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TECHNICAL NOTE

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Combining Autosomal and Y-Chromosomal Short Tandem Repeat Data in Paternity Testing with Male Child: Methods and Application*

ABSTRACT: Paternity testing is being increasingly requested with the aim of challenging presumptive fatherhood. The ability to establish the biological father is usually based on the genotyping of autosomal short tandem repeat (STR) in alleged father, mother and child, but the use of Y-chromosomal STR has gained interest in the last few years. In this work, we propose a new probabilistic approach that combines autosomal and Y-chromosomal STR data in paternity testing with father/son pairs taking into account mutation events. We also suggest a new two-stage approach where we first type Y-STRs and possibly autosomal STR for the putative father and son, conditional on Y-STR results. We applied this approach to 22 cases. Our results show that Y-STRs can identify nonpaternity cases with high accuracy but need to be validated with autosomal STR to establish paternity. Moreover, the two-stage approach is less costly than the standard approach and is very useful in motherless cases.

KEYWORDS: forensic science, paternity analysis, short tandem repeat, Y chromosome, autosomal loci, mutation

The use of genetic markers to analyze paternity began in the 1930s, shortly after the description of ABO blood groups. In the 1990s, short tandem repeat (STR) or microsatellite markers was widely used in the forensic community (1–3). DNA paternity testing, a highly accurate analysis of the genetic profiles of the mother, child and father, is based on the fact that child inherits half of its DNA pattern from the mother and half from the father, according to the Mendel's law of inheritance (4,5), as far as autosomal STR (A-STR) are considered.

With male children, Y-chromosomal STR (Y-STR) has gained more and more interest in paternity testing in the last few years (2,3,6–9). In fact, the Y chromosome, excluding the pseudoautosomal region, is not involved in meiotic recombination and is transmitted unaltered from father to son (10,11). Several studies have shown the enormous potential of Y-STRs haplotyping in forensic investigation. It was demonstrated that Y-STRs have enough power to allow interindividual discrimination with >99% probability (3). In the forensic community, a set of consensus markers have emerged and have been used in routine in parentage testing and individual identification (1,12–14).

The DNA analysis is based on the interpretation of similarities or differences at genetic marker loci. In paternity testing, differences at genetic marker loci between the putative father and the offspring, are used as evidence for nonbiological paternity, and thus, lead to the exclusion of paternity. However, mutation at STR can contribute to these differences, and an exclusion of paternity should not be based on the genetic inconsistency for a single marker locus. In the literature, recommendations are to use at least three STR loci (2,15).

Recently, probabilistic approaches that take into account the mutation in paternity exclusion have been proposed (7,16). With accumulating data on mutation rates for A-STR and Y-STR markers (17–19), it is now possible to take into account the event of mutation in the computation of paternity likelihood.

Combining evidence from A-STR and Y-STR to compute a joint paternity index may improve power of paternity testing, especially when data are available from father/son pairs with or without the mother. In this paper, we presented a probabilistic method that combines different sources of evidence from STR marker genotypes or haplotypes to calculate the paternity likelihood. We then assessed its potential power and performance by studying 22 cases of paternity testing.

Materials and Methods

Samples

DNA samples of 22 alleged father/son/mother trios were randomly selected from the database of tested cases in the Laboratory service of the Hospital Centre CHU Hedi Chaker of Sfax (Tunisia). All selected individuals originated from the South of Tunisia and especially from the region of Sfax and neighboring cities. The anonymity of the individuals investigated was protected according to the laws of individual data protection of the country.

DNA Analysis

The 22 alleged father/son pairs were genotyped for a set of Y-STR and A-STR markers. The mothers were also genotyped for A-STRs.

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For A-STR—PCR amplification was performed in Gene Amp PCR system 9600, using the AmpFlSTR-SGMPlus™ PCR Amplification Kit (Applied Biosystems, Foster City, CA), that amplifies 10 A-STRs (D3S1358, vWa, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, THO1, and FGA), according to the manufacturer's instructions. The PCR products were carried out in the ABI PRISM® 310 DNA analyser (Applied Biosystems). Genotypes were determined with GeneScan (version 3.5) (Applied Biosystems) and Genotyper (version 3.7) (Applied Biosystems) by comparison with supplied allelic ladders and an internal size standard (GS500 Rox).

For Y-STR—12Plex-amplification was performed by the commercial kit Y-Plex™12 (Reliagene, New Orleans, LA) that amplifies 11 Y-STR loci (comprising DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438, and DYS439) and a segment of the amelogenin gene, according to the manufacturer's instructions, but in a total reaction volume of 12.5 μL. The PCR products were analysed with the ABI PRISM® 3100-Avant Genetic Analyser (Applied Biosystems) using the Genemapper software (version 3.5) (Applied Biosystems). Allele designation was determined by comparing the PCR products with those of allelic ladders provided with the kit and an internal size standard (GS500 Rox). Nomenclature of loci and alleles is according to the International Society of Forensic Genetics (ISFG) guidelines reported by Gusmão et al. (20).

