Resolving Questioned Paternity Issues Using a Philippine Genetic Database

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ABSTRACT

The utility of the Philippine genetic database consisting of seven Short Tandem Repeat (STR) markers for testing of ten questioned paternity cases was investigated. The markers used were HUMvWA, HUMTH01, HUMCSF1PO, HUMFOLP23, D8S306, HUMFES/FPS, and HUMF13A01. These markers had a combined Power of Paternity Exclusion of 99.17%. Due to the gravity of some cases handled in the laboratory, routine procedures must be assessed to determine the capacity of the analysis to exclude a non-father or predict paternity. Clients showed a preference for only testing father and child to lower costs and reduce conflicts, particularly when the mother objects to the conduct of DNA tests, or when she is deceased or cannot be located. The Probability of Paternity was calculated with and without the mother's profile in each of the cases. In all instances, results were more informative when the mother's DNA profile was included. Moreover, variations in the allelic distribution of five STR markers among eight Caucasian, one African-American, and two Amerindian (Argentina) populations resulted in significant differences in Probability of Paternity estimates compared to those calculated using the Philippine database.

Based on the results of the present study, it is recommended that tests on alleged father-child samples be performed to screen for at least two mismatches. In the absence of these mismatches, further analysis that includes the mother's DNA profile is recommended. Moreover, it is recommended that a Philippine genetic database be used for DNA-based paternity testing in the Philippines.

Key words: Short Tandem Repeat markers, Philippine genetic database, inclusions, exclusions, paternity trios, motherless cases

INTRODUCTION

Paternity testing has changed from its early days, when conventional serum-based testing, such as ABO blood typing and protein polymorphism, was the norm, to the current DNA-based analysis using Restriction Fragment Length Polymorphism (RFLP) and/or the Polymerase Chain Reaction (PCR). DNA typing is based on the uniqueness of the genetic make-up of all individuals, except identical twins (Jeffreys et al., 1985). It is widely used in criminal investigations, in establishing familial relationships between individuals in simple paternity disputes and immigration cases, and identification of mass disaster and war victims. DNA-based systems offer a higher exclusion power than protein-based systems, thereby minimizing the chance

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of falsely including a non-father (Markowicz et al., 1990), as well as a more accurate inclusion probability for the identification of true biological fathers (Chakraborty & Stivers, 1996). DNA testing also allows greater flexibility in terms of the types of sample that can be submitted for testing. Blood, hair, tissues, buccal swabs, and exhumed materials can be used as sources of DNA (Chakraborty & Stivers, 1996). In addition, DNA-based systems are unaffected by blood transfusion (Huckenbeck & Rand, 1994) which has been known to result in erroneous conclusions in cases tested using conventional protein-based methods.

Numerous countries have reported the establishment of population databases relevant for DNA-based paternity testing. In keeping with recent developments in forensic DNA technology, a Philippine population database of the National Capital Region (NCR) consisting of Short Tandem Repeat (STR) markers was constructed (Halos et al., 1999). The seven STR markers, comprising the database, namely D8S306, HUMFOLP23, HUMTH01, HUMvWA, HUMCSF1P0, HUMFES/FPS, and HUMF13A01, with a combined Power of Paternity Exclusion of 99.17%, are used to evaluate the results of DNA tests employing well-established statistical parameters. In routine DNA-based paternity tests, samples from paternity trios – the mother, the child, and the alleged father (AF) – are obtained. However, to make testing more affordable, it was suggested that tests be performed using only samples from AF-child pairs, i.e., motherless cases.

In countries such as the United States, Canada, Germany, Australia, and the United Kingdom, where forensic DNA technology is established, paternity testing guidelines have been formulated to aid in interpreting DNA results and in using DNA evidence in court. These include requirements for a minimum number of mismatched markers prior to the exclusion of an AF as father of the child (paternity exclusions), or a minimum value of the Probability of Paternity (W) prior to the presumption of paternity (paternity inclusions). W provides a numerical estimate for the likelihood of paternity of an AF compared to the probability of a random match of two unrelated individuals. In many genetic testing laboratories, mismatches in at least two STR markers are required for paternity exclusions (Mertens et al., 1997). However, due to the probabilistic nature of paternity inclusions, W will never equal 100% and the minimum legally accepted value of W will vary in different localities/countries. Legally accepted minimum W values in the United States range from 95.0% in New York to 99.9% in Louisiana (www.cga.state.gov). In the Philippines, similar guidelines and laws are not yet in place.

