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Research Article

DNA Fingerprinting as an Aid in Justice Case Processing

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Abstract

Background: The use of DNA evidence in criminal investigations has gained worldwide acceptance in recent decades. During the last years in Mexico, reports of missing persons have increased dramatically due to the abrupt rise of violent crimes. The situation goes far beyond the current technological response capacity in the country and it exposed a second challenge in the form of increased social pressure on the government coming from the relatives of missing people: they seek to find any remaining of their beloved ones. Therefore, we implemented the FICHA project. Methods: A total of 190 blood samples from relatives of missing people in northeastern Mexico were genotyped and analyzed using a multiplex STR system. Results (statisticals) and conclusions: some of the profiles obtained served as an aid in the resolution of cold cases.

Introduction

'Genetic fingerprint' is the denomination for the laboratory test based on the DNA of individuals that allows the discerment of a particular person among all the inhabitants on the planet. Although approximately 99.9% of the human DNA sequence is the same in every person, forensic scientists take advantage of the 0.1 percent of the DNA that is unique to each individual. In fact, the likelihood of two unrelated individuals having the exact same DNA profile is ~10^{-15}, or about 1 in 594 trillion individuals [1].

DNA typing (profiling) or DNA fingerprinting was first described in 1985 [2]. It was found that certain regions of DNA contained repeated DNA sequences. These repeated DNA sequences come in all kind of sizes and are collectively referred to as variable number of tandem repeats (VNTRs). In addition to this variations, the human genome also possess nucleotide differences at single nucleotide positions, which are referred as single nucleotide polymorphisms or SNP [3]. Since the 80s, scientists have made an effort to design tests in order to analyze variable DNA regions applicable to human identification.

The first generation of DNA profiling consisted on VNTR analysis and Restriction Fragment Length Polymorphisms (RFLP) which generates a pattern due to the activity of restriction enzymes over their recognition sites. This test is no longer used by the forensic community, because of the implication of radioactive probes, the need of large amounts of DNA and the limited accuracy for the analysis of degraded samples. The second generation of DNA fingerprinting was based on PCR and mainly involved DNA detection by gel silver-staining, slot blot, and reverse dot blots [4,5]. However, it was not suitable in the analysis of longer strands of DNA. The third generation of DNA typing and current method of choice is the short tandem repeat or STR analysis. Despite its undoubted advantages, amplicon based genome variation analysis does not work as well for highly degraded DNA samples, such as in cases of mass disaster situations or accidents where an individual is too badly damaged to identify [6]. It was the discovery of Short Tandem Repeats (STRs), together with the introduction of automated sequencing technology that led to the current powerful systems for individual identification.

The STRs reflecting variation of DNA microsatellites repetition number constitute the basis of the current genetic fingerprinting of individuals [7]. STRs have simple Mendelian inheritance, are codominant (being able to differentiate individuals homozygous from heterozygous), and encompass a very large number of alleles in a single locus (highly polymorphic). Their genotyping is relatively easy and can be automated, with the possibility of processing multiple samples in parallel [8].

Another field of application of DNA typing is paternity testing and testing for relatedness on the maternal or the paternal lineage using autosomal, mitochondrial or Y-chromosomal DNA markers [9,10]. Either way, more effective, faster and cheaper DNA analysis techniques are continually being developed, which are addressing different targets for forensic applications. Hence, the application of these techniques introduces new factual evidence to criminal investigations and court cases.

DNA markers, especially STRs are quickly replacing or complementing genetic markers or other methods and applications in areas such as species conservation and cattle breeding, paternity analysis, identifying individuals, assigning individuals to certain breeds, planning mating and others of reproductive nature [11].

Thirteen STRs, referred to as "the CODIS STRs series", have been validated by the Federal Bureau of Investigation (FBI) in the United States and are used and endorsed worldwide for identification in humans. Using these markers, it is possible to obtain a unique profile of an individual with minimal sample amounts from various sources such as blood, saliva, semen, hair, chewing gum, cigarette butts and debris in clothing, among others. Forensic genetic fingerprinting is defined as the comparison of the DNA in a person's nucleated cells with that identified in biological matter found at the scene of a crime or with the DNA of another person for the purpose of identification or exclusion [12].

