence (allele 8) between alleged father and child for the locus CSF1PO

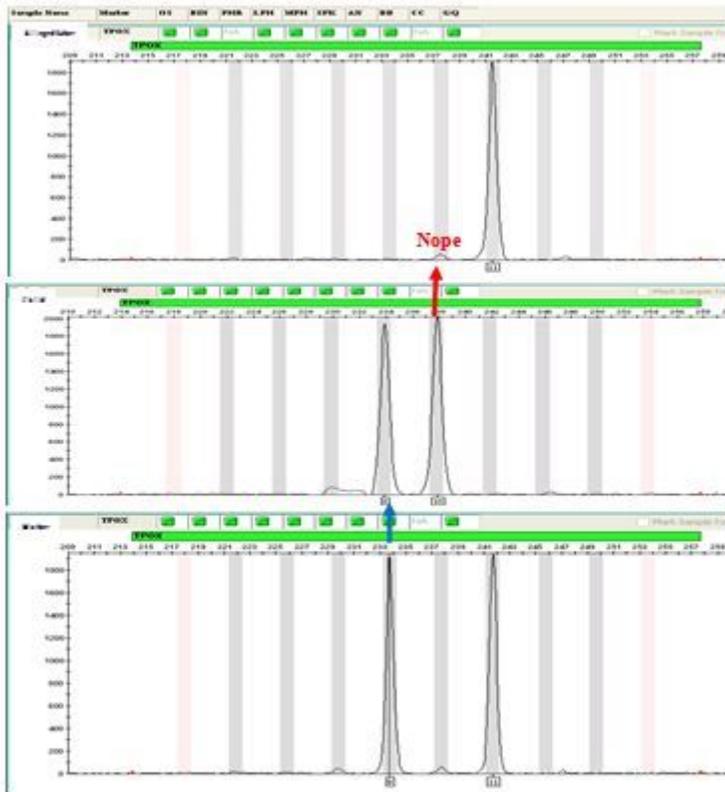


Figure 2

Exclusion of paternity for the trio 2: example of allele no correspondence between alleged father and child for the locus TPOX