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ORIGINAL ARTICLE

Discrimination Power Assessment of STR Genotyping in Parentage Investigation

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KEY WORDS: *paternity testing; forensic DNA typing; autosomal short tandem repeats (aSTRs); human leukocyte antigens (HLA)*

Abbreviations
AF(s) = alleged father(s)
aSTR = autosomal short tandem repeats
CPI = combined paternity index
HLA = human leukocyte antigens
MHC = major histocompatibility complex
PCR = polymerase chain reaction
PI = paternity index
SSOP = sequence specific oligonucleotide probes
SSP = sequence specific primers
W = probability of paternity

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ABSTRACT

OBJECTIVE Nowadays, the application of DNA-typing in laboratory medicine is increasing rapidly for paternity/maternity disputes. The goal of this study was to evaluate the use of polymorphic microsatellite marker DNA analysis and to establish this analysis as the method of choice for parentage investigations.

SUBJECTS AND METHODS Among 708 civil parentage tests addressed to our Laboratory previously examined for HLA class I (-A*, -B*, -Cw*), and class II (-DRB1*, -DQB1*, -DPB1*) alleles using PCR-SSOP and/or PCR-SSP methodologies, a cohort of 50 cases (137 individuals) of disputed parentage was selected. In these cases DNA-typing was generated from co-amplification of 15 autosomal STR DNA markers (D3S1358, HUMTH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, Penta D, HUMvWA, D8S1179, HUMTPOX, HUMFGA and the sex determining Amelogenin marker HUMAMEL), using fragment analysis methodology.

RESULTS The evaluation of the results showed that 15 out of 50 cases were sufficient for exclusion of fatherhood by both approaches (HLA and STRs). In all remaining 35 non-excluded cases, the PI value using HLA genotyping ranged from 76 to 6,452,794, whereas using aSTR genotyping ranged from 15,173 to 9.2×10^{10}. In one non-excluded motherless case the alleged father showed one genetic discrepancy with the child at D21S11 locus, due to a mutation event.

CONCLUSION The use of DNA-typing with 15 aSTR loci for parentage testing provides an accurate and high-sensitivity method which is simpler to perform and more rapid than an accepted standard technology, such as HLA genotyping. The analysis of aSTR loci offers a highly discriminating test suitable for trio paternity testing, increasing the W rate in comparison to HLA genotyping. Nevertheless, when a mutation event occurs in motherless cases, combination of HLA and STR polymorphisms offers high level of information, and also diminishes the possibility of false exclusion due to aSTRs mutations.

Conflict of Interest: none declared

INTRODUCTION

The need to establish family relationships arises not only for medical reasons (genetic counselling), but mainly in forensics for human identity testing, disaster victim identifications, as well as in parentage disputes.[1] Nowadays, the progress of the molecular techniques have greatly contributed to evaluate the relatedness between individual kinship analysis and civil parentage assessments, leading to solving the most complex cases, with high discriminating power.[2]

For a long time determination of the major histocompatibility complex in humans (MHC), known as human leukocyte antigen (HLA) region represented the standard procedure in forensic genetics. MHC is known as a cluster of genetic markers and consists of more than 220 genes of diverse function, encoded in a 3500 kbp segment on the short arm of chromosome 6p21.31.[3,4] With the introduction of DNA based methods of HLA typing, extensive molecular polymorphism has been discovered in each of the relevant HLA class I (HLA-A*, HLA-B*, HLA-Cw*) and class II (HLA-DRB1*, HLA-DQB1*, HLA-DPB1*) loci.[5,6] According to the World Health Organization Nomenclature Committee more than 13.700 HLA alleles have been documented in the international ImMunoGeneTics (IMGT/HLA database, http://www.ebi.ac.uk/imgt/hla released 3.22.0, 2015-10-10).[7] MHC loci are located on the short arm of human chromosome 6, and an individual inherits two alleles of each of the above loci, one each from either parent, according to Mendelian fashion. Due to their close proximity, a complete set of alleles of genes mapped in a row to the same chromosome is usually inherited as an haplotype, apart from the recombination events (crossing over).[8] However, in some complex paternity testing or deficiency cases, HLA typing could not provide enough discrimination power due to the linkage disequilibrium phenomenon and the predominance of certain HLA alleles in particular ethnic groups. The last two decades, new molecular markers located in the microsatellite regions have been introduced in the field of human identification in order the above problems to be solved.[9,10]

