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The Philippine Genetic Database of Short Tandem Repeats (STR) in DNA-based Paternity Testing

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The utility of the Philippine genetic database for paternity testing was investigated. The seven Short Tandem Repeat markers used were HUMvWA, HUMTH01, HUMCSF1PO, HUMFOLP23, HUMFES/FPS, HUMF13A01 and HUMD8S306 with a combined Power of Paternity Exclusion of 99.17%. The Probability of Paternity (W) of 50 volunteer mother-child-father (family trios) calculated using the DNAView™ software ranges from 96.49% to 99.99%. The range of the Probability of Paternity ($W_{-mother}$) of the same 50 volunteer families, albeit excluding the mother's DNA was 79.76% - 99.99%, which was lower than the range of W values.

A mismatch possibly due to an insertion or deletion of a tetra-nucleotide repeat was detected at the HUMD8S306 locus in one family. This result highlights the need for at least two DNA mismatches prior to positively excluding a man from being the father of a child.

DNA testing of the 50 family trios and simulated motherless cases (man-child) was compared using 11 non-Philippine population databases. Variations in W reflected the variation of the allelic distributions of the STR markers included in these databases which, in some instances, could lead to false presumptions of paternity. The results of the present study underscore the need to implement scientific and legal guidelines for DNA-based paternity testing in the Philippines.

Keywords: inclusions, exclusions, family trios, motherless cases, chance matches

Paternity testing has changed from its early days, when conventional testing was the norm, to the current DNA-based analysis using Restriction Fragment Length Polymorphisms (RFLP) (Jeffreys et al. 1985) and/or the Polymerase Chain Reaction (PCR) analysis. DNA-based paternity testing has proved useful in resolving disputed parentage issues in criminal and civil cases, e.g. claims for child support, inheritance disputes or immigration. DNA-based systems offer a higher exclusion power, so that the chance of falsely including a non-father is minimized (Markowicz et al. 1990) and a more efficient inclusion probability for the identification of true biological fathers (Chakraborty & Stivers 1996) than conventional testing. DNA testing also allows greater flexibility in terms of the types of sample amenable to testing. Consequently blood, hairs, tissues, buccal swabs, and exhumed materials may all be used for analysis (Chakraborty & Stivers 1996). In addition, DNA-based systems are unaffected by blood transfusion (Huckenbeck & Rand 1994).

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DNA typing relies on the fact that eukaryotic DNA contains up to 100 times more non-coding regions than coding regions, much of which is repetitive (Butler 2001). Currently, the most widely-accepted procedure for DNA typing involves the PCR amplification of Short Tandem Repeat (STR) markers (Gill et al. 2000). STRs are localized repetitive DNA sequences, 2 to 6 base pairs (bp) long that occur in tandem arrays. The human genome contains about 50,000 dinucleotide (2 bp) and 300,000 trinucleotide (3 bp) and tetranucleotide (4 bp) STRs. The number of repeats is highly variable among individuals making STRs very useful genetic markers for human identification (Li 1997).

STR analysis has several advantages over other DNA-based methods, including high genetic variability with some STR loci having as many as 30 alleles and the use of low molecular weight fragments that facilitate accurate sizing. STR analysis does not require highly purified or large quantities of DNA and validated procedures for analysis are readily available (Butler & Reeder 1997). Hence the use of STR in routine casework is well established in crime laboratories such as the UK Forensic Science Service (FSS) and the US Federal Bureau of Investigations (FBI).

DNA profiling to support paternity requires that the DNA profile of the child must be consistent with the DNA profile of the alleged father. A mismatch suggests that the alleged father is excluded as the biological father of the child. However, a match between the DNA profile of the alleged father and the child does not necessarily establish paternity, but may be attributed to chance matches of common alleles between totally unrelated individuals. To estimate the likelihood that a match is due to paternity over non-paternity (chance matches), a Probability of Paternity W is calculated (Weir 1996). W is used in many Courts of Law where W estimates greater than the legally accepted minimum value of W create a presumption of paternity. The minimum accepted value of W ranges from 95% in New York (USA) to 99.9% in Louisiana (USA) (National Conference of State Legislatures, www.cga.state.gov/). To date, no law regarding the minimum acceptable value of W exists in the Philippines.

