Int J Legal Med (2011) 125:817–824
DOI 10.1007/s00414-010-0535-4

ORIGINAL ARTICLE

Y-STR DNA analysis of 154 female child sexual assault cases in the Philippines

Sheena Marie B. Maiquilla · Jazelyn M. Salvador · Gayvelline C. Calacal · Minerva S. Sagum · Miriam Ruth M. Dalet · Frederick C. Delfin · Kristina A. Tabbada · Shiela Ann Edith L. Franco · Henry B. Perdigon · Bernadette J. Madrid · Merle P. Tan · Maria Corazon A. De Ungria

Received: 13 July 2010 / Accepted: 18 November 2010 / Published online: 3 December 2010

Abstract The laboratory evaluated 154 sexual assault cases from four Child Protection Units in the Philippines involving female child victims aged from 2 years to 18 years old. All child victims sought medical attention within 72 h after sexual contact. In 130 cases, the child victim knew the alleged offender and identified them during the interview with the social worker. Penile ejaculation was reported by 68 child victims with varying reports of washing after contact. Overall, 84 child victims admitted having wiped their genitalia prior to the collection of biological samples for DNA testing. Laboratory personnel examined vaginal smears in only 109 cases using a light microscope and reported 23 samples to be positive for sperm cells. Using the PowerPlex® short tandem repeat of the Y chromosome (Y-STR) DNA multiplex system, male DNA was detected in vaginal swab samples from 63 child victims. In 39 cases, positive amplification at 11 Y-STR DNA markers consistent with a single male DNA profile was observed. Twenty-eight of these full single Y-STR DNA profiles were found to be unique when searched in worldwide Y-STR DNA population databases (~40,000 haplotypes), eight haplotypes matching Filipinos and/or Asian haplotypes and one Y-STR DNA profile only matching European, Caucasian, and Latin American haplotypes. Y-STR DNA profiles generated will be compared with reference DNA profiles of alleged offenders once reference samples are submitted to the laboratory.

Keywords Child sexual assault · Vaginal swabs · Y-STR DNA typing · Female sexual abuse · Child protection unit · Philippines

S. M. B. Maiquilla · J. M. Salvador · G. C. Calacal · M. S. Sagum · M. R. M. Dalet · F. C. Delfin · K. A. Tabbada · S. A. E. L. Franco · H. B. Perdigon · M. C. A. De Ungria (✉)
DNA Analysis Laboratory, Natural Sciences Research Institute, Miranda Hall, University of the Philippines, Diliman, 1101 Quezon, Philippines
e-mail: mariadeungria@gmail.com

B. J. Madrid · M. P. Tan
University of the Philippines Manila Child Protection Unit, Philippine General Hospital, Taft Avenue, 1000 Manila, Philippines

B. J. Madrid
Department of Pediatrics, College of Medicine, University of the Philippines, Taft Avenue, 1000 Manila, Philippines

Introduction

Sexual assault of children is the most frequently reported type of child abuse in Southeast Asia [1]. In the Philippines, approximately 40% of child abuse cases reported to the Department of Social Welfare and Development (DSWD) from 1998–2007 involved sexual abuse of female victims [2]. When available, DSWD referred cases to designated Child Protection Units (CPU) of participating hospitals for medico-legal examination and treatment. In 2007 alone, 4,456 children had been evaluated by 24 CPUs nationwide [3]. Majority of cases do not proceed to trial because victims choose not to testify against the perpetrator, many of whom are related to them. Since cases are litigated largely based on testimonial evidence resulting in trials that take years to finish, victims and their families do not usually have the economic resources to finance the practical costs of pursuing their cases in court [4]. On the other hand, using DNA evidence that was generated after trial and evaluated post-conviction, one case involving the rape of a minor that

led to the conviction of the accused was shown to have been the product of erroneous identification [5]. Hence, there is a need to include other types of evidence, such as DNA evidence, as part of the routine litigation of sexual assault cases in the Philippines in order to accelerate the identification of the real perpetrators of abuse of child victims and to prevent wrongful conviction of innocent persons.