Statistical Methods

In paternity testing, one is interested in calculating the probability that the tested man (alleged father) is the father (or the ratio of that probability to the probability that a random men is the father) given data on genotypes or haplotypes of some DNA markers (21).

Let us denote by P the event ``the alleged father is the true father," by $\mathbf{G}$ the set of genotypes of the mother, alleged father, and son for a set of m A-STR markers, and by $\mathbf{H}$ the set of haplotypes of alleged father and son for a set of m' Y-STR markers.

The evidence for paternity is given by the posterior probability $W = \Pr(P/\mathbf{G},\mathbf{H})$ which, according to Bayes' theorem, is equal to $\Pr(\mathbf{G},\mathbf{H}/P)\ \Pr(P)/\ \Pr\ (\mathbf{G},\ \mathbf{H})$ where $\Pr(P)$ is the prior probability of paternity, thereafter denoted by π. In common practice, a value of $\pi = 0.5$ is considered, if no prior value is available about the probability of paternity.

The evidence for paternity versus nonpaternity is generally measured by the paternity index (PI), defined as the likelihood ratio $L = \Pr(\mathbf{G},\mathbf{H}/P)/\Pr(\mathbf{G},\mathbf{H}/\bar{P})$, where $\bar{P}$ denotes the complementary event to P. It is easy to show that the paternity probability is $W = L\pi/(L\pi + (1-\pi))$ which reduces to $W = L/(L+1)$ when $\pi = 0.5$. In practice, a value of $W > 0.999$ or $L > 1000$ is taken as an evidence for paternity (22).

For Y-STR Markers—Using the same arguments as in Rolf et al. (7), we can easily show that if the father and the son's haplotypes differ by $n > 0$ alleles among the m' alleles considered, the paternity index based on Y-STRs is given by:

$$L_{\mathrm{H}} = \prod_{i=1}^{n} \mu_i \quad \prod_{j\neq i}^{m'} (1-\mu_j)/f_{\mathrm{S}} \qquad (1)$$

where f_{S} is the frequency of the son's haplotype and μ_i is the mutation rate of locus i; the first product in Eq. (1) is over loci having alleles that differ between alleged father and son, and the second is over loci having identical alleles. We here assume that mutation rates are similar for all alleles of a given locus; a more detailed analysis of mutation will be given in the next section.

From expression (1) we can see that the L_{H} index decreases with the number of mutated/inconsistent markers and when the frequency of the son's haplotype increases. For a haplotype of 11 Y-STR with 5% frequency, we need only two discordant markers to get $L_{\mathrm{H}} < 10^{-4}$. As most of the Y-STRs haplotypes (90% of them) has <0.1% frequency (see YHRD database), we thus expect a very high exclusion power of Y-STRs markers.

For A-STR Markers—The analysis of A-STR markers for father/son duos or mother-alleged father–son trios, taking mutation into account, has been described in details elsewhere (see for example Dawid et al. (16). Paternity index L_{G} could be calculated using one of the available programs such as PATCAN (23), FINEX (24) or EASYPAT, a freely available program of the MKGST package (25).

Combining A-STR and Y-STR—Based on the independence of A-STR and Y-STR marker information, the combined paternity index L_{C} is:

$$L_{\mathrm{C}} = \frac{\Pr(\mathbf{G}/P)}{\Pr(\mathbf{G}/\bar{P})}\ \frac{\Pr(\mathbf{H}/P)}{\Pr(\mathbf{H}/\bar{P})} = L_{\mathrm{H}}\ L_{\mathrm{G}} \qquad (2)$$

where L_{G} and L_{H} are paternity indices calculated with A-STR and Y-STR markers, respectively. The combined posterior probability could be calculated by $W_{\mathrm{C}} = W_{\mathrm{H}}W_{\mathrm{G}}/(1-W_{\mathrm{H}})(1-W_{\mathrm{G}})$ or simply from L_{C}.

An Alternative Two-Stage Approach

According to expression (1), a discordance of two alleles or more for the Y-STR haplotype decreases substantially the PI, and thus gives strong evidence for exclusion. This led us to think that a two stage approach for paternity testing with male child might be optimal. The proposed approach proceeds as follows:

Step 1: first type alleged father and son for the set of Y-STR markers and decide for or against paternity based on L_{H}. If the evidence for exclusion is high (the threshold for exclusion could be here set to a small value) then exclude paternity; this will save money and time for the typing of the mother and alleged father/son for A-STR markers. If exclusion threshold could not be reached, then we go to step 2;

Step 2: genotype father/son and possibly the mother for a set of A-STR markers and decide based on L_{G} or the combined paternity index.

