DNA Tests for Maternity Determination

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ABSTRACT

This report describes the use of DNA analysis in resolving two cases of maternity disputes involving inheritance claims of an alleged child. In the first case, genetic comparisons of the 15 autosomal Short Tandem Repeat DNA (aSTR-DNA) profiles of a deceased woman, brother and the alleged child of the deceased confirmed that the woman is the sibling of her brother but disproved a maternal relationship with the alleged child. In the other case, mtDNA analysis was used to refute the matrilineal relationship between the person claiming to be the child of the deceased and a sister of the deceased.

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

Introduction

DNA typing is the most powerful method for human identification and evaluation of disputed parentage cases. There are several DNA typing methods based on length polymorphisms such as those targeting Short Tandem Repeat (STR)-DNA markers located on nuclear DNA; as well as DNA sequence polymorphisms in the hypervariable regions of the mitochondria.[1] Each of these tests relies on the characteristic pattern of inheritance at a particular DNA marker. For example, STR-DNA markers on any of the 22 pairs of human autosomal chromosomes of a person must be traceable to the person's biological parents. Each person possesses two copies of autosomal DNA, one copy of maternal origin and the other of paternal origin. In disputed parentage cases, the likelihood of parentage is evaluated based on the presence of shared DNA between the child and his biological parents. Exclusion from being a child's parents is made in the absence of shared DNA in at least three autosomal STR (aSTR) DNA markers between the alleged parent and child.[2-3] Notably, full siblings also share many common DNA since siblings obtain their DNA from the same set of parents.

Unlike autosomal DNA, which is passed on to children by both parents, mitochondrial DNA (mtDNA) is strictly inherited from the mother. MtDNA is separate and distinct from the nuclear genome and is passed from mothers to their sons and daughters without recombination, hence, barring mutations, siblings and maternal relatives have the same mtDNA sequence. Sequence variation at the hypervariable segment regions (HVR I and HVR II) within the mtDNA control region is analyzed to verify matrilineal relationship.[1] If the mtDNA sequences of two tested individuals are unequivocally different, i.e. a difference of two or more nucleotides, it is generally considered as an exclusion of a common maternal lineage. In cases where the resulting mtDNA sequences under comparison have a similar base at each nucleotide position, the significance of the mtDNA match needs to be assessed by determining the frequency with which that particular mtDNA sequence has been observed and the corresponding subpopulation structures in a relevant population.[4-5]

We report here the result of aSTR DNA typing and mtDNA analysis in resolving two cases of maternity disputes involving inheritance claims of an alleged child.

Case Background

Case 1

A dispute arose over ownership of properties of a deceased woman resulting in the filing of a civil case in court. The parties involved were her alleged illegitimate daughter against the siblings of the deceased. The judge ordered the conduct of DNA tests on the remains of the deceased woman and alleged child in order to resolve the issue. Prior to the exhumation, the grave of the deceased was identified by her brother and her alleged daughter based on its location within the cemetery and the inscription on the concrete vault showing the name of the deceased. Because of the three year post-mortem period, the body was fully skeletonised, hence, all the remains were packaged using the inner lining of the coffin. The body was transported to the College of Medicine, University of the Philippines, Manila for examination and collection of bone sample for DNA analysis. Blood samples were collected from the alleged daughter and brother of the deceased.

Case 2

Ownership of properties was disputed by a couple's

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legally adopted son and another person who claimed to be the couple's biological daughter. The judge ordered the conduct of DNA-based parentage determination. DNA-based parentage determinations involve routine procedures, particularly when handling fresh blood or buccal samples. However, since each alleged parent had been buried for approximately seven (alleged father) and two years (alleged mother), an alternative approach to aSTR DNA typing involving the use of mtDNA sequence data generated from blood samples collected from the alleged daughter and a maternal aunt was considered.