When biological samples are available during sexual assault investigations, male-specific short tandem repeat DNA (STR-DNA) markers located on the Y chromosome have been found to be useful because victims are usually female, while offenders are nearly always male [6]. A set of short tandem repeat of the Y chromosome (Y-STR) DNA that is used for male identification includes, but is not limited to, the core set of markers, namely, DYS19, DYS385a/b, DYS389 (I), DYS389 (II), DYS390, DYS391, DYS392, and DYS393, is commonly used to achieve a reasonably high level of discrimination [7, 8] and has been included in a number of worldwide reference Y-STR DNA databases maintained by several groups [9–11]. DNA kits, such as PowerPlex® Y (Promega Corporation) and AMPFlSTR® Yfiler® (Applied Biosystems Incorporated), which are commonly used for genotyping also include additional Y-STR DNA markers to increase the combined power of discrimination of the tests.

When handling young victims, medical examiners have observed significant delays in incident reporting and increased likelihood of loss of evidence through washing or bathing [12] that may affect the successful recovery of offender's DNA. In the Philippines, vaginal swab samples collected from victims 7 years old and younger involved the use of calcium alginate or 'calgi' swabs because of their fine tips and flexible handles that would make the collection of samples from the children's intimate parts less painful [13]. However, it is not known whether sufficient biological samples for Y-STR DNA profiling could be obtained using calgi swabs. In the present study, using PowerPlex® Y multiplex DNA typing kit, the laboratory evaluated the amplification of male DNA, if present, in biological samples collected from 154 child victims within 72 h post-contact. We described victim, offender, and other sexual assault incident characteristics to understand how these factors may affect the success of Y-STR DNA typing for the identification of the real perpetrator of the abuse and the exclusion of those who may have been erroneously identified.

Materials and methods

Collection of data and samples

From January 2002 to March 2006, vaginal swab samples from 154 female child victims who reported sexual contact within 72 h were collected at four Child Protection Units (CPUs). All child victims came from families with very low socio-economic status, as evaluated by the social worker who conducted the initial interview. Samples were collected from victims aged 2 to 18 years old. For children 7 years old and below, calcium alginate ('calgi') swabs were used while standard cotton-tipped pledgets were used for older victims. Oral swabs were also collected from victims as a source of reference DNA. All samples were submitted to the DNA Analysis Laboratory, Natural Sciences Research Institute, University of the Philippines together with the following information:

- Age of victim
- Time interval between the sexual assault incident and medical examination
- Victim's hygienic practice after sexual assault (genital wash or wipe)
- Reported number of offender(s)
- Relationship of offender(s) to the victim
- Presence and type (internal or external) of perpetrator's penile ejaculation in relation to the victim's person
- Cytological presence of sperm cells observed under a light microscope

Child victims and/or their legal guardians gave their consent for inclusion in the study.

DNA typing

DNA from vaginal swab samples was extracted using a one-step organic extraction procedure [13, 14]. Vaginal swab DNA was amplified in duplicates at 11 Y-STR DNA markers using PowerPlex® Y multiplex kit (Promega Corp., WI, USA) following manufacturer's recommendations. PowerPlex® Y multiplex system includes 11 Y-STR DNA markers namely, DYS391, DYS389 (I), DYS439, DYS389 (II), DYS438, DYS437, DYS19, DYS392, DYS393, DYS390, and DYS385a/b. DNA fragments were analyzed using the ABI Prism® 310 Genetic Analyzer with GeneScan® 3.7 and Genotyper® 3.7 softwares for automatic allele calling (Applied Biosystems, CA, USA). Single full profiles were searched in local [15, 16] and international [9–11] Y-STR DNA population databases (total of ~40,000 haplotypes).

To perform initial autosomal STR-DNA comparisons in the absence of reference samples from suspect/s, DNA from the victims' reference oral swabs was extracted using a standard organic extraction method [13, 14]. DNA from reference oral swabs and vaginal swabs were amplified at the autosomal STR marker HUMvWA as described by Halos and co-workers [17]. Fragment analysis was performed using the ALFexpress™ DNA sequencer with AlleleLinks™ software (Amersham Pharmacia Biotech, Uppsala, Sweden) using in-house allelic ladders [17].