Forensic analysis based on the amplification of the "CODIS" markers have achieved a public and professional acceptance worldwide as a reliable means of identifying individuals and has had a considerable impact on criminal justice systems [13]. The sensitivity of this technique has allowed the reopening of closed cases and led to the exoneration of prisoners in the US and the UK.

The recent wave of violence originated from organized crime groups in Mexico made evident that institutions responsible for security, especially the areas of criminology, are surpassed by the great challenge that represents the identification of a dramatically increasing number of victims. Atrocities like body parts found in clandestine graves, most of the times left no clear identification clues and represent a serious trouble for authority.

This involves an enormous social pressure reflected in the increasing reports of missing persons. It is therefore undeniable that such criminal acts go far beyond the current response capacity of the technology available in the country.

As scientists wishing to contribute to the solution of the problem, we obtained government funds and implemented the FICHA project ("*Fuente Inteligente de Cotejo de Huellas de ADN*"; spanish for "Intelligent Resource for DNA Fingerprinting Matching"), sponsored by the National Council for Science and Technology (CONACYT, project 185427), specially to aid relatives of missing people.

Methods

Study Population

Working with the Faculty of Medicine of the Autonomous University of Nuevo León and its Forensic Medical Service, relatives of missing people in the state of Nuevo León and the neighboring states of Coahuila and Tamaulipas (northeastern Mexico) were convened. For this purpose, we also received judicial help from non-governmental organizations and authorities. We made the call through advertisements in newspapers, local TV and announcements in Prosecutor's Offices. A total of 190 blood samples was collected during the process and classified using labels with a private identification code, so that the identity of the sampled person remained anonymous.

Sample collection

All samples from the relatives of missing people were collected by trained and qualified staff; written informed consent was obtained from each participant. The samples consisted of 5 ml of peripheral venous blood (draw in EDTA). These were stored at 4°C. During the process of collection, two abductions/murder cases were also investigated. In one case, the bone fragments were taken from the remains of a burned vehicle and a peripheral blood sample was taken from a couple who were thought to be the parents of the victim, as well as a child alleged to be the daughter of one of the missing men. In the other case, five blood stains were taken from a crime scene to be matched with two peripheral blood samples obtained from a couple who were thought to be the parents. These special samples were gathered by personnel from the Prosecutor's Offices in the custody of each crime scene; the bone fragments were sent to a facility to the Prosecutor´s Office in Zacatecas specialized in bone sample processing, whilst the blood stains were collected with cotton swabs for analysis in our laboratory.

DNA isolation

DNA from blood samples was obtained using the Thermo Scientific KingFisher® Blood DNA Kit (Thermo Scientific, Waltham, MA) and following the manufacturer's instructions [14] within the first week after reception. 250 µL of whole blood per sample were processed with the protocol KF_BloodDNA_Flex96. The purified DNA was stored for posterior analysis. The bone fragments were pulverized and a DNA extraction was performed with the QIAamp DNA Investigator Kit (Valencia, CA). DNA quantity and quality were assessed by NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, Delaware, USA), and stored at −20 °C until analysis.

Genotyping

After quantification by spectrophotometry, an amplification of the markers D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA, and gender marker Amelogenin was carried out using the AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, California, USA), according to the manufacturer's specifications. Primer sequences are commercial information, and cannot be disclosed for publication.

1 µl of PCR product was combined with 9.3µl formamide (Applied Biosystems) and 0.3 µl of the GeneScan™ -500 LIZ® standard (Applied Biosystems). The mixture was denatured for 5 minutes at 95°C and chilled for 3 min. Electrophoresis detection of PCR products and genotyping were carried out on the ABI 3130 Genetic Analyzer (Applied Biosystems) using ABI 3130 Data Collection Software and the GeneMapper® Software version 4.0 (Applied Biosystems).

Statistical analysis

Paternity index which measures the weight of the scientific evidence obtained from the paternity test was calculated for each STR using the method described by Brenner and Morris [15]. Then, the Combined Paternity Index (CPI) was estimated by multiplying the individual paternity index with the others.

Probability of Paternity (POP), a conditional probability of whether an alleged father is the biological father of a child, was calculated using the following equation: CPI / (CPI + 1), where CPI is the Combined Paternity Index.

