Overview of Forensic Services by the UP-NSRI DNA Laboratory: a 4-year Report

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ABSTRACT

This paper reports the use of DNA analysis in 342 cases that were handled by the DNA Analysis Laboratory of the Natural Sciences Research Institute, University of the Philippines, Diliman during the period from January 2005 to August 2008. Different DNA markers namely Short Tandem Repeats on autosomal and Y-chromosome as well as mitochondrial DNA sequences were used depending on the type and availability of samples that were submitted. The utility of the technology for human identification for diverse purposes was shown. The work presented here puts forward the importance of developing DNA laboratories to make this type of service available in the Philippines.

Key Words: Forensic Genetics, DNA Typing, Microsatellite Repeats, DNA, Mitochondria, Identification

Introduction

DNA testing is the most powerful tool for human identification. Since its introduction in the Philippines in 1998, the technology has been used to assist in criminal investigations, in resolving disputed parentage issues and in the identification of victims of mass disasters. Different DNA systems such as the autosomal Short Tandem Repeat (STR) DNA markers located on different autosomal chromosomes (aSTRs) and sex chromosomes such as X-STRs and Y-STRs; and mitochondrial DNA sequencing (mtDNA) have been used solely or in combination, for purposes of human identification[1]. Genotyping using autosomal STRs is the most utilized for most applications because this technology is based on the uniqueness of the individual genotype of a person, excepting identical twins. In contrast, STRs on the Y-chromosome are identical amongst males with the same patrilineal origin. Hence, brothers who have the same father will have identical Y-STR profiles. Likewise, mtDNA is characterized by maternal inheritance with all children of the same mother sharing the same mtDNA profile. Genotyping at X-STR markers is slightly complicated because of the variation in the number of copies between males and females.

Procedures for handling and DNA testing of diverse samples must be validated and the appropriate genetic databases of the Philippine population must be established prior to use for forensic applications.[2] Unlike other academic fields, these procedures and analyses must withstand both scientific as well as legal scrutiny because of the forensic aspect of this type of work.

In the Philippines, there are four local laboratories that conduct DNA testing for forensic applications. These laboratories are located at the Natural Sciences Research Institute, University of the Philippines, Diliman campus (UP-NSRI DAL); the St Luke's Medical Center (SLMC), the Philippine National Police (PNP) and the National Bureau of Investigation (NBI).

This paper reports an overview of 342 cases that were referred to the DNA Analysis Laboratory of the Natural Sciences Research Institute, University of the Philippines, Diliman (UP-NSRI DAL) during the period January 2005 to August 2008.

Methods

Handling of samples

The appropriate procedures for handling biological samples were observed following guidelines described by the American Association of Blood Banks.[3] Prior to genetic testing, the state of sample upon submission was documented. Samples were only handled as needed, and were stored at low temperatures in a designated area with restricted laboratory access. All information was treated as confidential, with concerned parties signing agreements for the release of information only to authorized persons, the courts when the appropriate order is issued, and for research and academic purposes, provided all information is anonymized prior to dissemination.

DNA Analysis

Samples that were submitted for DNA testing included blood, saliva, body fluids, tissues and bones. Procedures for extracting DNA, PCR amplification and DNA analysis of these samples had been validated for local applications

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and described previously.[4-6] In most instances, the best DNA typing results were generated with extracts obtained using the standard organic extraction procedure. However, other commercially available DNA extraction kits have also been validated and observed to be efficient in recovering DNA from routine biological samples without significantly reducing yield and quality.[7]

Downstream DNA analysis varies depending on the DNA marker technology that was used. Procedures for autosomal STR DNA typing used at UP-NSRI DAL[8-10] Y-STR typing[4, 11] and mitochondrial DNA sequencing[12, 16] had been described. Once genetic data was generated, all reports underwent a two-level technical and a two-level overall review process prior to release.

Databases

To assist in the statistical calculations of matching DNA profiles, several genetic databases are currently being maintained at UP-NSRI DAL. These databases include an autosomal STR,[8,13] Y-STR[11,14-15] and a mitochondrial DNA sequence[12, 16] database.

Results and Discussion

Distribution of cases

Cases were classified into categories depending on the nature of requests (Table 1). The most common types of requests that were made during the period of the study involved genetic testing of samples for direct identification (169 cases, 49.4%) and paternity determinations (133 cases, 38.9%).

The laboratory procedures used for those requiring complete DNA analysis are generally the same; with some modifications of the extraction procedures depending on the type of biological samples that were submitted for DNA testing. The types of DNA analysis may be grouped into relationship testing, direct identification and DNA profiling.

Relationship testing: paternity testing, maternity testing and kinship analysis

Relationship testing involves the comparison of genotypes of persons with parents or kin in order to establish the relationship between them. Many requests were driven by the need to utilize DNA technology in order to generate an objective piece of evidence for the resolution of disputes through amicable settlements or for use in courts of law when any type of negotiation was not possible.