Statistical analysis

Descriptive statistics of the cases are reported. Data were analyzed using GraphPad Prism® version 5.03 (GraphPad Software Inc., CA, USA). Data was summarized into 2×2 contingency tables and analyzed using Fisher's two-tailed exact test to evaluate the presence of association among factors initially believed to influence DNA results and the successful genotyping of male DNA in the vaginal samples. Factors tested include the effect of the victim's age, the time interval between contact and medical examination, the victim's hygienic practice after contact, the number of offenders reported by the child, the relationship of offender to child, the presence or absence of penile ejaculation, and sperm detection via light microscopy, on successful amplification of Y-STR DNA. For the purpose of statistical comparisons, 'successful Y-STR DNA analysis' was arbitrarily defined as repeated positive amplification of at least one Y-STR DNA marker. 'Unsuccessful Y-STR DNA typing' refers to DNA analysis that resulted in negative amplification. The children were also grouped into age <10 and age≥10 to represent the stage of prepuberty and puberty, respectively, based on the study of Christian and co-workers [18]. For time interval between last contact and medical examination, the samples were grouped into those that reported the contact within 24 h and those that reported the incident after 24 h. Case reports with missing information, e.g., child victim did not provide an answer, the particular case was removed for that particular analysis. P value <0.05 was considered statistically significant.

Results

Demographics and microscopic detection of sperm

Of the 154 child victims, 29 (18.8%) were <10 years old (prepubertal age), and 125 (81.2%) were 10 to 18 years old (Table 1). Majority of the children were examined within 24 h after sexual contact (68.8%). Information regarding ejaculation was recorded for 85 children, with 68 children (44%) reporting that the perpetrator ejaculated. Eighty-four children (54.6%) washed themselves after the assault. Sperm was detected via microscopic examination in 23 cases (15%). No sperm was detected in 86 cases (56%). In the remaining 45 cases (29%), cytological presence of sperm was not determined.

Suspect identification

Child victims were able to identify a suspect/s in 143 cases (92.8%). In 38 cases, the alleged offender/s was/were biologically related to the victim, i.e., father, brother, uncle, or cousin, or by marriage of the suspect to a family member, i.e., brother-in-law, mother's partner. One hundred twenty-six victims (81.8%) reported to have been assaulted by one person, while 25 victims (16.2%) claimed to have been assaulted by at least two individuals. Three victims (1.9%) did not know the number of persons responsible for the assault.

Y-STR DNA analysis

Male DNA was detected in vaginal swab samples collected from 63 children (41%) using Y-STR DNA analysis. Thirty-nine samples showed a single peak at 11 Y-STR DNA markers (except DYS385) which was consistent with a single male DNA profile (Table 2). In 12 of these 39 cases, child victims identified two or more perpetrators. In one report (case 78), the child victim identified the perpetrators as her father and a male cousin. It was not clear if the cousin was paternally related to the child's father which could explain the single male DNA profile that was detected.

Twenty-eight Y-haplotypes were found to be unique when searched in local [15, 16] as well as in international Y-STR DNA population databases [9–11]. In contrast, in four cases involving unrelated child victims who reside in different communities (cases 55 and 59; and cases 120 and 122), the sole male DNA profile detected at 11 Y-STR DNA markers were identical. The Y-STR haplotypes detected in cases 55 and 59 as well as case 109 were already found in the Philippine database whereas the haplotypes in cases 26, 49, 77, 111, and 149 were also detected in Asian and/or Philippine datababases. The Y-STR haplotype of case 144 was the only haplotype that did not match an Asian haplotype and was detected in several foreign databases (Caucasian, Hispanic, European, Latin American, and North American).

More than one peak in at least three Y-STR DNA markers other than DYS385 were observed in eight cases (5%) which indicate more than one male source for each of the samples. In two of these cases, the 14- and 16-year-old victims named more than one offender. However, the remaining six child victims, with ages ranging from 12 to 17 years old, only identified a single offender.

Partial profiles in ten or less Y-STR DNA markers out of the 11 markers included in the PowerPlex® Y multiplex kit were found in 16 cases (10.4%). Three cases involved partial mixtures whereas samples of 13 cases were consistent with a single Y-STR haplotype. Comparison of the sizes of Y-STR DNA markers used (except DYS385) showed no significant difference in successful amplification based on allele size of the markers included in the PowerPlex® Y multiplex kit (Fig. 1), albeit the lowest amplification success rate was observed at the longest Y-STR DNA marker (DYS392).