Microsatellites, also called short tandem repeat (STR) loci, are highly informative markers found in the eukaryotic nuclear genome in both coding and noncoding regions. They are defined as tandem repeats of 2-6 bp units and may be present as perfect or imperfect repeats. The number of repeats found in each individual are highly variable with as few as two, or as many as 50, copies in each microsatellite unit, depending on the locus, making these genetic markers effective for human identification purposes (details of published STR studies http://www.cstl.nist.gov/div831/strbase). During the past decade STR loci became a valuable tool in parentage investigations due to their high polymorphism and heterozygosity.[11,12] Nonetheless, STR loci are highly mutable as compared with point mutations in coding genes and mutation rates range from 10^{-6} to 10^{-2} events per locus per generation. These rates are highly affected by multiple factors such as repeat number, repeat unit, repeat structure, flanking sequence, sex, and age.[13,14] According to the American Association of Blood Banks (AABB) guidelines, for parentage investigations, the criterion of power of exclusion is at least two incompatibilities to report exclusion of an alleged father.[15] However, the last years the use of STR loci has replaced the HLA typing, as the method of choice for parentage testing.

In the present study, 50 cases of disputed parentage (37 trios, and 13 motherless cases), already evaluated by HLA typing, were retrieved from the archived records of the Immunology and Histocompatibility Department of "Evangelismos" General Hospital and investigated by the new technology ("DNA profiling" based on STRs loci polymorphism). The aim was to investigate and compare the usefulness of these two systems (HLA and STRs), and to discuss the advantages and drawbacks of each of these two methodologies in disputed paternity testing.

SUBJECTS AND METHODS

1. SAMPLE COLLECTION AND DNA EXTRACTION

Among 708 civil parentage tests addressed to our Laboratory, a cohort of 50 paternity cases, some in deficiency, was selected, including 37 trios consisted of the mother, one child and one alleged father and 13 duos consisted of one child and one alleged father (motherless cases). The process of recording and archiving of samples, as well as the privacy procedure, were carried out following the guidelines of the human and ethical research principles. Additionally, the informed consent for genetic studies was obtained from all participants. Peripheral blood samples (drawn in sodium citrate 2%) were obtained by venipuncture from 137 individuals, all of Greek origin. Genomic DNA was isolated from peripheral blood leukocytes of all individuals by automated purification procedure, using the Maxwell® 16 instrument (extractor) and the Maxwell® 16 Blood DNA Purification kit (Promega Corporation, Madison Wisconsin) following the user's manual recommendations. The purified DNA was quantitated by ultraviolet absorbance spectrophotometry (NanoPhotometer™ spectrophotometer, Implen GmbH, Germany) at 260 nm. Additionally, DNA purity was estimated by measuring the A260/A280 ratio, which was >1.7.

2. HLA TYPING

HLA class I (HLA-A*, -B*, -Cw*) and class II (HLA-DRB1*, -DQB1*, -DPB1*) low-resolution (two digits) and high-resolution typing (four digits) was performed by well-established techniques: polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSOP) and/or -sequence specific primers (-SSP).[16,17] In particular, for

PCR-SSOP methodology (multiplex bead array assay) commercial kits provided by One Lambda Inc., (Canoga Park, CA, USA), and for PCR-SSP technique commercial kits provided by Life Technologies and Olerup Corp. were used, according to the instructions provided by the manufacturer.[18] PCR was performed using TECHNE TC-412 thermal cycler (Techne Inc. Burlington, USA). HLA data were acquired on a dedicated flow cytometry-based platform (Luminex 100 IS, Inc.) for SSOP methodology, or electrophoretic mobility of targeted sequences on agarose gel 2% for SSP methodology. The majority of HLA Class I alleles can be discriminated by their exon 2 and 3 sequence, and for Class II alleles, exon 2 is generally sufficient. Amplified HLA DNA fragments were analyzed with HLA Fusion 3.0 software (bead array) or SSP UniMatch 6.0, Helmberg-Start Score™ (SSP methodology) for automatic allele determination.

3. ASTRS TYPING

DNA samples were co-amplified at 15 aSTRs DNA markers namely, D3S1358, HUMTH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, Penta D, HUMvWA, D8S1179, HUMTPOX, HUMFGA and the sex determining Amelogenin marker HUMAMEL, using the PowerPlex® 16 multiplex system. The characteristics of the 15 aSTR loci are shown in Table 1. 5-10ng of diluted target DNA were amplified using PowerPlex® HS 5X master mix including hot-start Taq DNA polymerase and PowerPlex® 16 HS 10X primer pair mix. Multiplex PCR reactions were carried out in TECHNE Rrime/elite/02 thermal cycler following the procedure recommended by the manufacturer (Promega, Madison, WI, Technical Manual PowerPlex® 16 HS System).[19] Then, 1μl of fluorescently labeled PCR product was heat denatured in 9μl of Hi-Di™ formamide (Applied Biosystems, Weiterstadt, Germany) containing Internal Lane Standard 600 (Promega Corp,) molecular marker, before electrokinetic injection to the ABI3130 Genetic Analyzer (Applied Biosystems).[20] In parallel, an allelic ladder (PowerPlex® 16 HS AL) for each STR locus containing all the most common alleles present in the Caucasian population was injected in the genetic analyzer. As separation matrix performance optimized polymer (POP-4™ polymer, Applied Biosystems) was used. Raw data were further analyzed with GeneMapper® IDX1.3 software for automatic allele calling/STR profiles (Applied Biosystems, Foster City CA).

