Original Article

Identification of an unknown person using Y-chromosome markers and mitochondrial dna typing

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Abstract:

Background: The present article is concerned within the scope of Forensic Medicine, specifically Forensic Genetics. The case was taken care of in the Genetic-Molecular Laboratory of the Odessa Regional Bureau of Forensic-Medical Examinations, in Ukraine, during January and February of 2014.

Objectives: The aim of our work was to identify an unknown person, using the techniques: Y-chromosome markers and mitochondrial DNA typing.

Materials and methods: The materials available for our procedure were: pieces of tissue in paraffin blocks, saved from the corpse of the unknown person; blood from a living male subject, who claimed to be the grandfather, and from two females, allegedly the sisters. From all of them we extracted nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) respectively, that was analyzed to compare and draw the proper conclusions.

Results: The analyzed genetic materials confirmed the kinship.

Conclusion: Using Y-chromosome markers we were able to establish the correspondence of the subject to his paternal line, and by mitochondrial DNA typing we could establish his relation to the proper maternal line.

Keywords: Forensic Genetics, genetic markers, Y-chromosome markers, mitochondrial DNA sequencing, identification of a corpse.

Fac Med Baghdad
2015; Vol.57, No.1
Received: Aug.,2014
Accepted: Dec,2014

Introduction:

The purpose of this article is to present an interesting case we came across in our work, to make the public aware of the scientific work, made in the field of Forensic Genetics, in Odessa-Ukraine. The aim of our participation in this case was to identify an unknown person, using the technique of Y-chromosome typing to determine his kinship by paternal line to the subject who claimed to be his grandfather and mitochondrial DNA (mtDNA) sequencing to establish the family relationship by maternal line to the alleged sisters.

The case went as follows: some time ago the cadaver of a young male subject (X) in very bad physical conditions (unidentifiable) was brought in to the morgue by the police. As it is the protocol, samples were taken from different tissues and kept in paraffin blocks.

Around the same time a senior male subject (R) reported to the police, that his grandson went missing; the characteristics of which fit the ones of X. The parents of R's grandson are dead and their corpses irretrievable. The missing person has two sisters. See Figure 1.

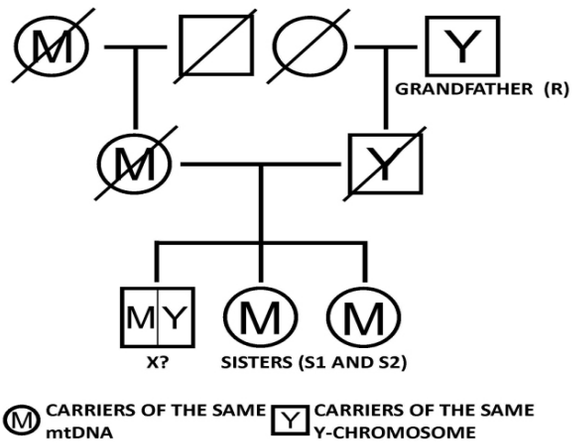

Figure 1: Hypothetical Genealogical Tree to be Confirmed with this Project

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The procedures were conducted in the Genetic-Molecular Laboratory of the Odessa Regional Bureau of Forensic-Medical Examinations with the help of the staff of the department of Forensic Medicine of the ONMedU. In Ukraine, the Odessa Bureau is the only one to use Y-chromosome typing and mtDNA sequencing as methods in Forensic Investigations.

Methods:

Y-chromosome typing. In order to achieve the goal, we used the following method:

First: Extraction of DNA from biological samples and quantification of the obtained material. DNA extraction was done using the MACHEREY-NAGEL NucleoSpin® Blood Kit from the blood sample of R and with the MACHEREY-NAGEL NucleoSpin® Tissue Kit from the tissue, preserved in paraffin blocks belonging to X. The goal of this step is to lyse the membranes and proteins in the cells in order to obtain pure DNA and to get rid of any elements that can inhibit the following processes; we then, by Fluorometry, measure the concentration of DNA in the tube contents. See Table 1.

Table 1: Concentration of the genetic material retrieved from the obtained samples

Units	Concentration	Subject
ng/mL	86	X
ng/mL	185	R
ng/mL	194	S1
ng/mL	162	S2

Second: Amplification of the loci in the extracted DNA by PCR. The polymerase chain reaction (PCR) was then done to amplify the number of DNA fragments, specifically the regions that we need to sequence, a range of short tandem repeat (STR) loci within the euchromatic region of the Y-chromosome: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438 and DYS448.

For this purpose we used the correspondent kit (AmpFLSTR® Yfiler® PCR Amplification Kit for the Y-chromosome).

Third: Visualization of the obtained copies by capillary electrophoresis. Each sample, a positive and a negative control were then introduced into the Analyzer (3130 instrument) to receive the electropherogram plots from the samples.

Fourth: Comparative analysis of the results. The electropherogram plots of X and R were compared to find that they both possess the same alleles with the same molecular weight for the analyzed loci.

Mitochondrial DNA Sequencing. The method for mtDNA typing consists roughly of seven steps:

First: Extraction of DNA from the biological samples. similar to the process for the Y-chromosome, the MACHEREY-NAGEL NucleoSpin® Tissue Kit4 was used for extracting DNA from the preserved tissues of X, and the MACHEREY-NAGEL NucleoSpin® Blood Kit3 to get the mtDNA from the blood samples of S1 and S2.

Second: Quantification of the obtained mtDNA. We quantified the contents of the three tubes we had to assess the amount of DNA that was obtained with the help of a fluorometer (Qubit® by life Technologies). See Table 1.