Twenty six cases conducted by UP-NSRI DAL were performed as ordered by Philippine courts to assist in the litigation of nine criminal cases that involved allegations of sexual assault leading to the birth of a child (known as "criminal paternity") and violence against women and children, and 17 cases involving civil disputes. Notably, in 20% of cases without court orders, parties expressed their intention to use the DNA test results for future court proceedings. The most common reason cited for DNA-based paternity determinations was child support since 128 (96.2%) of the 133 paternity cases presented here involved children born to couples who were not married to each other. However, other reasons cited include 1) inheritance claims; 2) issue of undisclosed pregnancy prior to marriage which could result in the nullification of the marriage; 3) petition to impugn legitimacy of a child who was born as a result of a woman's relation with a man other than the child's legal father; 4) medical negligence that could have resulted in the switching of children after birth and 5) possible errors in the use of sperms in artificial insemination procedures.

DNA test results for paternity cases are summarized in Table 2. Of the 133 paternity cases presented here, DNA excluded the man alleged to be the child's father from being the child's biological father in 30 cases (22.5%). Notably in 13 instances out of the 18 cases wherein both DNA and blood typing were performed, while blood typing resulted in false paternity inclusions, the DNA test results excluded the man as the biological father of the child. Prior to DNA-based paternity determinations, blood typing was the predominant scientific evidence used in courts for this purpose. The data presented here supports the need for more sensitive technologies such as DNA typing to evaluate paternity particularly when blood typing does not result in a paternity exclusion.

Unlike blood typing, DNA test results may also be used to estimate the weight of matching DNA over the possibility of a chance match resulting from a common genotype. When the alleged father is not excluded, a Probability of Paternity (W) is estimated based on matching DNA between a man and a child.[17] This value will only approximate but not equal 100%. The value of W is affected by the number of aSTR DNA markers that were tested and the availability of the mother's DNA profile in order to distinguish the maternal as well as the paternal alleles in the child's genotype.[18] During the early days of DNA technology, only a limited number of STR-DNA markers, e.g. seven to nine aSTR-DNA markers, were routinely used which resulted in lower W values.[19] In two cases out of 103 paternity inclusion cases presented here, the W value was lower than 95%, which was the minimum accepted W value in any state in the US.[20] The mother's DNA was not included in the analysis of these two cases.

There were 17 cases with W values which were greater than 95% but less than 99.9%. In the new Rule on DNA Evidence promulgated by the Philippine Supreme Court,[21] the court would presume paternity on the basis of DNA test results provided the value of W is 99.9% or higher. Hence, based on the new Rule, DNA test results in these 17 cases may still be used in court but only as a corroborative piece of evidence. These 17 cases include those wherein the mother's DNA was unavailable, or when DNA testing was conducted using nine aSTR markers prior to the promulgation of the new Rule. Given this development, UP-NSRI DAL now performs DNA-based paternity analysis by targeting 15 aSTR DNA markers using commercially available multiplex systems.

Table 1. Distribution of cases referred to UP-NSRI DAL during the period January 2005-August 2008

Request	Description	No. of cases
DNA typing services		
Paternity testing	Refers to the tests evaluating the biological paternity of a man with a child	133
Maternity testing	Refers to the tests evaluating the biological maternity of a woman with a child	6
Kinship analysis	Refers to the tests evaluating the complex kinship relationships of persons	5
Direct identification	Refers to the genetic profiling of samples that had been collected from mass disaster sites, crime scenes or victims' bodies for purposes of identification of the source of the biological sample	169
DNA profiling	Refers to the generation of DNA profiles of samples from known sources	15
Auxilliary services		
DNA extraction	Refers to the extraction of DNA from samples submitted to the laboratory	10
Storage of samples (5 years)	Refers to the collection and storage of biological samples in a secure environment for future use	3
Collection of samples	Refers to the collection of biological samples from persons to assist other agencies in their work	1
TOTAL		**342**

Table 2. DNA test results of paternity determinations

	DNA test results	No. of cases
Paternity exclusions		**(30)**
	Detected by ABO blood and DNA typing	5
	Detected by DNA typing alone	13
	Blood typing data not available	12
Paternity inclusions		**(103)**
a) using aSTRs	W values < 95%	2
	95% < W values < 99.9%	17
	W values > 99.9%	82
b) using Y-STRs	W values > 99.9%	2

Table 3. Background of cases where DNA testing was requested

Case background	Biological sample	No. of cases
Association to violent crimes	Bones	3
Significance of identifying source of sample did not justify the cost of DNA testing	Bones	1
Party decided not to pursue the case; reason undisclosed	Body fluid on clothing	1
Tests requested to aid in the resolution of family disputes	Body fluid on clothing	1
Unidentified victims of mass disaster	Bones	7
Samples collected by medical doctors from patients of the child protection unit of UP-PGH, Manila	Body fluid collected from the child using a sexual assault investigation kit	117
Samples collected by medical doctors from patients of four child protection units (Manila, Baguio, Cebu and Davao)	Body fluid collected from the child using a sexual assault investigation kit	39

Table 4. History and DNA results of 39 CPU cases

Case information	Positive result	Negative result	Result Unknown
Post-coital washing	28	8	3
Sperm detection via microscopic examination	6	21	12
Presence of male DNA	20	19	-

A special type of paternity test may be conducted when dealing with paternity determination of male children. This test makes use of the strictly paternal inheritance of the Y chromosome that result in fathers and sons sharing the same Y-STR DNA profiles. In two cases included here, Y-STR DNA typing was used to show that the alleged fathers were paternally related to the children.