4. STATISTICAL ANALYSIS

The HLA and aSTRs results within each trio or duo case were compared and a determination of inclusion or exclusion of the alleged father was made for each HLA and aSTR locus. Several statistical methods have been proposed to perform parentage analyses using data of HLA and aSTR genetic markers.[21,22] In this study for HLA analysis the Essen-Möller value has been applied.[23] For inclusion, in complete trios and motherless cases, the statistical parameters paternity index (PI) and probability of paternity (W %) were calculated based on HLA allele frequency of Greek population database.[24,25] The W value is attributed by the equation $W=PI/1+PI$ for each alleged father (AF), where PI tells how many times more easily the observed results are explained by relationship rather than by coincidence, using a prior probability of 0.5. According to the Essen Möller values obtained, different probability of paternity categories were estimated, as follows: <95%, non-useful; 95.1% to 99.0%, likely; 99.1% to 99.75%, extremely likely; 99.76% to 99.99%, practically proved.

For aSTRs analysis the statistical parameters combined paternity index (CPI) and probability of paternity (W) were calculated based on equations provided by Buckleton et al (2005)[26] in order to express the value of evidence, using STRs allele frequency of the Greece population published by the Laboratory of Forensic Medicine and Toxicology, School of Medicine of Thessaloniki.[27] PI is a statistical measure of how powerfully a match at a particular marker indicates paternity. The CPI value was calculated by multiplication of the PI values for all fifteen loci. The CPI indicates the overall probability of an individual being the biological father of the tested child as related to any random man from the entire population of the same race. Using the combined value of PI, we calculated the W value as follows: $W=CPI\times0.5/[CPI\times0.5+(1-0.5)]=CPI/CPI+1$, for each AF using a prior probability of 0.5.[28] The CPI and W values were interpreted according to the guidelines and recommendations of the International Society for Forensic Genetics (ISFG).[29-31] Additionally, in order to create pedigrees and calculating the W value, the "Familias" program (download free at http://www.nr.no/familias) was used.

RESULTS

Fifty paternity cases, previously examined by HLA typing, were additionally analyzed by aSTR technology. The results of the HLA and the STR analysis from the mother, child, and the AF were examined for inheritance of alleles. If the AF was indeed the biological father, the child would share one allele with the mother and one allele with the father at each tested marker (barring mutations). Incompatibilities in at least one HLA allele between parents and offspring indicated exclusion, while for aSTR DNA typing paternity was excluded in cases where apparently paternally inherited alleles were not present in at least two aSTR loci, due to the high rate of mutations.

In summary, 15 out of 50 cases (30%, 12 trios, and 3 motherless cases) were sufficient for exclusion of fatherhood by both approaches. For HLA analysis, exclusion was confirmed on at least one HLA locus (one trio case), while four exclusions were documented on five HLA loci. Exclusion on all six HLA loci was not found in any trio or duo case. The highest number of exclusions was observed at HLA-A* locus (10 cases) as

TABLE 1. Information on 16 commonly used autosomal STR loci present in PowerPlex® 16 multiplex system commercial kit.

STR Locus	Chromosomal Location	Repeat Sequence 5′→3′	Repeat Numbers of Allelic Ladder Components*	Size Range of Allelic Ladder Components (bp)	Label
Penta E	15q	AAAGA	5-24	379-474	FL#
D18S51	18q21.3	AGAA (22)	8-10, 10.2, 11-13, 13.2, 14-27	290-366	FL
D21S11	21q11-21q21	TCTA Complex (22)	24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38	203-259	FL
TH01	11p15.5	AATG(22)	4-9, 9.3, 10-11, 13.3	156-195	FL
D3S1358	3p	TCTA Complex	12-20	115-147	FL
FGA	4q28	TTTC Complex (22)	16-18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26-30, 31.2, 43.2, 44.2, 45.2, 46.2	322-444	TMR†
TPOX	2p24-2pter	AATG	6-13	262-290	TMR
D8S1179	8q24.13	TCTA Complex (22)	7-18	203-247	TMR
vWA	12p13.31	TCTA Complex (22)	10-22	123-171	TMR
Amelogenin	Xp22.1-22.3 Y	NA	X, Y	106, 112	TMR
Penta D	21q	AAAGA	2.2, 3.2, 5, 7-17	376-449	JOE§
CSF1PO	5q33.3-34	AGAT	6-15	32-357	JOE
D16S539	16q24.1	GATA	5, 8-15	264-304	JOE
D7S820	7q11.21-22	GATA	6-14	215-247	JOE
D13S317	13q22-q31	TATC	7-15	176-208	JOE
D5S818	5q23.3-32	AGAT	7-16	119-155	JOE