Methods Sample Source and DNA extraction

Fresh blood samples were collected on FTA® card and purified following manufacturer's instructions (Whatman® BioSciences, USA). To generate a DNA profile from the exhumed remains in Case 1, the right femur that was partially articulated at the pelvis was dissected by Dr. Raquel dR Fortun at the College of Medicine, University of the Philippines. Traces of soft tissues were removed prior to air-drying of the bone sample. Bone fragments were cleaned following methods described previously.[6-7] Bone DNA was extracted using an organic procedure.[6]

DNA Analysis Autosomal STR-DNA analysis (Case 1)

DNA extracts were amplified at 15 autosomal STR-DNA markers namely, D3S1358, HUMTH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, Penta D, HUMvWA, D8S1179, HUMTPOX and HUMFGA using the PowerPlex® 16 multiplex system (Promega Corporation, Madison WI). A sex-determining marker, Amelogenin (HUMAMEL) was also included in the system. Amplification was carried out on a PE 9700 thermocycler (Applied Biosystems, Foster City, CA) using PCR conditions recommended by the manufacturer (Promega Corporation, Madison WI). Amplified fragments were analyzed on the ABI Prism® 310 Genetic Analyzer with GeneScan 3.7 and Genotyper 3.7 softwares for automatic allele calling (Applied Biosystems, Foster City CA). Alleles and peaks were evaluated based on published guidelines and recommendations.[1,8]

Mitochondrial DNA Analysis (Case 2)

DNA was amplified using primer pair L15971/H484 to produce a 1083bp fragment covering the entirety of HVR I and HVR II of mtDNA. Purified amplicons were sequenced using the Big Dye® Terminator Cycle Sequencing System v3.1 and detected in an ABI Prism® 310 Genetic Analyzer (Applied Biosystems, Foster City CA). Six (6) to seven (7) internal sequencing primers were used to cover the entire 1083bp region. The consensus sequence was aligned with the revised Cambridge Reference Sequence (rCRS)[9] using the DNA Alignment software (Fluxus Technology Ltd., England). Differences between the case samples and rCRS were reported following the international guidelines recommended for the analysis and interpretation of mtDNA sequences.[4, 10,11]

Results

Case 1

A complete 15 autosomal STR-DNA profile was obtained from exhumed bone sample and blood samples collected from the deceased woman's brother and her alleged daughter. The genotype of the remains at the amelogenin marker was XX which confirmed that these remains were those from a female person. In addition, the deceased and her brother shared alleles in 14 out of 15 aSTR DNA markers which are consistent with the fact that they are full siblings. The calculated likelihood ratio for sibship[12] was greater than 100,000, which means that the results are 100,000 times more likely that the remains were from a sibling of the man who was tested than if it were from a random person. In contrast, the absence of shared DNA in three different autosomal STR DNA markers namely D21S11, Penta E and HUMFGA between the deceased and the alleged child clearly showed that these two persons were not related.[2] The information derived from autosomal STR DNA typing of the deceased woman's femur bone and the alleged daughter's blood showed that these two persons were not maternally related.

Case 2

A 970-bp mtDNA sequence was generated using DNA from blood samples that were collected from the deceased woman's sister and a woman claiming to be the couple's biological daughter. This region includes the two hypervariable regions namely HVR I and HVR II as well as adjacent sequences. Comparison of the 970 bp mtDNA sequence between the alleged daughter and sister of the deceased showed six mismatches at nucleotide positions 16183, 16189, 16261, 16362, 195 and 199 (Table 1). Additional mismatches were observed in three other nucleotide positions namely, 16182, 16217 and 16223. However, the resulting mismatches in the three nucleotide positions were not reported since the complementary sequence that was generated using the reverse primers did not cover these three sites. A minimum requirement of two independent – preferably forward and reverse – sequence strands covering each control region positions is necessary to reduce ambiguities in the resulting mtDNA sequence.[11] However, the presence of six mismatching sites between the mtDNA of a putative biological daughter and a maternal relative is sufficient to negate any claim of maternal relationship between the deceased woman and the alleged child.

Discussion

The development of different DNA marker systems has further expanded the utility of DNA technology for human identification, particularly in situations wherein one or more of the persons involved are already deceased. The formulation of a strategy to generate the information required to resolve disputes in such cases must be made in consultation with the requesting party or parties. Factors

Table 1. Sequence analysis of the 1083bp region covering the mitochondrial Hypervariable Regions I and II (HVR I and II).

revised Cambridge Reference Sequence (rCRS) at certain nucleotide positions	Child	Alleged Maternal Aunt
A16182*	C	A
A16183	C	A
T16189	C	T
T16217*	C	T
C16223*	C	T
C16261	T	C
T16362	T	C
T195	T	C
T199	T	C

*Sequence of only one strand was available and resulting mismatch was not reported

which must be considered include the type and availability of biological samples for DNA testing, the cost and time requirements of each procedure in relation to the genetic information required, and the overall impact of DNA evidence in the whole context of the case in court. In this report, two different DNA typing procedures were used to resolve issues of maternity.