In this study, we report the use of the Philippine database in resolving questioned paternity issues in ten cases submitted to our laboratory, the result of testing only AF and child pairs (motherless cases) and the effect of using population databases other than the Philippine database in evaluating probabilities of paternity. The results of the present study will be used in formulating specific guidelines for DNA-based paternity determination in the Philippines.

MATERIALS AND METHODS

Sources of samples

Ten questioned paternity cases submitted to our laboratory, consisting of five paternity exclusions and five paternity inclusions, were used in the present study. Brief case descriptions are listed in Table 1. Motherless cases were simulated using only the DNA profiles of the AF and child for statistical analysis.

DNA extraction

Blood samples were collected from the AF, the mother, and the child, blotted on FTA™ cards (Flinders Technologies Pty Ltd., Fitzco Inc.), and processed following manufacturer's instructions.

PCR amplification

For amplification at seven STR loci, unlabeled primers (Gibco-BRL, Life Technologies, Gaithersburg, MD) and Cy5-labeled fluorescent primers (GenSet Oligos, Singapore) were used. For each 25 μL reaction, two FTA™ discs (2 mm in diameter) were placed in a 0.2 mL PCR tube and amplified as described earlier (Halos et al., 1999).

Table 1. Description of questioned paternity cases

Cases	Nature of Case	Case Descriptions
1	Criminal	Sexual assault/ Accused imprisoned > 5 years/ Child > 7 years
2	Criminal	Sexual assault/ Accused imprisoned > 1 year/ Child > 2 years
3	Civil	Recognition of illegitimate child/ Child > 15 years
4	Civil	Woman has two lovers, needs to know the real father of her child/ Child < 5 years
5	Civil	Petition for illegitimate child (immigration)/ Child > 3 years
6	Civil	Child support (annulled marriage)/ Child > 5 years
7	Civil	Recognition of illegitimate child by the father's family/ Child < 1 month
8	Civil	Girlfriend is suspected of having another relationship/ Child < 2 years
9	Civil	Recognition of illegitimate child by the father/ Child > 4 years
10	Civil	Continued support for illegitimate child/ Child > 12 years

DNA fragment analysis

Amplified products were separated by size using a High Resolution ReproGel™ (Amersham Pharmacia Biotech, Sweden) and the ALF Express™ unit (Amersham Pharmacia Biotech) according to manufacturer's instructions. Sizes of PCR products were compared with those of allelic ladders as previously reported (Halos et al., 1999).

Statistical analysis

The Probability of Paternity (W) of paternity trios and simulated motherless cases in instances of paternity inclusions were calculated using DNAVIEW™ program (Brenner, 1997). The W values of paternity trios and the corresponding motherless cases (designated as $W_{\text{-mother}}$) were compared.

Calculations of probability of paternity using various population databases

The W and $W_{\text{-mother}}$ of each of the paternity inclusion cases were calculated using published genotypic frequencies of 11 other populations compiled by the DNA Serology Group at the University of Duesseldorf, Germany (http://www.uniduesseldorf.de/WWW.MedFak/Serology/dna.html). The population databases included in the study were from the autochthonous Basque region (Garcia et al., 1998a; Garcia et al., 1998b); Brescia region of North Italy (Cerri et al., 1998); Pomerania-Kujawy region of Poland (Miscicka-Sliwka et al., 1998); North Portugal (Gusmao et al., 1995; Lurdes-Pontes et al., 1998); Northeast Spain (Crespillo et al., 1997); USA Caucasoid and African American populations (Smith, 1997); French Caucasoid population of Quebec, Canada (Busque et al., 1997); and Caucasoid (Buenos Aires), Mapuche (Rio Negro Province), and Wichi (Salta Province) populations of Argentina (Sala et al., 1998). These population databases were selected based on the availability of the five STR loci HUMF13A01, HUMFES/FPS, HUMvWA, HUMCSF1PO, and HUMTH01. Population databases with the D8S306 and HUMFOLP23 markers were not available so W and $W_{\text{-mother}}$ values were calculated using only the DNA profiles at five STR markers of each population including the Philippines. The 11 population databases were grouped as nonPhil, and the modal W and $W_{\text{-mother}}$ values were compared with those of the Philippine database.