*Report published at the U.S. National Institute of Standards and Technology (NIST) web site at: www.cstl.nist.gov/div831/strbase/
† carboxy-tetramethylrhodamine (TMR)
§ 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxy-fluorescein (JOE)
# fluorescein (FL)

opposed to HLA-DPB1* (6 cases). On the other hand, HLA data agreed with the results reported using analysis of aSTR genetic polymorphism both for inclusion and exclusion. More specifically, in all cases, exclusion was confirmed on at least five genetic aSTR loci (one duo case), while evidence for one exclusion was obtained by twelve loci, whereas exclusion based on 15 aSTR loci was not observed in any case. The most powerful excluding locus of the PowerPlex® 16 multiplex system kit was D21S11 and D18S51 with 11 exclusions each, followed by D3S1358 and D16S539 (10 exclusions each), Penta E, D13S317, and FGA (9 each), D7S820, Penta D, and D8S1179 (8 each), THO1, CSFIPO and TPOX (7), while D5S818 (6) and vWA (5) turned out to be the least informative loci. Figure 1 illustrates the number of cases in which exclusions were observed for various numbers of HLA and STR loci, while the distribution of exclusions for each HLA and STR locus is presented in figure 2.

In all remaining 35 non-exclusion analyzed cases, the AF could be determined as the biological father. All cases gave STR probability statistics closer to 100% than did the HLA

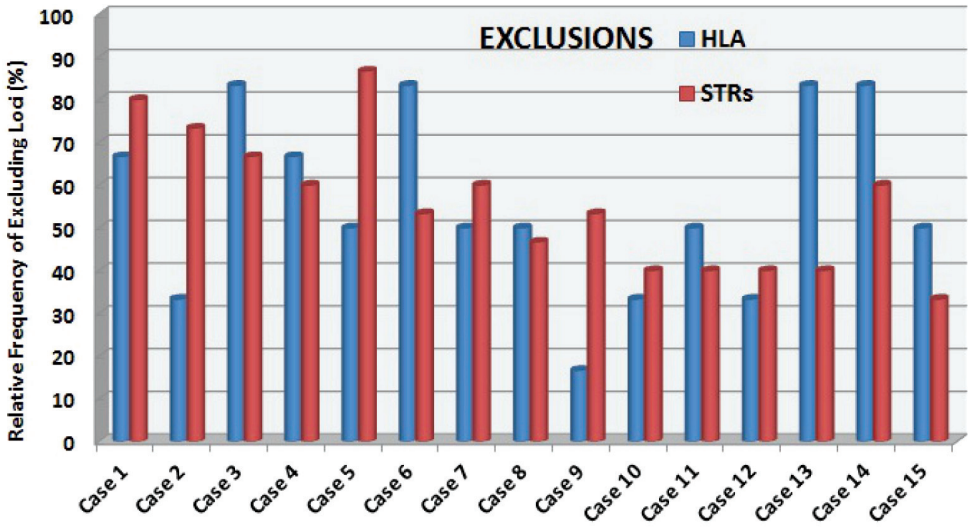

FIGURE 1. Comparison of the rate of mismatch for all HLA and STR loci concluded from 15 excluded paternity cases.

probabilities calculated. The PI value for the 35 non-excluded cases using HLA genotyping ranged from 76 to 6,452,794, whereas using aSTR genotyping ranged from 15,173 to 9.2×10^{10}. Additionally, the W rates ranged from 98.69990663% to 99.99998145% using HLA genotyping were increased to 99.99999999% using aSTR analysis. The results are summarized in table 2. A comparison with the probabilities of paternity calculated for the alleged father of each matching trio or duo based on HLA and aSTR data is given in figure 3. Furthermore, concerning CPI values between paternity trios and duo cases calculated by STR, statistically significant difference was observed.

In general, using aSTR loci CPI values were estimated to be $>10^5$ in all inclusion paternity trios cases and $>10^6$ in 96% of the inclusion paternity trios cases, whereas CPI values of the inclusion paternity duos cases were estimated to be $>10^6$ in only 10% of the tested cases (Table 3).