Mutation Model

Overall mutation rates are now available for many A-STR and Y-STR loci (26,27). For Y-STR loci mutation, rate is on average 2 10^{-3} as estimated by Gusmão et al. (28). Many studies have shown that the stepwise mutation model is better suited to explain conversion between alleles at STR loci (e.g., 16,29,30). Following the procedure described in Dawid et al. (16), we computed the mutation transition matrix for each of the A-STR and Y-STR markers (available on request to the authors). This matrix gives the specific mutation rates from any given allele to any other observed allele, calculated using a stepwise model. In this model the mutation rate from allele i to allele j ($i \neq j$) is expressed as:

$$\mu_{i\to j} = \frac{\lambda\ \alpha^{|i-j|}}{p_i}$$

where p_i is the frequency of allele i in the studied population, α is a parameter to be chosen (generally $\alpha = 0.5$) and λ is scale

parameter calculated by equating the overall mutation rate of the locus to the weighted sum of the $\mu_{i \to j}$ [see Dawid et al. (16)]. For the computation of mutation matrices, we used allele frequencies estimated in our population (31–33).

Calculation of Paternity Indices

Paternity index L_H and corresponding posterior probability W_H were calculated using an excel worksheet implementing the method described above with allele specific mutation rates. We used haplotype frequencies from YHRD database (all populations), which is by far the largest Y-STR database available with more than 41,000 different haplotypes (27).

Paternity index L_G and the corresponding posterior probability W_G were also computed using an excel worksheet based on the approach described by Dawid et al. (16). For validation we also used EASYPAT 25 and PATCAN (23) which implement standard methods and compute paternity indices taking mutation into account.

Combined paternity index L_C was then calculated as the product of L_H and L_G.

Results and Discussion

Haplotype Analysis

In the set of the 44 males (22 alleged father/child pairs), we identified 26 different Y-haplotypes, 14 of which were not found in the YHRD database (release 18). Duplication was observed at locus DYS19. We also identified a new allele for D21S11 (allele 36).

Exclusion of Paternity Using Y-STR

Among the 22 alleged father/son pairs tested, we found 12 cases of clear exclusion with values of L_H ranging from 6×10^{-6} to 2.9×10^{-27} (Fig. 1). The number of markers showing genetic inconsistency varied from 1 to 9, with seven cases having six or seven inconsistent Y-STRs markers. As expected, the paternity index decreases when the number of inconsistent markers increases. Case #13 is problematic because $L_H = 37.9$ is below the inclusion threshold (1000), but there is a single discordant marker (DYS385b) between alleged father and son. Case #15 has an inclusion profile (father and son's haplotypes are identical), but the PI is relatively low (<1000) because of the fact that the son's haplotype is common. The eight other cases showed no evidence for exclusion with high L_H values ranging from 5467.9 to 38194, and in these cases the alleged father could not be excluded.

Exclusion of Paternity Using A-STR

All 12 nonpaternity father/son pairs based on Y-STR showed many genetic inconsistencies based on A-STR markers. Among these, one case has genetic inconsistency for three A-STR, and 11 cases have inconsistencies for five STRs or more. Paternity indices L_G ranged from 9.7×10^{-9} to 3.1×10^{-27} when only father/son data are used and from 5.4×10^{-8} to 6.2×10^{-27} when genotype of the mother is also used (Fig.1).

Among the eight cases of paternity inclusion based on Y-STR, one case (case #14) showed an exclusion profile with A-STRs, while the seven others were concordant having L_G values that indicate no evidence for exclusion. We see a decrease in L_G of about 100% when we compare duos with trios. In fact, Wenk et al. (34) showed on 25 cases that omission of maternal typing reduces evidence for or against paternity from 30% to 40%. Based on this, they recommended that cases involving one parent and child (e.g., immigration) would require examination of five additional loci to compensate for absent maternal data. However, as we will see in the next section, combining Y-STR and A-STR compensates much better the absence of the mother. Paternity inclusion for case #15 was confirmed by A-STR markers (L_G = 760,000).

The discordant case #14 (Table 1) showed genetic inconsistency for five A-STR markers, which is the average number of inconsistent markers in cases of exclusion. The paternity index L_G was equal to 1.2×10^{-14}. This discordance could be explained by the fact that the true father is very likely to be a close relative (brother, father) of the alleged (excluded) father so that they share the same Y-STR haplotype, but not the same genotype for A-STR markers. However, we do not have any historical evidence supporting this assumption. This is, actually, the typical circumstance where the Y-STR will clearly fail to detect exclusion and where the use of A-STR is needed.