RESULTS

Paternity exclusion cases

The absence of common alleles (also called an incidence of a mismatch) as shown in Fig. 1, was detected between the AF and the child when the mother's DNA profile was known (Cases 1 to 5). The presence of at least two mismatches in each of these cases results in the exclusion of the AF from being the biological father of the child (W=0%). On the other hand, fewer mismatches between AF and child were detected without the mother's genotype (Table 2) due to the presence

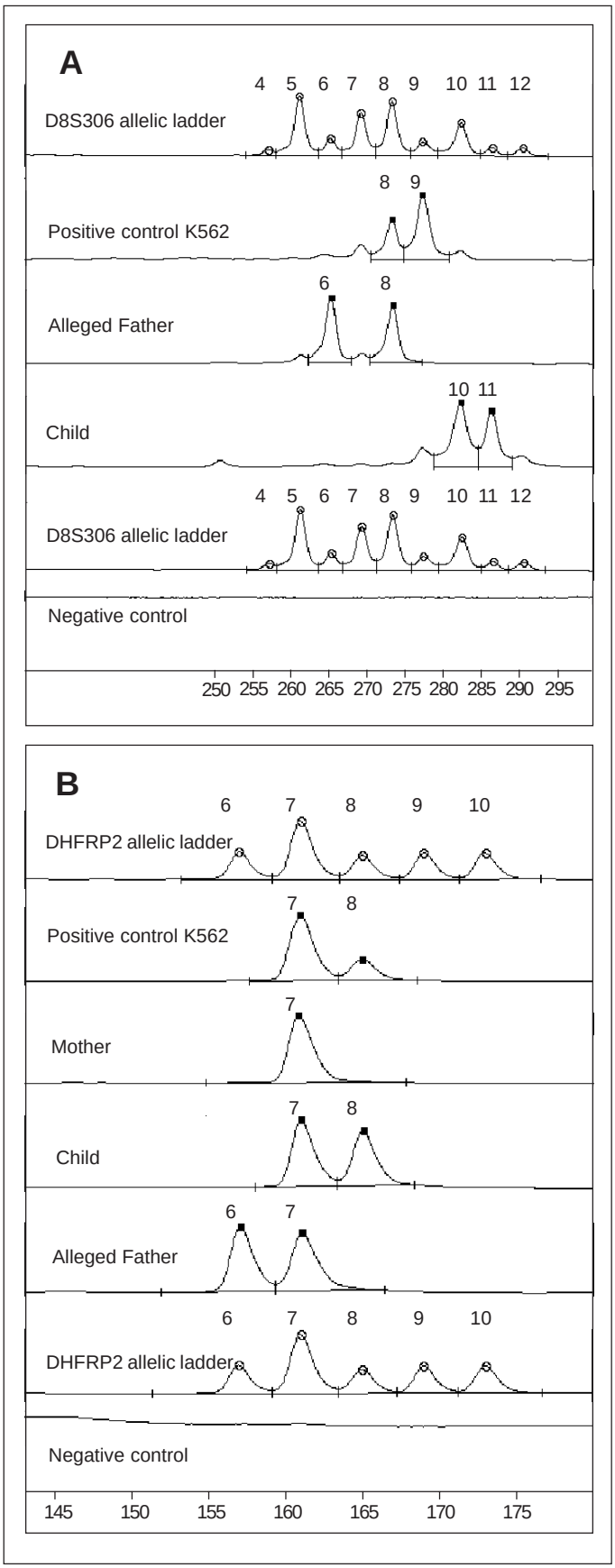

Fig. 1. (A) Paternity exclusion at STR locus D8S306. The absence of any allele-sharing between AF and Child (mismatch) indicates non-paternity. In this situation, the mother's DNA profile is no longer needed to exclude the AF as the biological father of the Child. (B) Paternity exclusion at STR locus HUMFOLP23/DHFRP2. This case illustrates the necessity of obtaining the Mother's profile in some cases. Without the Mother's profile, it appears that the Child and the AF share allele 7. With the Mother's profile it becomes evident that since the Child shares allele 7 with the Mother, then the Child does not share any allele with the AF. The AF is therefore excluded as being the biological father of the Child.

by chance of similar alleles in the AF and the child's mother. It is possible that a random match between the AF and child alleles masks a paternity exclusion if the paternal alleles are not properly identified in the child's DNA profile. Moreover, although a mismatch in a single STR marker does not automatically exclude an AF (Case 3) the presence of a mismatch is more consistent with a paternity exclusion, than a paternity inclusion.