Third: PCR Amplification of Hypervariable Regions 1 and 2 (HV1 and HV2). During this stage HV1 and HV2 are amplified to obtain millions of copies of each one, with the use of the AmpliTaq Gold® PCR Master Mix by Applied Biosystems for mtDNA.

Fourth: Purification of the PCR products. While sequencing mtDNA we need much higher resolution for proper visualization of the nucleotides, and considering that within what we got from the PCR are not only the mtDNA fragments, but also the byproducts of the reaction; consequently it is necessary to purify the tube contents before the actual sequencing can be done. This was achieved with the application of available commercial kits (ExoSAP-IT® For PCR Product Cleanup by Affymetrix).

Fifth: Setting the sequencing reaction. The performance of DNA sequencing reaction to incorporate fluorescent dideoxynucleotide triphosphates (ddNTPs), with each reaction containing a different primer to dictate which strand is sequenced. At the end of this phase it is important to remove the unincorporated fluorescent dye terminators from the completed sequencing reaction through spin column filtration. (BigDye® Terminator Cycle Sequencing Kit by Applied Biosystems).

Sixth: Electrophoresis. The dilution of purified sequencing reaction products in formamide is done and then the separation through electrophoresis in a capillary system in the same device we used before: Analyzer 3130 Instrument.

Seventh: Analysis and interpretation of the results. DNA sequencing is performed in both the forward and reverse directions so that the complementary strands can be compared to one another for quality control. A software called SeqScape is used for the analysis of the data. For reporting purposes, sequences are listed in a minimum data format as differences relative to the revised Cambridge

Reference Sequence. When differences are observed, the nucleotide position is cited followed by the base present at that site. Results from the edited and reviewed sequences for X, S1 and S2 samples were compared for a portion of HV1. All 610 nucleotides (positions 16024–16365 and 73–340) are normally evaluated between samples being compared. See Table 2 for a didactic example of the process.

Table 2: Didactic example of the process of comparison between the samples and the revised Cambridge Reference Sequence (rCRS)

Match between samples	Sample S2	Sample S1	Sample X	rCRS	Position
Yes	T	T	T	C	16294
Yes	T	T	T	C	16296
Yes	T	T	T	C	16304

Results:

The application of Y-chromosome typing allowed the identification of X, satisfactorily, as a relative, by paternal line, of R; see Figure 2. The comparison of the three mitochondrial DNA sequences resulted in a perfect match. Knowing the two facts above mentioned we tracked the vital records of the involved subjects and analyzed all pertinent documents provided to us by the civil registry, and we could conclude that X, effectively, was the grandson of R and the brother of S1 and S2. We confirmed the genealogical tree proposed in Figure 1

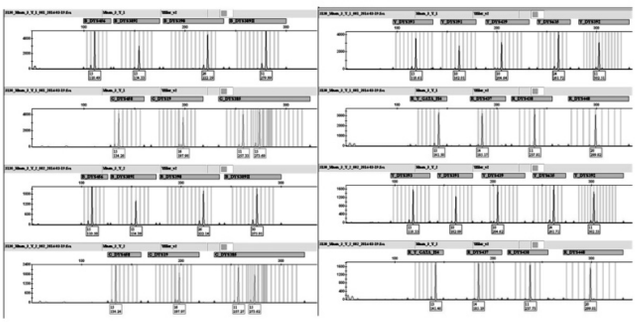

Figure 2: Screenshot of the identified loci

Discussion:

The application of usual DNA sequencing techniques allows the determination of family relationship on a direct basis, only between parents and offspring. This case presented us the impossibility, thus, to apply this methods.

So, we saw the opportunity to apply Y-chromosome markers sequencing, since, we have two male subjects, who hypothetically, would belong to the same paternal line and mtDNA sequencing to confirm the kinship by maternal line to the supposed sisters. We came up with this method, that gave us the possibility, to prescind from the genetic material belonging to the parents of the subject in question.

Separately, a reference of the genetic profile of the father and of the mother were obtained, but these two were incomplete, given the fact that we sequenced the loci of the Y-chromosome permitted by the used kits on one hand, and on the other the same mtDNA is shared by those with the same maternal line as the individual and his sisters.

The explained case is a very specific and peculiar one. Rarely, are these techniques used for identification of unknown people (or corpses). Mostly, as explained by Butler JM, the areas of use in Y-chromosome testing are limited to: forensic casework on sexual assault evidence, verification of amelogenin Y deficient males, paternity testing, missing persons investigations, human migration and evolutionary studies, as well as, historical and genealogical research. To the same degree, the uses of mtDNA sequencing extend to: genealogical studies, evolutionary studies of humans, cases in which little amount of DNA can be retrieved or when it is very degraded, medical studies of mitochondrial diseases and some famous real life examples, like the identification of Tsar Nicholas II's remains.

Y-chromosome (paternal) markers and mitochondrial (maternal) markers are jointly known as lineage markers, which by definition are passed down from generation to generation without changing (except for rare mutations) and are shared by family members with the same lineage.

Situations like the one cited are seldom to come across with, literature of similar cases could not be found in the months we spent on the project, so it becomes an unprecedented case we deliver to your consideration for general knowledge and for professionals who may find it useful.

Authors contributions:

Y-chromosome markers sequencing: Dr. Aymen Abdullah with the assistance of the staff of the Genetic-Molecular Laboratory of the Odessa Regional Bureau of Forensic-Medical Examinations.

Mitochondrial DNA sequencing: Dr. Tatiana B. Fernandez T. with the assistance of the staff of the Genetic-Molecular Laboratory of the Odessa Regional Bureau of Forensic-Medical Examinations.
Supervision of the work by the Dr. Ruslan Grigorevich Krivda, Head of the Department.
Redaction of the article: Dr. Aymen Abdullah and Dr. Tatiana Belen Fernandez Trokhimtchouk

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