In conclusion, our data show that the core of 15 aSTR loci gave acceptable results, as 100% of cases gave CPI values >10,000 and it is in accordance to the global guidelines, where

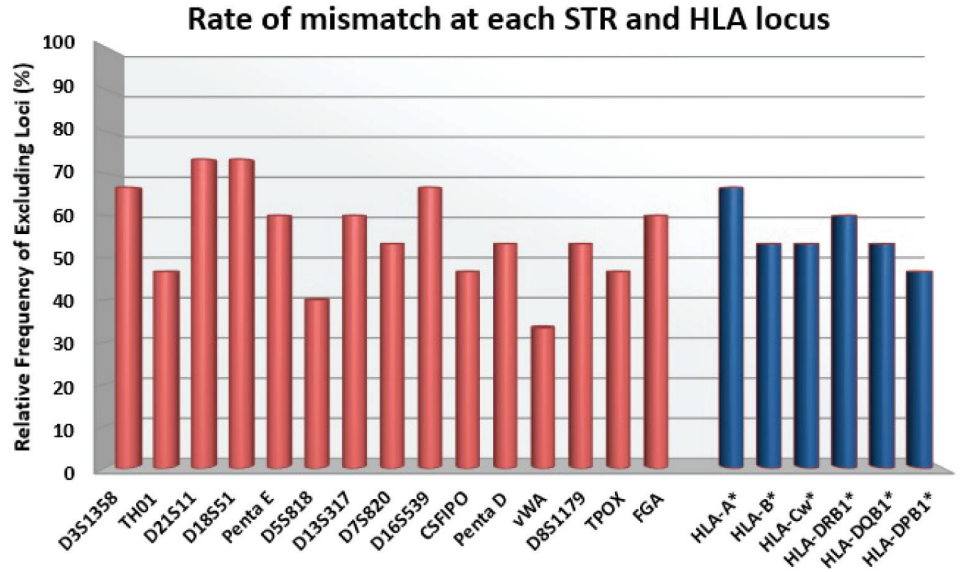

FIGURE 2. The distribution of STR and HLA loci according to the number of exclusions. For parentage investigations, three excluding STR loci are required to prove non-paternity. Additionally, incompatibilities in at least one HLA allele between parents and offspring indicate exclusion.

TABLE 2. The paternity index (PI), the combined paternity index (CPI), and the probability of paternity (W) by HLA and STR loci respectively for twenty-five trio and ten duo (motherless cases) non-excluded paternity cases.

Case no	Case type	PI by HLA*	W by HLA (%)	CPI by STRs	W by STRs (%)
Case 1	Trio	5,390,081	99.99998145	517,054,819	99.99999981
Case 2	Trio	152	99.34595638	714,736	99.99986009
Case 3	Trio	76	98.70041262	5,710,242	99.99998249
Case 4	Trio	84,220	99.99881265	1,009,541	99.99990094
Case 5	Trio	3875	99.97420098	113,311,778	99.99999912
Case 6	Trio	304	99.67190525	5,072,937	99.99998029
Case 7	Trio	8300	99.98795322	90,512,864	99.99999889
Case 8	Trio	642	99.84451514	91,657,841,253	99.99999999
Case 9	Trio	2465	99.95944137	6,507,788	99.99998463
Case 10	Trio	70,982	99.99859121	1,371,661,427	99.99999993
Case 11	Trio	162,738	99.99938552	48,943,755	99.99999796
Case 12	Trio	224,578	99.99955472	7,039,306	99.99998579
Case 13	Trio	11,142	99.99102551	77,275,750	99.99999871
Case 14	Trio	45,069	99.99778124	15,322,435	99.99999347
Case 15	Trio	21,891	99.99543203	3,635,636	99.99997249
Case 16	Trio	76	98.69990663	5,826,893	99.99998284
Case 17	Trio	897	99.88858005	470,473,480	99.99999979
Case 18	Trio	3356	99.97021558	59,709,569	99.99999833
Case 19	Trio	72,893	99.99862815	34,852,378	99.99999713
Case 20	Trio	173	99.42537659	9,268,964	99.99998921
Case 21	Trio	11,088	99.99098196	7,508,945	99.99998668
Case 22	Trio	2,089,890	99.99995213	49,429,109	99.99999798
Case 23	Trio	6,452,794	99.99998450	2,247,801	99.99995551
Case 24	Trio	28,466	99.99648718	3,817,747	99.99997381
Case 25	Trio	51,320	99.99805149	2,345,470	99.99995736
Case 26	Duo	4497	99.97776811	31,986	99.99687373
Case 27	Duo	4497	99.97776811	128,456	99.99922153
Case 28	Duo	4779	99.97908034	965,787	99.99969646
Case 29	Duo	2210	99.95476452	118,540	99.99915641
Case 30	Duo	72,366	99.99861815	529,736	99.99981123
Case 31	Duo	659	99.84839830	15,173	99.99340978
Case 32	Duo	665	99.72684949	190,507	99.99947509
Case 33	Duo	5654	99.98231744	207,965	99.99951915
Case 34	Duo	5654	99.98231744	8,775,980	99.99998861
Case 35	Duo	481	99.79248510	67,829	99.99852573