In Case 2 no mismatch was detected between the AF and child in the absence of the mother's DNA profile since the AF and the child's mother possessed similar alleles (Fig. 1B). The absence of a mismatch between AF and child already suggests possible paternity. However, the low $W_{\text{-mother}}$ value ($W_{\text{-mother}}$ = 73.26%) indicates that the AF and the child share alleles which commonly occur in the Philippine population, and

Table 2. Number of mismatches between AF and child in paternity exclusion cases using seven STR markers

Case no.	No. of mismatches between AF and Child	
	With mother's DNA profile	Without mother's DNA profile
1	4/7	3/7
2	2/7	0/7
3	3/7	1/7
4	3/7	2/7
5	4/7	2/7

the weight of DNA evidence to support paternity is considerably less compared to higher $W_{-mother}$ values.

Paternity inclusion cases

In cases where no mismatches were detected with and without the mother's DNA profile (Fig. 2), the Probability of Paternity estimates using seven STR markers range from 96.48% to 99.98% and 79.71% to 99.49%, respectively (Table 3). W values were higher than the corresponding $W_{-mother}$ values in all five cases that demonstrate the greater accuracy of DNA test results when the maternal genotype is known. In Cases 6 and 9 for example, $W_{-mother}$ was significantly lower than the corresponding W.

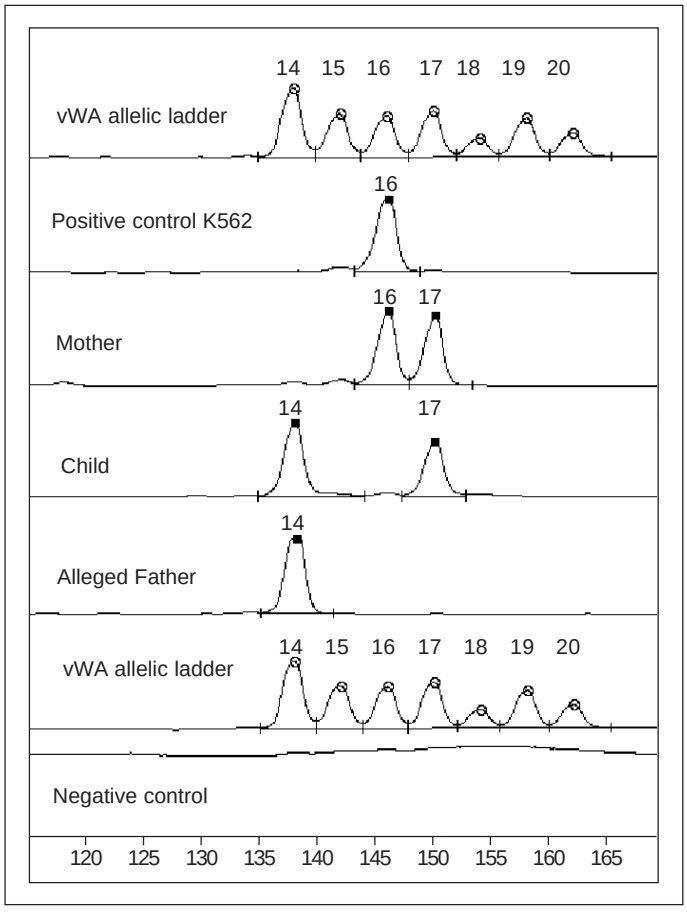

Fig. 2. Paternity inclusion at STR locus vWA. In this case, the AF is not excluded as the biological father of the Child. The weight of the DNA evidence is subsequently assessed by calculating Probability of Paternity (W).