Table 1: Comparison of STR loci from a crime scene blood sample and alleged parents.

Marker	Alleged Mother		Blood Sample		Alleged father	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D8S1179	14	**13**	**13**	**13**	**13**	10
D21S11	30	**31.2**	**31.2**	**30**	**30**	28
D7S820	11	**12**	**12**	**12**	**12**	9
CSF1PO	12	**11**	**11**	**12**	**12**	13
D3S1358	17	**15**	**15**	**17**	**15**	14
TH01	8	**6**	**6**	**6**	**6**	7
D13S317	13	**8**	**8**	**11**	**11**	12
D16S539	10	**11**	**11**	**13**	**13**	11
D2S1338	17	**17**	**17**	**18**	**18**	22
D19S433	**13**	**14**	**14**	**13**	**13**	**14**
vWA	16	**17**	**17**	**19**	**19**	16
TPOX	9	**8**	**8**	**8**	**8**	12
D18S51	17	**12**	**12**	**15**	**15**	12
D5S818	11	**10**	**10**	**11**	**11**	11
FGA	23	**23**	**23**	**25**	**25**	25
AMEL	X	**X**	**X**	**Y**	**Y**	X

Results

Of all the samples collected both from relatives and human remains, 94.4% were direct relatives of the abductees (43.4% of biological mothers, 16.7% of biological fathers, 9.6% of siblings and 24.7% of offspring). The remaining 5.6% included partners and human remains (1.5 and 4%, respectively).

From the profiles obtained and sent to the Prosecutor's Offices of the participating states, it was possible to make progress in certain cases.

Table 2: Genetic markers from three bone fragments analyzed.

Marker	PM025-1		PM025-2		PM025-3	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D8S1179	12	15	12	15	12	15
D21S11	29	32.2	29	32.2	29	32.2
D7S820	11	11	11	11	11	11
CSF1PO	10	10	10	11	10	10
D3S1358	15	17	15	15	15	17
TH01	6	8	8	9	6	8
D13S317	9	11	9	9	9	11
D16S539	11	12	11	12	11	12
D2S1338	23	24	18	20	23	24
D19S433	13.2	15	13	15.2	13.2	15
vWA	16	16	16	16	16	16
TPOX	11	12	8	11	11	12
D18S51	15	17	15	17	15	17
D5S818	11	13	12	13	11	13
FGA	23	24	23	24	23	24
AMEL	X	Y	X	Y	X	Y

Murder case 1

A case of kidnapping of a young man resulted in a visible trail of blood in his home; bystanders had reported to the authorities the abduction of an unknown individual. Analysis of the residence led to the identification of several bloodstains present in certain furniture. All the blood stains collected yielded the same genetic profile, whilst the individual was catalogued as N/N. The parents of the inhabitant of the house reported the disappearance of their son, the alleged abductee, and therefore, their genetic profile was analyzed (Table I). The comparison of genetic profiles revealed an inclusion of blood samples with both parents with a Combined Probability of Paternity of 99.9996108072% and a Combined Paternity Index of 256941.0675, identifying the abductee as the son of the couple.

To date, the abducted individual has not been located, but his profile is available in the Prosecutor's Office.

Murder case 2

Three related individuals (siblings) were reported missing. Two of them were allegedly located in a burning vehicle; however, identification of the bodies by physiological characteristics was not viable. Three bone fragments identified as PM025-1, PM025-2 and PM025-3 were found on site and analyzed. The comparison of their genetic profiles indicated that two of them (PM025-1 and PM025-3) came from the same person (Table 2).

In order to identify these two individuals as some of the missing siblings, DNAs from the alleged parents were analyzed. The comparison stated that both individuals were the couple's offspring (Table 3).

Table 3: Genetic profiles of bone fragments PM025-1, PM025-2 and alleged parents.