Table 1 Reported victim or assault characteristics and outcome of Y-STR typing

Variables	Y-STR DNA typing				Total (n =154)
	Complete[a] (n=39)	Mixture (n=8)	Partial[b] (n =16)	Negative[c] (n=91)	
Age of victim (years)					
2–3	1	0	0	3	4
4–6	0	0	1	12	13
7–9	1	0	1	10	12
10–12	3	1	1	9	14
13–15	15	3	7	33	58
16–18	19	4	6	24	53
Time between sexual assault and medical examination (h)					
24	29	5	8	64	106
48	6	1	5	19	31
72	4	2	3	8	17
Victim's practice after sexual assault (genital washing)					
With washing	14	6	10	54	84
Without washing	22	2	6	31	61
No data	3	0	0	6	9
Presence and type of offender penile ejaculation					
Internal	16	3	7	29	55
External	2	1	0	10	13
None	3	1	1	12	17
Unknown	18	3	8	40	69
Cytological presence of sperm					
Positive	11	3	2	7	23
Negative	16	4	13	53	86
Undetermined	12	1	1	31	45

[a] Full Y-STR DNA profile at 11 markers

[b] Y-STR DNA profile at 1–10 markers

[c] Negative amplification

No male DNA was detected in 91 (59%) vaginal swab samples tested. When amplified using a singleplex reaction targeting the HUMvWA marker, PCR products were generated for 87 vaginal swab samples that were consistent with the genotype of the child victim's reference oral sample. In two cases that involved a 15- and 16-year-old victim, only the reference oral swabs generated a DNA profile. No DNA was detected in samples tested in two cases.

Factors associated with successful Y-STR DNA typing

To determine whether a victim's background and behavior affect subsequent Y-STR DNA typing, data collected based on the child's report were compiled and compared with DNA profiling results (Table 1). Three factors, namely, age of victim (P value=0.0008), number of offenders identified by the child (P value=0.0006), and sperm detection using microscopy (P value=0.0096) showed significant associations with successful DNA amplification. All other factors tested such as the time interval between contact and medical examination (P value=0.7176), the victim's hygienic practice after contact (P value=0.1252), the relationship of offender to child (P value=0.1780), and the presence or absence of penile ejaculation (P value=0.4112) did not show any significant correlation with successful amplification of Y-STR DNA.

Discussion

Studies on medical examinations of sexual assault cases have been reported previously [12, 18–22]. In this study, the laboratory focused on evaluating factors which may affect successful amplification of Y STR-DNA markers from vaginal swab samples collected from 154 child victims of sexual assault in aid of revising current medico-legal procedures for the examination of children.

All cases included here involved the collection of vaginal swab samples from child victims who were examined within 72 h from the last contact. The 72-h cut-off period was based on data generated from adult victims that showed that sperms survived in the vagina up to 72 h after sexual intercourse [23]. In this report, sperm cells were

Table 2 Summary of single Y-STR DNA profiles generated at 11 markers using vaginal swab samples from sexually assaulted victims

Case no.	Y-chromosome STR markers										
	DYS391	DYS3891	DYS439	DYS389II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385
6	10	12	12	28	10	15	15	13	13	23	12, 17
7	10	12	12	28	10	15	15	13	13	24	12, 17
10	10	12	12	28	10	15	15	12	12	23	12, 17
11	10	12	12	28	10	14	15	15	13	23	13, 14
13	10	13	11	31	10	14	15	11	12	24	19
21	9	13	11	29	10	16	15	13	14	22	12, 16
25	10	13	12	29	10	14	16	11	13	23	12, 19
26	10	12	13	27	10	14	15	14	13	23	13
32	10	12	13	28	11	15	15	13	13	24	12, 16
35	10	13	11	28	10	14	16	12	13	23	15, 17
44	10	13	14	29	10	15	15	13	13	24	12, 16
46	9	11	13	27	10	15	16	13	13	24	13, 16
47	10	13	12	29	10	14	15	13	13	24	12, 15
49	10	12	13	28	10	14	15	14	13	23	12, 13
55 and 59	10	13	13	29	10	14	15	13	12	24	12, 16
58	11	12	13	28	11	14	15	14	12	24	13, 20
66	10	12	11	28	11	15	15	13	12	23	15, 20
77	10	12	13	28	10	15	15	13	13	24	12, 16
78	11	13	12	29	10	14	15	12	12	21	11, 16
84	10	13	12	29	10	14	16	14	14	23	13, 14
91	11	14	11	30	10	14	15	13	15	25	13, 18
92	11	12	11	30	10	14	15	13	11	23	12, 20
93	11	13	12	31	11	15	16	13	14	25	14, 18
95	9	12	13	29	10	14	15	13	14	24	12, 16
100	11	13	13	30	10	14	16	13	13	24	13, 19
105	10	12	12	29	10	14	16	14	13	23	14
109	10	12	12	29	10	15	15	13	13	24	12, 16
111	10	12	12	28	10	15	15	13	13	24	12, 16
114	10	13	10	29	10	14	17	15	13	23	10, 15
120 and 122	10	12	13	28	10	14	15	13	13	25	12
124	11	13	12	31	11	15	13	13	13	26	13, 17
130	10	12	11	28	10	14	16	14	13	23	13
135	11	13	11	30	10	14	16	14	13	23	13
136	11	13	11	29	10	14	15	13	14	25	13, 19
139	11	13	12	29	10	14	17	14	13	22	13
144	11	14	12	30	12	15	14	13	13	23	11, 14
146	10	14	11	30	9	15	14	13	13	24	12, 19