Table 3. Probability of Paternity (W) estimates in five paternity inclusion cases using seven STR markers

Case no.	Probability of Paternity (%)	
	W	$W_{-mother}$
6	96.48	79.71
7	99.98	99.49
8	99.98	99.34
9	98.70	83.97
10	98.60	98.10

Probability of paternity values calculated using different population databases

Using 11 population databases, W and $W_{-mother}$ can be calculated for Cases 6 to 10 (inclusion cases) in five STR loci, namely, HUMvWA, HUMTH01, HUMCSF1P0, HUMFES/FPS, and HUMF13A01 (Tables 4 and 5). Some W and $W_{-mother}$ values derived from non-Philippine databases were found

Table 4. Comparison of probability of paternity (W) values calculated using 12 population databases and five STR markers

Population databases+	Probability of Paternity W (%)				
	Case 6	Case 7	Case 8	Case 9	Case 10
Philippines	81.97*	99.89	99.07	97.04	86.77*
Basque	98.91	99.50	99.88	98.99	97.25
Italy	99.99	99.99	99.99	98.59	97.53
Poland	98.72	98.93	99.86	98.78	95.76
Portugal	98.84	98.52	99.58	98.39	97.50
Spain	98.19	98.60	99.75	98.25	96.80
US-Cau	99.35	98.67	99.86	98.08	96.88
US-Afr	99.77	99.56	99.74	98.17	99.13
Canada	99.22	98.88	99.80	98.77	98.26
Argentina-C	99.28	98.78	99.60	98.47	97.55
Argentina-M	99.98	99.55	99.98	98.97	99.84
Argentina-W	99.98	99.52	99.99	99.70	99.73

+ Population databases include the Philippine (NCR); autochthonous Basque region; Brescia region of North Italy; Pomerania-Kujawy region of Poland; North Portugal; Northeast Spain; USA Caucasoid and African American populations; French Caucasoid population of Quebec, Canada; and Caucasoid (Buenos Aires), Mapuche (Rio Negro Province), and Wichi (Salta Province) populations of Argentina;
* Values are less than the minimum legally accepted W (=95.0%).

Table 5. Comparison of probability of paternity (motherless) $W_{-mother}$ values calculated using 12 population databases and five STR markers

Population databases+	Probability of Paternity $W_{-mother}$				
	Case 6	Case 7	Case 8	Case 9	Case 10
Philippines	72.20*	98.40	93.57*	81.81*	87.42*
Basque	97.91	96.67	99.44	96.13	95.21
Italy	99.99	99.99	99.99	94.00*	92.44*
Poland	94.38*	94.87*	99.12	94.45*	91.97*
Portugal	94.28*	91.41*	98.63	94.13*	92.11*
Spain	93.49*	90.68*	99.31	91.52*	92.56*
US-Cau	96.87	93.65*	99.27	92.48*	89.63*
US-Afr	98.11	96.66	99.22	90.74*	93.58*
Canada	96.57	91.44*	99.14	93.75*	93.49*
Argentina-C	94.45*	91.01*	98.35	92.95*	89.81*
Argentina-M	99.77	95.62	99.93	95.12	99.05
Argentina-W	99.79	98.93	99.99	98.26	96.86

+ as for Table 4.

to vary significantly from values calculated using the Philippine database. For example, W values in Cases 6 and 10, and $W_{-mother}$ values of Cases 6, 8, 9, and 10 calculated using the Philippine database are less than 95%. In contrast, W and $W_{-mother}$ values obtained using databases other than the Philippine database, were significantly higher than the 95% minimum legal threshold value which in some localities, are sufficient proof of biological paternity.