Marker	Alleged Mother		Alleged Father		PM025-2		PM025-1	
	Allele 1	Allele 2	Allele 2	Allele 1	Allele 1	Allele 2	Allele 1	Allele 2
D8S1179	13	12	15	12	12	15	12	15
D21S11	30	29	32.2	28	29	32.2	29	32.2
D7S820	11	11	11	10	11	11	11	11
CSF1PO	12	10	10	11	10	11	10	10
D3S1358	15	15	17	15	15	15	15	17
TH01	9	6	8	7	8	9	6	8
D13S317	9	11	9	11	9	9	11	9
D16S539	12	12	11	13	11	12	12	11
D2S1338	20	23	24	18	18	20	23	24
D19S433	13	15	13.2	13.2	13.2	15	15	13.2
vWA	16	16	16	16	16	16	16	16
TPOX	11	11	12	8	8	11	11	12
D18S51	17	17	15	11	15	17	17	15
D5S818	12	11	13	13	12	13	11	13
FGA	21	24	23	24	23	24	24	23
AMEL	X	X	X	Y	X	Y	X	Y
Combined Paternity Index					33977644.99		618859329.4	
Combined Probability of Paternity					99.9999970569%		99.9999998384%	

Table 4: Comparison of genetic profiles from samples PM025-1 and PM025-2 with that of alleged daughter.

Marker	PM025-1		Alleged Daughter		PM025-2	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D8S1179	15	12	12	13	12	15
D21S11	32.2	29	29	33.2	29	32.2
D7S820	11	11	11	12	11	11
CSF1PO	10	10	10	12	10	11
D3S1358	17	15	15	15	15	15
TH01	6	8	8	7	8	9
D13S317	9	11	11	13	9	9
D16S539	12	11	11	12	11	12
D2S1338	23	24	24	20	20	18
D19S433	13.2	15	15	15.2	15.2	13
vWA	16	16	16	16	16	16
TPOX	11	12	12	8	8	11
D18S51	17	15	15	14	15	17
D5S818	13	11	10	11	12	13
FGA	23	24	24	19	24	23
AMEL	X	Y	X	X	X	Y

As an attempt to determine the identity of the siblings, the genetic profile of the daughter of one of them was performed; no other relative was available. The analysis of the genetic profile of the child (Table 4) indicated a concordance of fifteen markers with PM025-1, achieving a Combined Probability of Paternity of 99.9757797593% and a Combined Paternity Index of 4127.77812; whilst only a concordance of thirteen markers with PM025-2.

Discussion

The use of DNA evidence in criminal investigations has gained worldwide acceptance in recent years. DNA testing (DNA profiling or DNA analysis) has helped to identify criminals and solve difficult crimes. On the other hand, DNA evidence has also helped to prove that many convicted people are actually innocent. Since every person has unique DNA, the discovery of particular DNA evidence at a crime scene can help law enforcement to determine who was involved in the crime. The more DNA markers examined and compared, the greater the chance that two unrelated individuals will have different genotypes. Alternatively, each piece of matching information adds to the confidence in connecting two matching DNA profiles from the same individual.

The practical application of DNA technology to the identification of biological material has had a significant impact on forensic biology, because it enables much stronger conclusions of identity or non-identity to be made [16]. It ought to be pointed out that DNA profiling can assist in the identification of a body only if DNA samples known to come from the deceased person, from the parents of the deceased, or from a child of the deceased are available.Concerning the crime cases presented in this research, the STR analysis was very useful. In the first case, all markers examined in the blood stains' DNA profiles were found to match with the alleged parents, indicating that the abductee and the couples' missing son is in fact, the same individual. Regarding the second case, although the soft tissue was almost completely decayed due to fire, STR loci could be detected from DNA extracted from bone fragments. The fact that bone and teeth are in many cases the only surviving material for DNA testing is evidence that these tissues are to a certain extent able to resist the damaging effects of time and environmental insults [17]. In fact, it is believed that inorganic hydroxyapatite, which constitutes approximately 70% of bone tissue, provides a physical barrier or protection for DNA enclosed within the bone cells (osteocytes) [18]. It is also an advantage that the STR loci detected are relatively small in size (117–345 bp) for forensic specimens, which often render severely damaged DNA.

Despite the notable advantages of this methodology, it has not yet been able to establish a national database of DNA in Mexico due to administrative discrepancies and slow adoption of forefront technology. It is still clear that it is a critical need to support the resolution of the unfortunate recent explosion of forensic cases.

In conclusion, STR typing is a reliable and robust genetic tool which has an important central role in society to solve problems of family relationships and forensic caseworks.

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