* PI by HLA corresponds to CPI by STRs

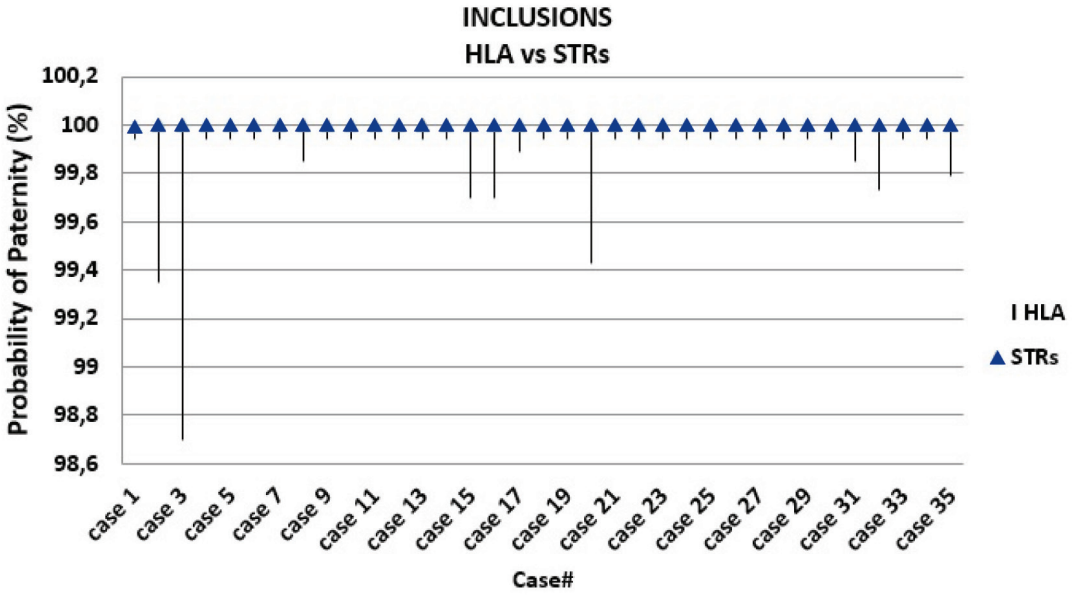

FIGURE 3. Comparison of the probabilities of paternity for the biological father calculated for the 35 inclusion cases. All of the tested cases have higher probabilities of paternity for the STR loci in comparison to HLA loci.

CPI of 10,000 is recommended as minimal for fatherhood determination.

UNEXPECTED OBSERVATIONS

Among 35 parent/child allele transfers at 15 aSTR loci, where the paternity is not excluded using aSTR loci, one AF showed one genetic discrepancy with the child at D21S11 locus (case 32), the most heterozygous and complex locus, which was scored as inconclusive result. It was a case where the mother was missing from the triplet (motherless case) (Fig. 4 and 5). In practice, one locus mismatch is considered as a mutation event and is therefore ignored. Statistical analysis was conducted considering the 14 remaining loci only. The D21S11 incompatibility was classified as a repeat loss single-step mutation (putative mutation 31→30) or double-step mutation (31→29), possibly as a result of slippage of the DNA replication complex during DNA synthesis. Initially, the motherless case was reanalyzed from the original samples to confirm the unexpected result, and as a final supplement, full HLA-A*, -B*, -Cw*, -DRB1*, -DQB1*, -DPB1 typing and allele subtyping was added. The AF was typed as HLA-A*02, *32; -B*18, *27; -Cw*02, *07; -DRB1*11, *16; -DQB1*05, *03; -DPB1*02:01,*04:02, while the child in

TABLE 3. Reported values of paternity index (PI) and the probability of paternity (W) for 35 paternity trios and motherless cases using HLA and aSTR genetic polymorphism respectively.

PI-values	W-values	Families by HLA (%) N=35	Families by STR (%) N=35	
		Trio & Motherless cases*	Trio cases (N=25)	Motherless cases (N=10)
<100	<99%	5.71	0	0
100-1000	99-99.9%	22.86	0	0
1000-10,000	99.9-99.99%	28.57	0	0
10,000-100,000	99.99-99.999%	28.57	0	30
100,000-1,000,000	99.999-99.9999%	5.71	4	60
>1,000,000	>99.9999%	8.57	96	10

* The W value is the same in both trio and motherless cases as the calculated equation does not take into account the genotype of the mother.