The construction of the population STR database of the National Capital Region (NCR), the country's main urban center, was reported previously (Halos et al. 1999). Of the eight microsatellites available in the database, we routinely use seven STR markers (HUMD8S306, HUMFOLP23, HUMTH01, HUMvWA, HUMCSF1P0, HUMFES/FPS and HUMF13A01) to analyze samples obtained from the alleged father, the child and the mother. Another STR marker, HUMTPOX that was included in this database is not ideal for DNA typing in the Philippines due to its relatively low genetic polymorphism, with one allele (repeat 8) having a frequency of 0.468. The ninth microsatellite, D1S80 is a Variable Number Tandem Repeat Locus (VNTR), and is characterized by longer repeats (16 bp) (Hayes et al. 1995). DNA typing using D1S80 involves different and more cumbersome procedures, e.g. use of slab gels and silver-staining, and hence are not routinely used in paternity testing.

To make testing more affordable, it was suggested that DNA testing of man-child samples without the mother's DNA be adopted. Hence, the objectives of the present study are to evaluate the utility of the Philippine database in confirming paternity of 50 volunteer families using seven STR markers for family trio and simulated motherless cases. The present study also seeks to compare the use of the Philippine database against 11 other population databases for paternity testing in the Philippines.

Materials and methods

Sources of samples

Fifty volunteer family trios (father-mother-child) from the National Capital Region (NCR) were included. These families were recruited based on common knowledge on the part of the researchers that these were true families. Motherless cases were simulated using only the DNA profiles of the father and child for statistical analysis.

DNA extraction

Blood samples were collected from the father, the mother and the child and blotted directly 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 fluorescence primers (GenSet Oligos, Singapore) were used. For each 25 µL reaction, two FTA™ discs (2mm in diameter) were placed in a 0.2 mL PCR tube and amplified as described earlier (Halos et al. 1999).

DNA fragment analysis

Separation of amplified products was performed using a High Resolution ReproGel™ (Amersham Pharmacia Biotech, Sweden) and the ALFexpress™

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).

Paternity Analysis using the Philippine Population Database

The Power of Paternity Exclusion (PE) for seven STR loci was determined based on the equation $PE_{n\ loci} = 1 - \Pi(1-PE_i)$ (Brenner & Morris 1989). The Probabilities of Paternity (W) of 50 family trio volunteers and 50 simulated motherless cases were calculated using DNAVIEW™ software (Brenner 1997). The Probabilities of Paternity in trio and motherless cases were designated as W and $W_{-mother}$ respectively.

Paternity Analysis of Random Pairs with Common Alleles

The sharing of common alleles between unrelated individuals included in the Philippine (NCR) database in seven loci was investigated. These randomly matching pairs were treated as motherless cases and the probability of paternity based on shared alleles was calculated as described by Brenner (Brenner 1993). The $W_{-mother}$ of these pairs were compared with the minimum $W_{-mother}$ value accepted in some courts (95.00%) and with the normal value accepted by many DNA testing laboratories (99.00%) (Butler & Reeder 1997).

Calculations of Probability of Paternity using various population databases

The W and $W_{-mother}$ of each of the 50 volunteer family trios 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. 1998), Brescia region of North Italy (Cerri et al. 1998), Pomerania-Kujawy region of Poland (Misciccka-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 (Salaet 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 HUMD8S306 and HUMFOLP23 markers were not available; hence W and $W_{-mother}$ values were calculated using only the DNA profiles of the 50 families in five STR markers. These 11 population databases were grouped as nonPhil, and the modal W and $W_{-mother}$ values were compared with those of the Philippine database.

Results

Power of Paternity Exclusion (PE), Probability of Paternity for family trios (W) and simulated motherless cases ($W_{-mother}$)

The PE of seven loci was calculated to be 99.17%. The ranges of W and $W_{-mother}$ values were 96.48% - 99.99% and 79.76% - 99.99%, respectively. These values were grouped and summarized in Table 1. Values for W were generally greater than the corresponding $W_{-mother}$ of the man-child pair.