detected in vaginal smears prepared from swabs collected 72 h from two 17-year-old victims (data not presented) which was consistent with the earlier study. Using light microscopy, no sperm cell was detected in samples collected from younger children within 48 or 72 h post contact. Because of the limited sensitivity of microscopic detection of sperms [12], particularly in samples collected from children more than 24 h after sexual intercourse, DNA technology provides a more promising alternative. DNA testing not only detects the presence of male DNA in female children but also provides a DNA profile for more accurate comparisons with a reference DNA profile of suspect/s. However, the use of light microscopy remains to be useful as a preliminary screen for the detection of sperm cells prior to DNA testing. Data derived from analyzing 109 vaginal swab samples which were examined under light microscopy showed a significant correlation between positive sperm detection and successful amplification of male DNA.

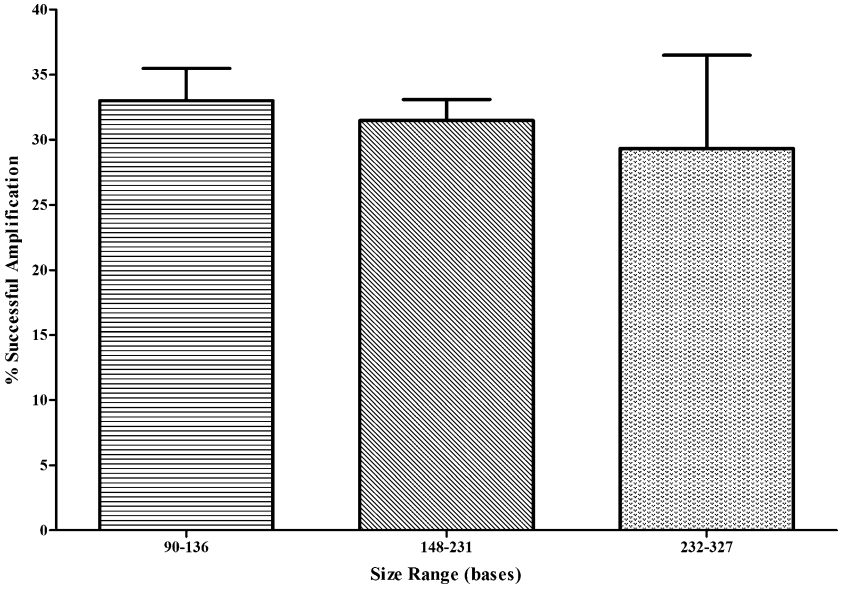

Fig. 1 Size range of Y-STR DNA markers versus rate of successful amplification. Size range 90–136 bases include DYS391, DYS438, and DYS393; 148–231 bases include DYS389I, DYS437, DYS393, and DYS439; 232–327 bases include DYS19, DYS389II, and DYS392. Percentage of successful amplification per size range is the average of the individual marker success rates included in that size range. *Lines on top of the bars* represent the binomial 95% upperbound

To date, DNA technology is not incorporated in routine examinations of children, particularly prepubescent children in the Philippines. Problems due to delays in reporting, lack of trained medical personnel and facilities for the collection and storage of biological samples for DNA testing, unavailability of sexual assault investigation kits in most government hospitals, the reliance of trial courts on testimonial evidence rather than scientific evidence, and the high cost of DNA testing contribute to the continued minimal utilization of this powerful technology for the identification of the real perpetrator/s of the crime and the extent of the abuse. In fact, none of the 154 cases submitted a reference sample from any of the suspect/s that was collected during the investigation or when the case was filed in court, if at all.