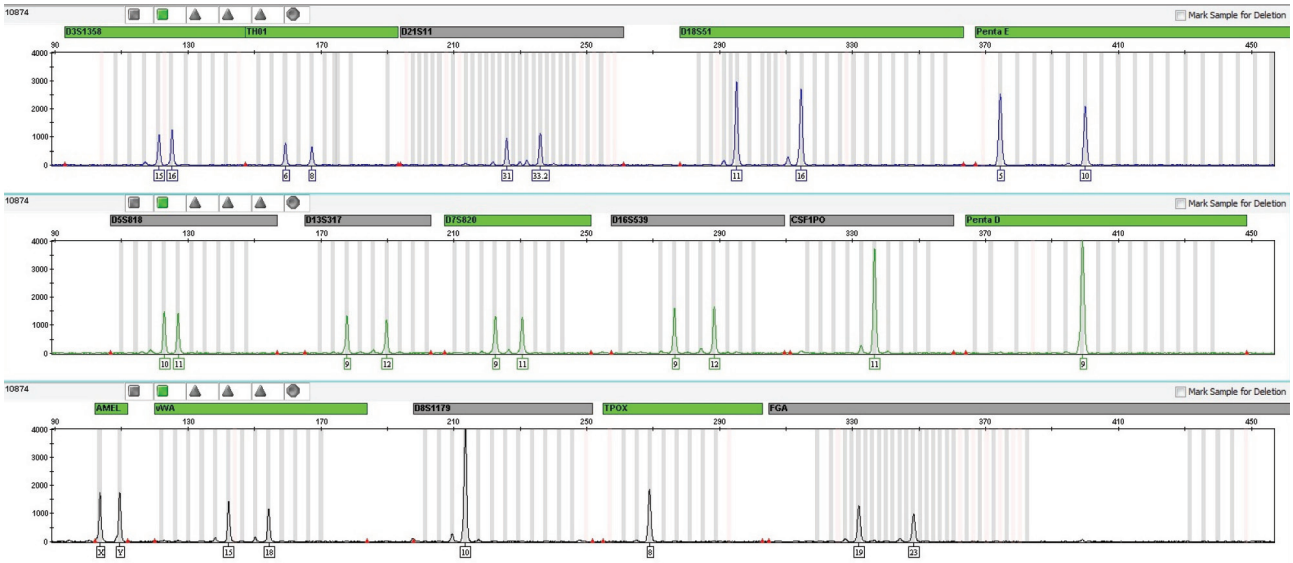

FIGURE 4. Electropherogram data depicting the DNA profile of the alleged father (ID:10874) produced with the PowerPlex® 16 multiplex system (case 32).