Table 1. Probability of Paternity in 50 Family trios (W) and Father-child pairs ($W_{-mother}$) using the Philippine genetic database in 7 STR markers (HUMvWA, HUMCSF1PO, HUMTH01, HUMFES/FPS, HUMF13A01, HUMFOLP23 and D8S306)

Values	Family Trios (W)		Father-Child pairs ($W_{-mother}$)	
	No.	%	No.	%
<95.00	0	0	13	26
95.00 – 99.00	9	18	19	38
99.01 – 99.99	41	82	18	36
Total	50	100	50	100

Detection of a mismatch between a parent and a child

A mismatch at the HUMD8S306 locus was detected in one family. The paternal or maternal and child alleles responsible for the mismatch differed by a single tetra-nucleotide repeat. This suggests the possibility of an insertion or deletion event resulting in the variant spermic or egg DNA. Since the mother and the father possess identical genotypes at this locus, either parent may be the source of the mismatch. The DNA profiles of this family trio at the HUMD8S306 locus are shown in

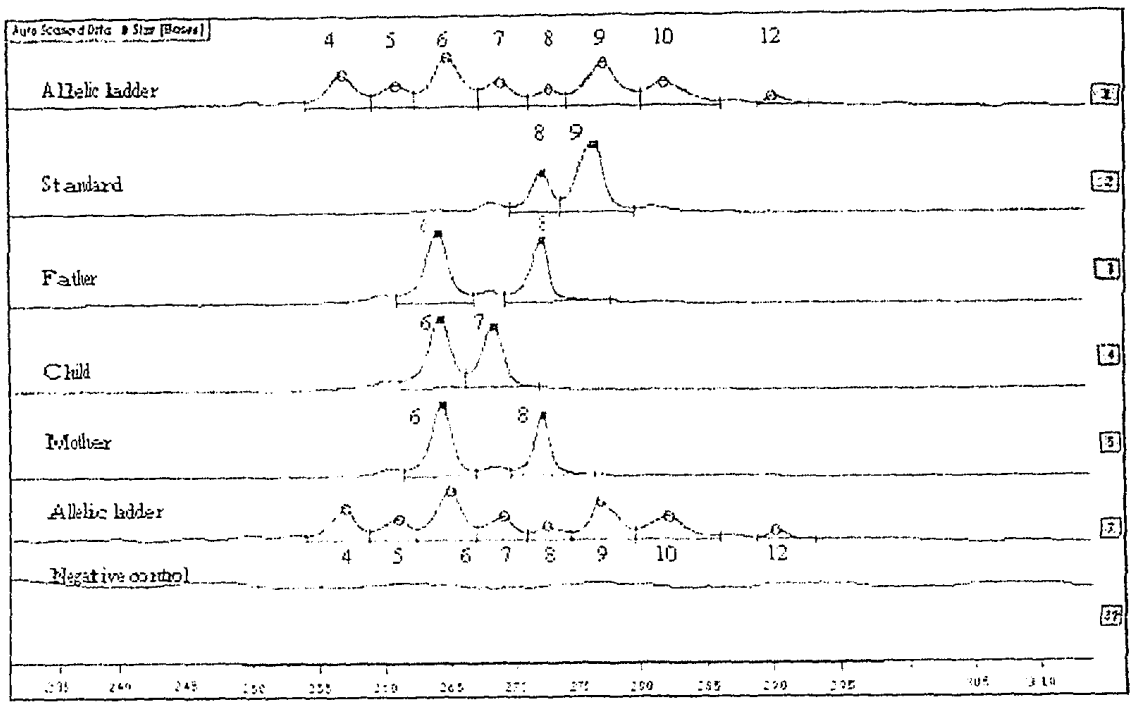

Figure 1. Mismatch detected at the D8S306 locus. ALFexpress™/Allelelinks™ electropherogram showing the profiles of a family trio at STR locus D8S306. The father and mother of this family trio are heterozygous for the same alleles (6 and 8) at D8S306. The child has a genotype of 6/7, which suggests a paternity exclusion. However because the profiles of this family trio matched at six other loci (W=99.70%), it is possible that the mismatch detected at this locus is the result of a recombination event during meiosis leading to the production of spermic/egg DNA with a variant allele. Based on this result, we recommend that mismatches between the DNA of a man and child must be found at two or more loci before the case is declared a paternity exclusion.

Figure 1.

Random sharing of alleles of unrelated individuals in the Philippine database

Direct visual examination of the Philippine database (number of persons n = 103) revealed 195 pairs of unrelated individuals sharing alleles in seven STR markers. These random pairs were treated as motherless cases and the $W_{-mother}$ values were summarized in Table 2.