Requests for DNA-based maternity tests are significantly less than requests for paternity tests because in most situations, the mother is certain of her relation to her own children. The six maternity cases in the present study were requested for the following reasons: 1) to acquire child custody because two women claimed to be the mother of the child (1 case); 2) to include DNA test results in the petition for the child to migrate in another country (1 case); 3) to evaluate possible switching of children at birth (2 cases) and 4) to claim inheritance from deceased mothers (2 cases). The use

of different DNA technologies for maternity determinations in two cases is discussed in a separate paper.[22]

Kinship analysis is performed to indirectly address parentage issues via DNA typing of biological samples from relatives. Autosomal STR DNA typing may still be used for this purpose, albeit statistical analysis of DNA data is more complex than in routine paternity or maternity determinations. In more recent years, alternative approaches using the strictly paternal inheritance of Y chromosome STR markers and/or the strictly maternal inheritance of mtDNA markers have been used. Three of the five cases included in the present report, involved the determination of paternal relationship between half-brothers using Y-STR profiling of their DNA. The other two cases involved the mitochondrial DNA sequencing of blood samples from putative maternal relatives in order to establish the maternal relation of a child to a deceased woman. In all five cases, the exhumation and laboratory analysis of decomposed human remains that are time-consuming and require the use of more costly chemical reagents were avoided.

Direct identification

Direct identification involves the generation of the genotype of a biological sample in order to identify the source of the biological sample that may have been collected from mass disaster sites, crime scenes or bodies of victims of violent crimes, such as sexual assault and/or homicide. A summary of the types of identification cases included in the study is shown in Table 3.

Many of these cases are still on-going because of the unavailability of ante-mortem genetic information from the deceased and/or the absence of parents, children or close relatives who could provide biological samples in order to reconstruct the possible genotypes of the source of sample. There is also a need to develop more sensitive DNA procedures for handling challenged biological samples that underwent different harsh environmental conditions or exposure to chemicals that could interfere with downstream DNA analysis.

We also tested samples collected from the internal genitalia of female patients of four child protection units for the presence of male specific DNA. Samples were collected by medical doctors only when the child's last contact with the perpetrator was within 72 hours from the time of examination. Positive results were generated in 76 out of 156 cases (48.7%) with 46 of these samples showing a full 11 Y-STR DNA profile of the perpetrator of the crime. Background information on 39 cases was compiled in order to determine the impact of DNA results in the whole context of the case (Table 4).

Washing after contact did not sufficiently remove all biological samples left by the perpetrator on the body of the child patient. This information supports the need to collect samples from patients even after a person has washed herself. In addition, this study reports the lower rate of sperm detection under microscopy (15%) as compared to the detection of male DNA (51%). It was not clear why sperm detection was not performed in 12 cases (30%) included here.

DNA profiling and sample archiving

There were 15 requests for DNA profiling of samples from known sources. Twelve of these samples were collected from medical professionals who were handling human samples that may potentially be submitted for DNA testing in relation to actual forensic cases. In two instances, the genotypes of persons were generated as required by private companies. In another case, a person actively sought to have his ante-mortem DNA profile and providing his family access to his genetic data, if ever there was any need for this information in the future.

There were 10 requests for DNA extraction of biological samples whose sources were known but without proceeding to the genotyping of these samples in order to minimize cost. In three cases, biological samples were stored without proceeding to DNA extraction. The main concern was to store ante-mortem DNA or biological samples, which may be subjected to analysis in case there is a need in the future. This would markedly reduce the cost of DNA testing because processing of blood DNA is less labor and cost intensive. In one instance, an overseas agency contacted the laboratory for assistance in the collection of forensic samples from an individual in the Philippines who was involved in a civil dispute.

Conclusion

There is an increasing demand for DNA testing to settle civil disputes and to assist in the litigation of criminal cases. Medical professionals have used DNA typing in their work in situations when identification of sources of tissues/biological samples was required. There is also a growing interest for persons to provide biological samples for DNA profiling and/or archiving for future applications. With the recent promulgation of the Rule on DNA Evidence, there is official recognition of the utility of DNA technology to address legal, social and humanitarian concerns. The current paper underscores the need to support the development of forensic DNA laboratories so that this type of technology can be made more readily available locally.

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