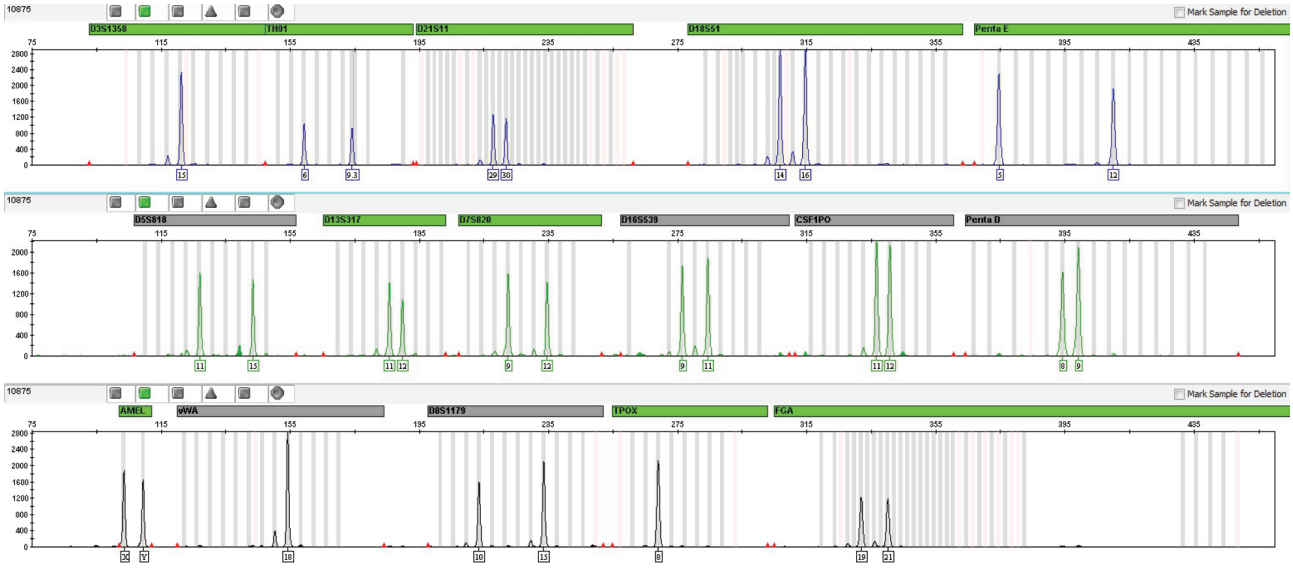

FIGURE 5. Electropherogram data depicting the DNA profile of the child (ID:10875) produced with the PowerPlex® 16 multiplex system. The child share one allele with the alleged father (ID:10874) at each tested STR marker, barring D21S11 STR loci due to mutation event (Putative single-step mutation: D21S11 31→30 or double-step mutation 31→29). Paternity is not excluded (case 32).

question was typed as HLA-A*03,*32; -B*18,*35; -Cw*04, *07; -DRB1*11, *15; -DQB1*05, *03; -DPB1*04:01,*04:02. We did not rule out fatherhood and a sufficient paternity probability was achieved even after the mutation calculation, where CPI and W values by aSTR analysis were calculate to be 190,507 and 99.99947509%, while PI and W values by HLA approach were calculate to be 665 and 99.72684949% respectively. This by STR calculated probability was sufficient for inclusion of the father. However, the probability of paternity for the biological father was low by HLA methodology due to the inheritance of very common HLA alleles: HLA-A*32, -B*18, -Cw*07, -DRB1*11, -DQB1*03, -DPB1*04:02.

DISCUSSION

Nowadays, the application of STR DNA-typing in laboratory medicine is increasing rapidly, with uses in paternity and

maternity assessment. As the most DNA-typing applications frequently have legal and ethical implications, there is a particular need for high reliability, and high discrimination power.[32,33]

Data from the present study lead to the conclusion that aSTR genotyping is a powerful tool for the analysis of parentage disputes. From the practical perspective, easily performed standard methodology with high reproducibility and low cost as well as abundance of STR loci in human genome, makes them ideal genome markers for paternity/maternity assessment.[34,35] Specifically, in all paternity trio cases, 15 forensic loci were effective in excluding paternity or sufficient to provide positive proof (strong evidence) of paternity, and offer high discriminating power with W rate ranged from 99.99340978% to 99.99999999%. Using a well-qualified database, we can obtain a PI likelihood ratio as high as 10^6 in usual trio cases. Additionally, the application of STR DNA-typing in parentage testing provides more flexibility in samples handling and testing, as well as greater discrimination efficiency in comparison to conventional HLA genetic typing.

The W value in the trio case is higher than that in the motherless cases, and the difference becomes higher when the paternal obligatory alleles are presented with high frequency in the population study.

It should be noted that, motherless case results are usually reliable, although the absence of the mother's genotype increases the possibility of false paternity inclusions, especially in cases analyzed with limited number of aSTR loci.[36,37] Many studies have shown that duo cases were inclusive for paternity, but after consideration of the mother's DNA typing, the kinship was rejected and it was so mainly due to the use of a limited number of aSTR loci.[38,39] Thus, the laboratory experts should include the mother's genotype in every paternity case. If that is impossible, for many reasons, investigation of more polymorphic aSTR loci must be considered. In the present study the analysis of father-child pairs without investigation of the mother resulted in lower likelihood ratios in comparison to trio cases, although the W remains higher compared to that calculated using HLA typing.

We emphasize the necessity for greater caution when dealing with motherless cases, especially in cases where mutation events occur and also in motherless cases without any incompatibilities using a limited number of aSTR loci.[40,41] In such deficiency cases, where fatherhood cannot be confirmed or ruled out with statistical certainty additional relatively stable aSTR loci with low mutation rates, or other markers (Y-STR loci, X-chromosome specific STRs, alternative mitochondrial genome (mtDNA) markers) would be analyzed in order to include the AF or to reveal additional exclusions.[42-44] Thereby, every clinical Laboratory involved in paternity disputes investigation should have the ability to increase the number of analyzed STR loci, or when it is not feasible other methods, such as investigation of HLA analysis, should be performed in order to come to a sound conclusion or exclusion.[45]

CONCLUSION

The use of DNA-typing with 15 aSTR loci for parentage testing provides an accurate and high-sensitivity method which is simpler to be performed and more rapid than the accepted HLA standard technology. Analysis of aSTR loci offers a highly discriminating test suitable for trio paternity testing. It is also useful in cases where the maternal DNA is not available, or when individuals originate from the same lineage are tested. Furthermore, when a mutation event occurs in motherless cases, combination of HLA and STR polymorphisms offers high level of information and also diminishes the possibility of false exclusion due to aSTRs mutations, or minimizes the risk of wrong inclusion due to the absence of the mother's genotype.

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