In the first case, the brother of the deceased woman was provided with two options in order to evaluate the maternal relationship of his deceased sister and an alleged daughter. These were 1) aSTR-DNA typing of bone sample from the deceased and blood sample from the alleged child; or 2) comparison of the mtDNA sequences of blood samples from the brother of the deceased and the alleged child. Between these two options, mtDNA analysis appears to be simpler, faster and less expensive because it does not involve exhumation and DNA analysis of the human remains. However, subsequent consultations with the living relatives of the deceased revealed that there might be a possibility that the alleged daughter is a distant maternal relative to the deceased woman and brother. If this is the case, the mtDNA sequence of the alleged child will most likely match with the mtDNA of the brother of the deceased. This mtDNA evidence can be used to argue that the alleged daughter is indeed the biological child of the deceased. Hence, given the uncertainty of the relationship of the alleged daughter with the family of the deceased, autosomal STR-DNA analysis using bone sample from the deceased and blood sample from the alleged daughter (direct maternity determination) was recommended as more appropriate and relevant for the case. However, there was a need to establish that the bone sample is from the deceased woman prior to the conduct of maternity determination. Comparison of the aSTR DNA profiles of bone sample with the brother of the deceased confirmed that the remains are that of the deceased woman. Autosomal DNA typing of at least 13 or more STR markers is currently the method of choice in parentage determination due to its high power of discrimination and relative ease in data interpretation. Moreover, the utility of autosomal STR DNA typing for parentage determinations has been recognized by the Philippine Supreme Court since 2001.[13]

In the second case, two options were also provided to the daughter of the legally adopted son to determine if the alleged daughter is the biological child of the deceased couple. These options were 1) aSTR DNA analysis of bone samples from the deceased parents; or 2) mtDNA sequence analysis of blood samples from the sister of the deceased woman and alleged child. In determining the type of DNA analysis to be carried out, cost and time required to perform the DNA analysis as well as length of time the alleged parent had been buried, were taken into consideration. Exhumation, sample collection, sample processing and DNA analysis of bone sample from two human remains requires a longer period to complete and is expensive. In addition, there is a possibility of obtaining insufficient amount of DNA with poor quality from the remains of the alleged parent, which had been buried for seven years. The parties also wished to expedite the resolution of the case. Based on these issues and concerns, proving the maternal relationship between the deceased woman and putative biological daughter by analyzing the mtDNA of a sister of the deceased woman and the alleged child appeared to be more suitable for this case. As mentioned previously, mtDNA is identical for all maternally-linked relatives, hence, this genetic system can prove if individuals tested are related by common descent through maternal lines. Therefore, the availability of a biological sample from a living sister of the deceased and subsequent mtDNA analyses of this sample and that of the alleged daughter is sufficient to determine maternal relationship between the alleged daughter and the deceased woman. It is important to note however, that mtDNA can never provide the resolution of individuality that aSTR DNA typing can. Hence, it should only be used for cases or samples for which analysis of autosomal DNA is impossible or not feasible, or when distant relatives are used as reference samples.[14]

We have demonstrated in this report the utility of two DNA typing systems namely, autosomal STR-DNA (aSTR-DNA) analysis and mitochondrial DNA (mtDNA) sequence analysis, for maternity determination. Based on the two maternity cases presented here, choosing which DNA typing system to use depends on the nature, background and relevance to the case at hand as well as, limitations afforded by each system. However, regardless of DNA typing procedure used, the DNA evidence generated either by aSTR-DNA or mtDNA tests was conclusive in excluding both women as maternally-related to the alleged daughters claiming inheritance. This provides very strong objective evidence that could aid our Courts of Law resolve these cases and similar cases, in support of other corroborating evidence.

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