Combining Y-STR and A-STR

In Fig. 1, we report the values of paternity indices based on Y-STR (L_H) or A-STR (L_G) or combined (L_C), represented on a logarithm scale. We see that for the cases of no-exclusion (upper part of Fig. 1), the highest evidence is given by combined indices with or without mother data, while the weakest evidence is

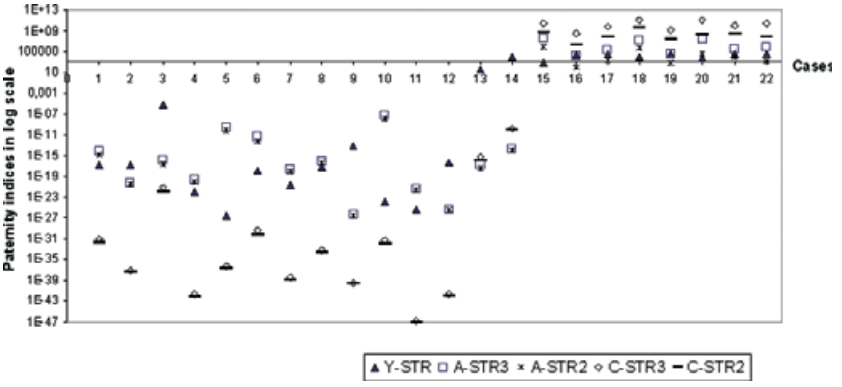

FIG. 1—*Paternity indices (represented on a log scale) for the 22 cases considered. Y-STR, Y-chromosomal STR; A-STR3, autosomal STR involved in trios (father, child, and mother); A-STR2, autosomal STR involved in duos (child and father); C-STR3, combined Y-STR and A-STR3; C-STR2, combined Y-STR and A-STR2.*

TABLE 1—*Haplotype of one discordant case in paternity testing (case #14).*

		A-STR									
Case	Y-STR*	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	THO1	FGA
Father	14-13/19-13-30-23-11-11-12-10-11	14-17	17-18	9-12	20-23	13-14	30-35	13-13	14-14,2	6-7	24-26
Child	14-13/19-13-30-23-11-11-12-10-11	16-17	16-18	12-12	18-20	13-13	29-35	13-14	12-12	6-6	21-21
Mother		16-17	15-18	11-12	20-20	13-14	30-35	12-14	12-14	6-9	19-21

*Haplotype of DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438 and DYS439, in order.

provided by Y-STR or A-STR data alone without the mother. In five cases over seven, Y-STRs were more informative for inclusion than A-STRs, but have less or equal informativeness than these when the mother data are considered. This stresses the advantage of combining both marker systems.

For the 12 exclusion cases (cases 1–12 in Fig. 1), we see that combined paternity index performs much better than the others but almost equally regardless to whether the mother is included or not. For these cases, paternity indices calculated with Y-STR are on average more informative for exclusion than those calculated with A-STR.

For case #14, showing discordant conclusions from Y-STR and A-STR markers, the combined index L_C was 9.2×10^{-11} when mother is not included showing a clear exclusion without the need for mother typing. Cases #13 and #15 were resolved using combined index showing high evidence for exclusion for the first and inclusion for the last.

Towards a Two-Stage Approach

The probability of exclusion of Y-STRs seen in our data suggests that a two-stage approach, where we first type Y-STR markers, will be very efficient and cost-effective. In fact, 11 Y-STRs loci can accurately exclude paternity with a male child and needs only typing of the alleged father and the son. This is particularly very useful for motherless cases (e.g., immigration, deceased mother). In this two-stage approach, only when Y-STRs do not give high evidence for exclusion, we need to genotype the alleged father and son for A-STR markers. Again, this could be done without the information from the mother because combining both sources of information would compensate the missing mother data.

From an economical point of view, based on the data we have at the Hospital of Sfax (Tunisia), we estimated that the typing cost per individual, using typing method described in this paper, is about a = \$12 for Y-STR and b = \$27 for A-STR. This means that paternity testing of a father/child duo with the two-stage approach will cost $\$2a+\alpha(2b)$ where α is the proportion of cases showing no evidence for exclusion with Y-STR markers. From our experience we estimated α to be about 0.70 so that the cost of the two-stage testing approach is about \$61 per case. On the other hand, testing trios with the classical A-STR approach will cost \$81 per case. This gives an expected gain of \$20 per case for the two-stage approach. Moreover, in our service, paternity cases with male children represent about 60% of all paternity cases, on average for the last 3 years. This shows that a substantial gain is expected from using a two-stage approach combining the typing of Y-STR and A-STR.

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