DNA technology is able to provide objective evidence to support a child's allegation of sexual contact. In 63 cases (41.9%), the children's vaginal swab samples were positive for male DNA, regardless of whether the child was able to identify a suspect or not. In fact, in 11 cases, the child victims were not able to identify a suspect. Full Y-STR DNA profiles were generated in seven of these cases without a suspect, including one which involved a 3-year-old child (case 21). Interestingly, a calgi swab was used to collect the sample from this child who was not able to provide information as to the number of offenders, the time and location of the incident, and the manner of ejaculation, if any, of her assailant. The child only reported that she did not wash herself after the incident. Another calgi swab sample collected from a 6-year-old child provided a partial DNA profile at two Y-STR DNA markers (case 118; DYS437 and DYS438). The partial amplification of Y-STR DNA markers in the latter sample necessitates further optimization of procedures to increase the success rate when handling this type of samples collected from very young children.

The use of calgi swabs may partly explain the significant decrease in successful amplification observed in younger children. Medical examiners use a blind swabbing technique when collecting samples from prepubescent children to avoid hurting the young victims, which could decrease the amount of recovered DNA. The shortened survival period of sperm cells and semen on the genitalia of prepubertal children because of the absence of cervical mucus may also be a contributing factor [18].

Although the data consisting of one full (case 136, 8-year-old child) and two partial DNA profiles (case 8, 9-year-old child; and case 118) support the recommendation of Christian and co-workers [18] that children should be swabbed for evidence provided the incident was reported within 24 h post contact, the concept of time may not be clear to some children (case 21). Hence, in situations when young children could not provide information or are uncertain on the actual time of the last contact within the 72-h cut-off time, we still recommend that the children be swabbed and the swabs be submitted for DNA typing. Statistical analysis of the data shows a lack of significant correlation between time interval and successful Y-STR DNA amplification. DNA amplification is therefore not dependent on the time interval between contact and examination, provided that the report was made within 72 h after the incident.

The Y-STR haplotype which was generated from the child's sample in case 21 and 27 other Y-STR haplotypes reported here (Table 2) did not match any Y-STR haplotype in the Philippines, Asian, and world databases which suggested the relative uniqueness of these haplotypes. These DNA profiles are now available for comparison

once reference samples from suspects are submitted for DNA typing. Other than wait for more samples, no further work to identify the male sources of these DNA could be made. The Philippines does not have a DNA criminal database which could have been used to identify possible suspects, given the availability of the Y-STR haplotypes from the children's samples. The promise of mining DNA databases for information to generate investigative leads is illustrated in four cases included here. In cases 55 and 59, the Y-STR haplotype detected in both samples only matched a single profile in the Philippine database. In cases 120 and 122, the Y-STR haplotype detected did not match any haplotype in all databases searched. Interestingly, the child victims in cases 55 and 122 did not know their assailants whereas the child victims in cases 59 and 120 provided names of the persons who allegedly assaulted them. The locations of the assault in all four cases are reasonably close to each other. It may be that the same perpetrator or persons who are paternally related are responsible for the assaults on the child victims in cases 55 and 59, as well as those in cases 120 and 122.

Interestingly, the only Y-STR haplotype detected here that matched non-Asian haplotypes in the databases searched involved the abuse of a 10-year-old minor (Case 144). The child identified her own father and a neighbor as her assailants, albeit only a single Y-STR haplotype was generated. The possibility that the child's father was involved in prostituting his own child to the 'neighbor' was suggested. However, reference samples from both the child's father and the neighbor are needed to further evaluate the significance of the DNA evidence.

In casework investigations, a child's account of sexual assault may not always be accurate. In our study, there was a poor correlation between the child's knowledge of her assailant having ejaculated during intercourse and successful amplification of Y-STR DNA. Five children reported no ejaculation occurred, but three full, one mixed, and one partial DNA profiles were generated using their vaginal samples. In addition, 30 children tested positive for male DNA who have admitted to having cleaned themselves after contact. These observations highlight the need to swab the child within the 72-h cut-off limit in spite of the child's account of the sexual assault and attempt to clean herself after the incident.

In this study, the laboratory generated partial male DNA profiles in 16 cases. When using PowerPlex® Y, Y-STR DNA markers DYS19, DYS385, DYS392, and DYS438 are more likely to drop-out while the more robust markers DYS391, DYS393, and DYS437 have a greater chance of being included in the partial DNA result [24]. The inability to generate full Y-STR haplotypes in these cases suggests either the absence of a sufficient amount of male DNA or the presence of a significant amount of child victim's DNA that inhibited the amplification. Comparison of the sizes of Y-STR DNA markers (except DYS385) and successful amplification showed no significant difference based on allele size (Fig. 1).