DISCUSSION

The present study investigated the utility of seven STR markers, namely, D8S306, HUMFOLP23, HUMCSF1P0, HUMvWA, HUMTH01, HUMFES/FPS, and HUMF13A01, in the Philippine population database for resolving paternity disputes of paternity trio and motherless cases (Table 1). Most clients showed a preference for DNA testing of father and child (motherless) to lower cost and reduce conflicts, particularly when the mother is unavailable or refuses to participate in DNA testing. However, DNA results of paternity exclusion and inclusion cases presented here show the decreased capacity of DNA analysis to distinguish fathers from non-fathers when the mother's DNA profile is absent.

Paternity exclusions were definitive in all five cases (Cases 1 to 5) when the mother's DNA profile was included in the analysis (Table 2). To exclude a man as a possible father, many genetic testing laboratories (including ours) require two mismatches between AF and child. False paternity exclusions due to a single mutation across one generation, e.g., from a man to his child have been reported (Mertens et al., 1997). Hence, this guideline was formulated to take into account the possibility of false paternity exclusion due to a mutation. The occurrence of two simultaneous mutations in separate locations of the father's DNA in a single meiotic event is highly improbable, and the presence of mismatches in two STR loci is considered sufficient to prove non-paternity (W=0%).

When cases 1-5 were analyzed as simulated motherless cases, two out of the five paternity exclusion cases presented here (Cases 2 and 3) had inconclusive results. The absence of at least two mismatches between AF and child in both cases is due to the presence of common alleles between the AF and the child's mother (Fig. 1b). Hence, the maternal alleles in both instances were erroneously identified as paternal alleles and the AF in each case could not be excluded. Generally, the number of excluding markers is lower in motherless cases (Table 2).

Although a mismatch in a single STR locus does not automatically exclude the AF in Case 3, this sole mismatch is more consistent with non-paternity (exclusion) than paternity (inclusion). To rule out the probability of a mutation, further testing, such as the use of additional STR markers or testing the mother's sample, must be conducted. In this instance, the initial result supporting non-paternity was confirmed when the maternal genotype was included in the analysis (Table 2).

Case 2 qualifies as a paternity inclusion case since no mismatch was detected in all seven STR loci without the mother's DNA profile, albeit the $W_{-mother}$ value (=73.26%) is low. The low $W_{-mother}$ value is due to shared alleles of the AF and the child, which commonly occur in the Philippine population. The result of the

DNA tests for Case 2 is particularly significant since the AF was charged with sexually assaulting a mentally-retarded woman, resulting in the birth of a child. Without the maternal genotype, the AF would not have been excluded as a suspect and his conviction for a heinous crime was highly probable, although in this case, clearly erroneous.

In the five paternity inclusion cases, the W values are greater than the minimum legally accepted value of 95.0% (Table 3). However, the absence of the mother's DNA profile decreases the probability of paternity estimates ($W_{-mother}$) that in some instances would significantly affect the result of DNA tests. Since without the maternal genotype, it is not possible to assign a paternal and maternal allele in the child's genotype, the equation to derive $W_{-mother}$ assumes the paternal allele to be either one of the alleles found in the child (Brenner, 1993). Due to the additional uncertainty in the identity of the paternal and maternal alleles in the child's genotype, $W_{-mother}$ values in cases 6 and 9 were less than the minimum legally accepted value of 95.0%. In these two cases, the issue of paternity was resolved only upon submission of the mother's sample for further testing.