Table 2. Probability of Paternity $W_{-mother}$ of 195 unrelated pairs with common alleles using the Philippine genetic database in 7 STR markers (HUMvWA, HUMCSF1PO, HUMTH01, HUMFES/FPS, HUMF13A01, HUMFOLP23 and D8S306.

$W_{-mother}$	Man-Child Pairs	
	No.	%
<95.00	142	72.8
95.00 - 99.00	43	22.1
99.01 - 99.99	10	5.1
Total	195	100.0

Probability of Paternity using different population databases

Using 11 population databases, over 11 00 W and $W_{-mother}$ of 50 volunteer family trios in five STR loci were calculated, some of which were found to vary from corresponding values calculated using the Philippine database. Comparisons of W and $W_{-mother}$ values using the Philippine and non-Philippine databases were summarized in Table 3. False positive and false negative values were calculated based on the capacity of the Philippine database to determine paternity compared with non-Philippine databases. The frequency of false positives (W_{Phil} <95 and mod $W_{nonPhil}$>95) ranges from 20% to 42%, as opposed to false negatives (W_{Phil} >95 and mod $W_{nonPhil}$<95), 0% to 6%. These findings highlight the high error rates of DNA testing resulting from the use of an inappropriate population database.

Discussion

The present study investigated the utility of seven STR markers, namely: HUMD8S306, HUMFOLP23, HUMCSF1PO, HUMvWA, HUMTH01, HUMFES/FPS

Table 3. Comparison of Paternity Probabilities in 50 family trios when using the Philippine genetic database (W_{-Phil}) and non-Philippine genetic database (Modal $W_{-nonPhil}$) in 5 STR markers.

W of father-child-mother trios		$W_{-mother}$ of father-child pairs	
Condition	% of trios	Conditiion	% of pairs
When minimum W = 95%			
W_{Phil}[a]<95 and mod $W_{nonPhil}$[b]<95	2	W_{Phil}<95 and mod $W_{nonPhil}$<95	10
W_{Phil}<95 and mod $W_{nonPhil}$>95	20	W_{Phil}<95 and mod $W_{nonPhil}$>95	42
W_{Phil}>95 and mod $W_{nonPhil}$<95	0	W_{Phil}>95 and mod $W_{nonPhil}$<95	4
W_{Phil}>95 and mod $W_{nonPhil}$>95	78	W_{Phil}>95 and mod $W_{nonPhil}$>95	44
Total	100	**Total**	100
When minimum W = 99%			
W_{Phil}<99 and mod $W_{nonPhil}$<99	24	W_{Phil}<99 and mod $W_{nonPhil}$<99	56
W_{Phil}<99 and mod $W_{nonPhil}$>99	38	W_{Phil}<99 and mod $W_{nonPhil}$>99	22
W_{Phil}>99 and mod $W_{nonPhil}$<99	6	W_{Phil}>99 and mod $W_{nonPhil}$<99	2
W_{Phil}>99 and mod $W_{nonPhil}$>99	32	W_{Phil}>99 and mod $W_{nonPhil}$>99	20
Total	100	**Total**	100

[a] W_{Phil}: probability of paternity based on Philippine database;
[b] mod $W_{nonPhil}$: modal value of the probability of paternity based on 11 non-Philippine databases. The STR markers used for database comparisons were HUMvWA, HUMTH01, HUMCSF1 P0, HUMFES/FPS and HUMF13A01.

and HUMF13A01, in the Philippine population database for resolving paternity disputes in family trio and simulated motherless cases.

The Probability of Paternity W values of 50 volunteer families were determined for seven STR markers (Table 1). Assuming a prior probability of 0.5, the lowest W value is 96.48%. Since the lowest legally accepted value for W is 95.00%, the use of seven STR loci for paternity inclusion cases provides W estimates that already support paternity. However, in Courts of Law which require W ≥ 99.0%, a lower W will not lead to a presumption of paternity. In these cases, the alleged father is not excluded as a possible father until more tests are conducted. To increase W values or the possibility of detecting mismatches, the use of more STR markers is required. With the rapid development of DNA technology and the availability of commercial kits, many genetic laboratories are already using multiplex PCR to type samples in 15 STR loci to resolve paternity issues (Gill et al. 2000). Work is underway in our laboratory to validate more polymorphic STR markers for routine analysis.