A considerable percentage of possible offenders identified by the child victims were related to the child (24.7%) either biologically or by association, i.e., mother's partner, cousin's husband, brother in-law. If the offender is biologically related to the child victim such as child and father (six cases) or child and brother (one case), victim and offender share or are highly likely to share common autosomal alleles. This would make the analysis of this type of DNA data more complex. In contrast, Y-STR profile analysis is useful as a preliminary DNA screen to identify the real offender and to facilitate the release of innocent persons. We therefore recommend the use of Y-STR DNA marker technology as a preliminary screen to assist in the timely identification of male suspects and the exclusion of those who have been erroneously identified. This would then be followed by autosomal STR-DNA profiling of evidentiary samples and reference samples of male suspects who were identified by the child victims and who were not excluded using Y-STR DNA profiling.

Cases which involve more than one offender pose a greater challenge for investigators and DNA laboratory analysts. In the present study, only two cases involving a child that identified two alleged perpetrators resulted in DNA profiles that are consistent with at least two male individuals. In 16 cases included here, the child victims identified more than one offender but only a single Y-STR haplotype was detected in their samples. In these situations, other factors such as the use of condoms, the non-penetration of an offender/s, or the paternal relations of two offenders thereby sharing the same Y-STR haplotype could explain the absence of a second Y-STR haplotype in the child's intimate samples. The use of autosomal STR-DNA profiling to complement the initial Y-STR DNA profiles is expected to provide additional information that would differentiate paternally related offenders.

Conclusion

Male DNA was successfully detected in vaginal swab samples from 63 female child victims who reported sexual contact within 72 h. The generation of male DNA profile/s from intimate samples of female children provides convincing evidence of sexual contact, and hence, exploitation of a minor. Y-STR profiles were successfully generated from internal vaginal samples collected using calgi swabs for prepubescent child victims and cotton swabs for older children that will be useful for offender identification once reference samples are submitted for DNA testing. Although a significant correlation between a child's age and successful genotyping of male DNA from vaginal samples means that older child victims are likely

to have DNA evidence, the amplification of a full Y-STR profile from child victims aged 3, 8, and 10 years old underscores the importance of DNA evidence for very young and vulnerable children who are not able to comprehend the full extent of the abuse. The inclusion of Y-STR DNA typing in the routine evaluation of child sexual abuse cases in the Philippines is therefore recommended. Y-STR DNA profiling should be performed regardless of the child victims' hygienic practices and knowledge of whether the offender ejaculated or not, provided that the sample for DNA testing is collected within 72 h after sexual intercourse.