However, it is worth noting that in the remaining six cases (excepting Cases 2, 3, 6, and 9), DNA tests on the AF and child were sufficient to exclude a non-father (Cases 1, 4, and 5) or to predict probable paternity (Cases 7, 8, and 10). For developing countries such as the Philippines, where the cost of DNA testing is prohibitive, initial routine analysis of AF-child samples in at least seven STR markers is recommended. In the absence of the required two mismatches (probable paternity inclusions), or $W_{-mother}$ value $\geq$ 99.0%, the mother's sample should be obtained for testing. Because of the uncertainty introduced by the unfeasibility of assigning paternal and maternal alleles in motherless cases, setting the minimum $W_{-mother}$ at 99.0% is recommended. Likewise, it is also ideal that minimum value of W should be set at 99.0% to reduce the risk of false matches. When W=95%, there exists a 5% probability that the matching profiles of the alleged father and child may be attributed to chance, for example Case 2. A higher minimum W value (=99.0%) prior to the presumption of paternity requires testing to be conducted using additional STR markers in some cases. Work is underway in our laboratory to add more markers to the existing database in order to increase the discriminatory power of routine DNA tests.

The use of the appropriate population database to evaluate W and $W_{-mother}$ is also important. The Philippines is a multicultural country, with over 100 ethno-linguistic groups, all of which belong to the Austronesian family of language groups (Hagelberg et al., 1999), a rich history of Spanish, American, and Japanese colonization and a large Chinese population. Due to their unique cultural background and colonial history, the profile of Filipinos is likely to differ from that of other more well-studied races such as Caucasians and Blacks, and this may affect the results of DNA tests. In two of the five cases presented here (Cases 6 and 10), the use of non-Philippine databases resulted in W estimates that were significantly higher than those calculated using the Philippine database (Table 4). In these cases, which can be seen as false positives, use of non-Philippine databases to evaluate the results of DNA analysis leads to the recommendation that the alleged father is the biological father of the child. False positives can be attributed to variation in the distribution of alleles in different populations; since W values are dependent on allelic frequencies, high allele frequencies result in lower W values, while low allele frequencies give rise to higher W values. For example, allele 9 is the most common allele at locus HUMTH01 in Filipinos (frequency = 0.3933), whereas this allele is not common in other populations included in the present study (frequency = 0.0140 to 0.2100). Sharing of alleles between alleged father and child that are common in Filipinos, but relatively uncommon in other populations explains the significant variation in W values in Cases 6 and 10.

As a result of the decrease in the discriminatory power of DNA tests without the mother's DNA profile, variations in $W_{-mother}$ become more pronounced as a result of using different databases (Table 5). In all five cases presented here, the alleged father may or may not be the father of the child depending on the population database used to calculate $W_{-mother}$. These results support the use of the Philippine genetic database as a reference database for DNA-based

paternity testing in the Philippines, particularly in DNA tests for motherless cases.

CONCLUSION

The present study demonstrated the utility of a seven-STR Philippine genetic database to resolve ten questioned paternity issues. Results of the DNA tests conducted on complete paternity trio cases either supported paternity (W values are greater than the minimum legally accepted value of 95.0%), or excluded the AF as the biological father of the child due to the presence of at least two mismatches. However, DNA results of exclusion and inclusion cases show the decreased capacity of DNA analysis to distinguish fathers from non-fathers when the mother's DNA profile is absent. Hence to reduce the overall cost, it is recommended that initial tests should be conducted on AF-child pairs to screen for at least two mismatches or $W_{\text{-mother}} \geq 99.0\%$. If the results remain inconclusive, further testing to include the mother's sample should be conducted to increase the level of certainty of the DNA tests. Moreover, due to variations in the allelic frequencies observed in different populations, it is highly recommended that a Philippine genetic database be used as the reference database for paternity determination in the Philippines.

There is no law regulating the use of DNA-based paternity testing for paternity trio and motherless cases in the Philippines. However, more and more cases have been filed in court requesting DNA tests. Clearly, current legislation for paternity determination needs to be amended to incorporate scientific advances in the field of DNA-based paternity testing. Recommendations such as those presented here will be used in the formulation of appropriate national legislation that reflect the current developments in DNA-based paternity testing in the Philippines.

ACKNOWLEDGMENTS

This work was funded by the Natural Sciences Research Institute, University of the Philippines. The authors would also like to thank Ms. Liza P. Faustino for technical assistance and Ms. Nota F. Magno for editing the manuscript.

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