A single mismatch at the HUMD8S306 was detected in a family trio included in the present study (Fig.1). The father and mother are heterozygous for the same alleles, e.g. 6 and 8 whereas the child has a genotype 6/7 which suggests a paternity exclusion. However, in this case the paternal/maternal allele in the child's genotype differs from the father's/mother's genotype by a single repeat unit, i.e. a single tetranucleotide sequence. This situation suggests the occurrence of a single recombination event that lead to the production of spermic/egg DNA with a variant allele. To further lend support to the assumption of paternity, the samples of this family trio were analyzed in the remaining six STR loci resulting in W=99.70%. Moreover, genetic recombination, e.g. loss or acquisition of a single repeat unit across one generation in established families, has been reported previously (Mertens et al. 1997). The detection of a possible mutation across one generation in the present study is consistent with current requirements in many genetic testing laboratories, including ours, for a minimum of two mismatches prior to the exclusion of a man as the biological father of a child (Gunn et al. 1997). This is based on the assumption that the probability of two recombination events occurring in separate locations of the father's DNA during meiosis would be very low.

The use of more STR markers is of particular importance in cases where the mother's DNA profile is not available or not included in the analysis (motherless cases). Motherless cases are normally requested when the mother of the child is deceased, her location is unknown, when concerned parties want to reduce the cost for DNA testing or when the mother refuses to provide biological samples for testing. Notably, the probability of paternity without the mother ($W_{-mother}$) is generally lower than the corresponding W values in family trios. Of the 50 man-child pairs tested, 26% did not reach the minimum legally accepted value of $W_{-mother}$ = 95.00% whereas all the corresponding W estimates were greater than 95.00% (Table 1). The decrease in the capacity of DNA analysis to distinguish fathers from non-fathers in motherless cases is brought about by the uncertainty in identifying the maternal and paternal alleles in the child's DNA profile.

To further evaluate the feasibility of testing only man-child pairs, the error rate due to chance matches in the Philippine database (n=103) was determined

by screening for possible chance matches between unrelated individuals (Table 2). Of the 5253 possible pair-wise combinations, 3.71% showed putative paternity inclusions, e.g. pairs matching in 7 STR loci due to chance. This value means that four out of every 100 random males (non-fathers) will not be excluded as putative fathers in motherless cases. In contrast, when dealing with family trios, the Power of Paternity Exclusion (PE) is 99.17% (Halos et al. 1999) that means that 1% or one out of 100 non-fathers are not excluded as putative fathers, a four-fold difference from motherless cases. Moreover, 27.18% of the chance matches had $W_{.mother}$ values > 95.00%, which in some Courts of Law are considered to support the presumption of paternity (Table 2). These pairs are unrelated persons based on our laboratory sampling records. The error due to chance matches is decreased (=0.05%) when the admissible value of $W_{.mother}$ is increased to >99.0%.

Based on these results, we do not recommend routine testing of man-child pairs using only the seven STR markers currently comprising the Philippine genetic database. Work is underway to add more markers in the database that would increase the overall discriminatory power and decrease the error rate due to randomly matching pairs. DNA testing of motherless cases may be conducted if there is grave reason, e.g. mother is deceased or her location is unknown, but we recommend that $W_{.mother}$ cut-off should be greater than 99.0% prior to the presumption of paternity. Although this procedure is likely to provide inconclusive results in some instances involving real fathers, the use of a higher cut-off for $W_{.mother}$ reduces the probability of false positives. In our judgement, it is better to arrive at an inconclusive result (false negative) the probability of which is higher when W_{min} is set at 99.0%, rather than obtaining a conclusive result that is erroneous (false positive). The probability of false positives is lower when W_{min} is set at 99.0%. Simply stated, $W_{.mother}$ < 99.0% does not exclude the man from being the father of the child since exclusion requires the occurrence of 2 DNA mismatches. In this instance, evidence other than DNA, e.g. existence of relations between man and child's mother, must be considered by the Court to evaluate paternity. In contrast, when minimum value is set at $W_{.mother}$ = 95%, false positives would immediately create a presumption of paternity that is difficult to contest, and is therefore not acceptable. To resolve this issue, work is underway to validate more STR markers that would increase the utility of the Philippine database for statistical evaluations of motherless cases.