Acknowledgements The authors acknowledge the assistance in the collection of samples from the Child Protection Units of the University of the Philippines, Manila, the Davao Medical Center, the Baguio General Hospital Medical Center, and the Vicente Sotto Memorial Medical Center and the technical support provided by Lilian Villamor, Angeline Moya, and Angelo Porciuncula of the UP-NSRI DNA Analysis Laboratory. The authors are also grateful to Atty. Jose M. Jose, Atty. Jose Maria A. Ochave, and Dr. Saturnina C. Halos for interesting discussions. The financial support from the Natural Sciences Research Institute and the Office of the Vice-Chancellor for Research and Development (090906 PNSE), both located at the University of the Philippines, Diliman and the Development Innovation Marketplace with funding from the World Bank, are also acknowledged.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. World Health Organization, Regional Office for South-East Asia (2009) Child sexual abuse and violence: violence prevention in South-East Asia. Available via internet. www.searo.who.int/LinkFiles/Disability,_Injury_Prevention_&_Rehabilitation_child.pdf. Accessed 13 Mar 2009
2. Romulo RA, Balamban BB (2008) Statistics on violence against women and children: a morally rejuvenating Philippine society? National Statistical Coordination Board. Available via internet. http://www.nscb.gov.ph/headlines/StatsSpeak/2008/090808_rav_wedc.asp. Accessed 13 Oct 2010
3. Child Protection Network (2008) Annual report on cases handled by CPU from 2005–2007. Available via internet. http://www.childprotection.org.ph/factsfigures/index.html. Accessed 31 October 2010
4. De Ungria MCA, Calacal GC, Magno MMF, Delfin FC, Tabbada KA, Frani AM, Halos SC (2001) DNA evidence in Philippine courts: a case report. Philipp Law Gaz 13(3):46–49
5. De Ungria MCA, Sagum MS, Calacal GC, Delfin FC, Tabbada KA, Te DMRM, TO DJI, Diokno MSI, Asplen CA (2008) Forensic DNA evidence and the death penalty in the Philippines. Forensic Sci Int Genet 2:329–332
6. Collins KA (1998) The laboratory's role in detecting sexual assault. Lab Med 29(6):361–365
7. Roewer L (2009) Y chromosome STR typing in crime casework. Forensic Sci Med Pathol 5:77–84
8. Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidron F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling MA, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, de Kniff P, Roewer L (1997) Evaluation of Y-chromosomal STRs: a multicenter study. Int J Leg Med 110:125–133
9. PowerPlex® Y database (2004). Available via internet. www.promega.com/techserv/tools/pplexy. Accessed 2008–2009
10. Yfiler database (2004). Available via internet. www.appliedbiosystems.com/yfilerdatabase. Accessed 2008–2009
11. Willuweit S and Roewer L (2007). YHRD (Y chromosome Haplotype Reference Database). Available via internet. www.yhrd.org/search%20Database/Search%20Haplotypes;;s. Accessed 2008–2009
12. Janisch S, Meyer H, Germerott T, Albrecht UV, Schulz Y, Debertin AS (2010) Analysis of clinical forensic examination reports on sexual assault. Int J Legal Med 124(3):227–235
13. Delfin FC, Madrid BJ, Tan MP, De Ungria MCA (2005) Y-STR DNA analysis for detection and objective confirmation of child sexual abuse. Int J Leg Med 119:158–163
14. Federal Bureau of Investigation Crime Laboratory (1994) PCR-based typing protocols. United States Department of Justice, Federal Bureau of Investigation, Washington
15. Maiquilla SMB, Salvador JM, De Ungria MCA (2008) Update: Philippine Y-STR DNA haplotype database at 11 Y-STR DNA markers' DNA Analysis Laboratory Report, University of the Philippines Natural Sciences Research Institute
16. Lessig R, Willuweit S, Krawczak M, Wu FC, Pu CE, Kim W, Henke L, Henke J, Miranda J, Hidding M, Benecke M, Schmitt C, Magno M, Calacal G, Delfin FC, de Ungria MC, Elias S, Augustin C, Tun Z, Honda K, Kayser M, Gusmao L, Amorim A, Alves C, Hou Y, Keyser C, Ludes B, Klintschar M, Immel UD, Reichenpfader B, Zaharova B, Roewer L (2003) Asian online Y-STR haplotype reference database. Leg Med (Tokyo) 5(Suppl):S160–S163
17. Halos SC, Chu JY, Ferreon ACM, Magno MMF (1999) Philippine population database at nine microsatellite loci for forensic and paternity applications. Forensic Sci Int 101:27–32
18. Christian CW, Lavelle JM, De Jong AR, Loiselle J, Brenner L, Joffe M (2000) Forensic evidence findings in prepubertal victims of sexual assault. Pediatrics 106(1 Pt 1):100–104
19. Grossin C, Sibille I, Lorin de la Grandmaison G, Banasr A, Brion F, Durigon M (2003) Analysis of 418 cases of sexual assault. Forensic Sci Int 131(2–3):125–130
20. McCall-Hosenfeld JS, Freund KM, Liebschutz JM (2009) Factors associated with sexual assault and time to presentation. Prev Med 48:593–595
21. McGregor MJ, Du Mont J, Myhr TL (2002) Sexual assault forensic medical examination: is evidence related to successful prosecution? Ann Emerg Med 39(6):639–647
22. Laraque D, Demattia A, Low C (2006) Forensic child abuse evaluation: a review. Mt Sinai J Med 73(8):1138–1147
23. Willott GM, Allard JE (1982) Spermatozoa—their persistence after sexual intercourse. Forensic Sci Int 19(2):135–154
24. Krenke BE, Viculis L, Richard ML, Prinz M, Milne SC, Ladd C, Gross AM, Gornall T, Frappier JR, Eisenberg AJ, Barna C, Aranda XG, Adamowicz MS, Budowle B (2005) Validation of a male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex. Forensic Sci Int 148(1):1–14