Another important aspect that needs to be addressed is the use of the appropriate population database to evaluate W and $W_{.mother}$. The Philippines is a multicultural country, with over 100 ethno-linguistic groups (Hagelberg et al. 1999), a rich history of Spanish, American and Japanese colonization and a large Chinese population. Due to its particular cultural background and colonial history, it has been hypothesized that the genetic profile of the Filipino people is likely to differ from that of other races. In 50 volunteer family trios, a high percentage of false positives equal to 20% (W_{Phil} <95 and mod $W_{nonPhil}$>95) and 38% (W_{Phil} <99 and mod $W_{nonPhil}$>99) were identified when minimum W was set at 95% or 99% respectively (Table 3). The occurrence of false positives is attributed to variation in the distribution of alleles in different populations since W values are dependent on allelic frequencies. For example, allele 9 is the most common allele at locus HUMTH01 in Filipinos (frequency = 0.3933), whereas this allele is not common in the other populations included in the present study (frequency = 0.0140 to 0.2100). Common alleles result in lower W values. Moreover, when the minimum W was set at 99%, the error derived from the use of the wrong database in testing family trio cases increased from 20% to 38%.

On the other hand, the presence of alleles that are common in other populations but not equally common or even rare in Filipinos leads to false negatives. For example, allele 7 of HUMF13A01 is one of the most common alleles in the 11 non-Philippine databases (frequency = 0.2218 to 0.3990) included here, whereas this allele is rare amongst Filipinos (frequency = 0.0240). In family trios presented here, false negatives (W_{Phil} >99 and mod $W_{nonPhil}$<99) were detected (=6%) when minimum W was set at 99% compared to when minimum W was lowered to 95% (W_{Phil} >95 and mod $W_{nonPhil}$<95) when no false negative was identified. False negatives do not pose matters of great concern in paternity testing. A false negative does not mean that the alleged father is excluded as the real father, but rather that further tests or other evidence is required prior to the presumption of paternity.

The results presented here support earlier studies that suggested the occurrence of population-specific genetic profiles (Smouse & Chevilion 1998) by focusing on the effect of allelic variations in paternity testing results using DNA technology. To avoid errors (false positives and false negatives) resulting in the use of foreign databases, it is necessary to use the Philippine genetic database for paternity testing in the Philippines.

Conclusion and Recommendations

In conclusion, our results support the utility of the seven STR database of the Philippine population for DNA-based paternity testing of family trio and (to some extent) motherless cases. Clearly, with the rapid development of DNA-based paternity testing, guidelines for the appropriate DNA analysis of complete family trio and motherless cases, and the subsequent legal acceptance of testing results as proof for the presumption of paternity, must be put in place in Philippine Courts of Law. DNA testing of motherless cases should be accepted only when there is grave reason, e.g. when the mother is deceased or when her location is unknown. It should also be made clear that although motherless cases can be tested, the percentage of non-fathers that is not excluded is higher than when the mother's DNA profile is included. Hence results derived from DNA testing of motherless cases should carry less weight in Court compared to those derived from DNA testing of complete family trios. In this instance, evidence other than DNA, e.g. man's access to child's mother, existing relation between man and child's mother, a man's sperm count and whether man has undergone vasectomy prior to the birth of the child, must be considered by the Court to evaluate the issue of paternity.

We have also shown that variation in the distribution of alleles in different populations included in the present study affected the W and $W_{.mother}$ estimates that can potentially lead to erroneous conclusions. It is therefore necessary to use the appropriate population database for estimating W and $W_{.mother}$ values. This is particularly important when paternity testing is performed for immigration purposes, when the individuals involved are migrants to the area or in places where a population database is as yet to be established.

The results of the present study will be used in the establishment of specific scientific and legal guidelines for DNA-based analysis of complete family trio and motherless cases using the genetic database of the Philippine population. With the increasing demand for DNA-based paternity testing, DNA evidence will inevitably be utilized to support or argue against paternity in Courts of Law. Clearly, empirical data such as those presented in the current study are needed to better outline procedures for the use of the Philippine population database for DNA-based paternity testing in the Philippines.

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