

Genetic Identification of Victims in a Bus Fire by the Use of NoorGIS Software

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Abstract

During 2015, a fatal incident happened in Iran. A terrible explosion was happened by a bus fire and in this incident 11 victims burned. Some of the victims could be identified with their medical case history and their external signs. But six victims due to severe burns were identified with genetic analysis. For genetic identification was used 16 plex identifier kit. The identified victims were from different families and for that reason parents and children of victims were used for genetic analysis. Result of that analysis identified all of the victims with the highest amount of paternity index (PI). For DNA matching used novel software that called NoorGIS. Likelihood ratio (LR) and Probability of Paternity (PP) test were performed using Familias software for efficiency evaluation NoorGIS.

Keywords: DNA Analysis; Short Tandem Repeat (STR); Paternity Index (PI)

Introduction

An explosion happened by a bus fires at 24 km of Damghan Road in Iran. In this incident 11 victims burned. Only five victims could be identified from medical history and their fingerprints [1,2]. Samples of parents, children and siblings were used for genetic analysis and among them there were a parent with lost child and six children as family's samples.

Genetic identification of individuals in forensic assemblies is known as an exact molecular method. For example using PowerPlex16 and AmpF ℓ STR Identifier were 1 in 10^{18} (with applying 16 loci Random Match Probability is 7.2×10^{-19}) in different incidents [3]. In this method, it is possible to use molecular indicators like STRs, mtDNA and Single Nucleotide Polymorphisms (SNPs) [4-6]. Considering multiple loci simultaneously produces a single and unique combination for everyone. Those loci can be considered with different compositions in commercial kits but one common case is 15 Short Tandem Repeat (STR) loci including D2S1338, D16S539, D13S317, TH01, D3S1358, CSF1PO, D7S829, D21S11, D8S1179, D5S818, D18S51, TPOX, VWA, D19S433, FGA and amelogenin as one sexuality indicator [3]. In Disaster Victim Identification (DVI), there are substantial numbers of genetic data and other information that must be analyzed. For genetic analysis been used some tools like: MDKAP, DNA-View, PATPCR, CEPOL software and CODIS [7]. In this research, results analyzed by novel software that called NoorGIS. In this software Paternity Index (PI), Combined Paternity Index (CPI) and posterior probability are calculated according to allelic frequency of Iranian [8].

Material and Methods

Initially, to acquire genetic information of samples collected from victims and their family members. A centimeter of samples were collected. Then 17 blood samples from families of victims were collected to the genetic laboratory.

DNA Extraction

DNA purification from biological samples can be accomplished using different methods [9-12]. In this study was used RGDE method for DNA extraction [13].

For rapid genomic DNA extraction method pour 500 μ l of blood or 0.5 g of fresh tissue into a 1.5 ml microfuge tube and add 1000 ml of cell lysis buffer. Shake microfuge tube gently, and then centrifuge it for 2 min at 6000 rpm. Remove and discard supernatant

and repeat this step two or three more times according to the sample. Then to that white sediment nucleu lysis buffer was added and it was completely mixed with buffer using mixed peptide sediment. Then using salting out method some proteins and wastes go out of the samples.

The next step was supplying DNA with water by adding cold and absolute alcohol to the samples. In that case, alcohol molecules were connected to the DNA which makes DNA heavy and its string visible.

Then applying centrifuge makes DNA sediment and after making it dry, DNA samples were dissolved in TE buffer.

Quality Control

This step was done by light absorption using nanophotometer machine and Agarose electrophoresis gel for samples. After DNA purification and before performing PCR, for quantity control of samples used nanophotometer machine with IMPLAN model. That machine uses ultra-violet spectrometer to search for very small DNAs, RNAs and proteins. That machine calculates different absorption wavelengths of DNA, RNA and protein. It is possible to perform quality control for samples using electrophoresis method for 15 minutes with 1% of Agarose gel in a tank with 0.5 molar TBE and 110 W voltages. DNA yield calculated 4.36 ± 2.53 μg and light absorption A260/A280 ratio was 1.91 ± 0.13 for appropriate quality control of samples.

PCR

This step was done by applying PowerPlex16 AmpF ℓ STR Identifiler kit which is the product of Applied Biosystems Company. After quality control, PCR was used to amplify the number of STR sequences. Reaction components for this step are 1ng of DNA, specific primers, Master Mix and Taq polymerase enzyme. Steps involved in thermocycler machine's activity are denaturation, Annealing and Extension. In denaturation, temperature was increased to 950 °C. In Annealing process, the temperature was reduced to 550 °C such that primer was attached to the DNA sample. Finally, in Extension step, the temperature was again increased to 720 °C and Taq enzyme starts replication. Those three steps were usually repeated for 25-35 times depending on different applications they may have. Then the reaction was ended at 4 °C temperature. This step is called PrePCR. After that PostPCR is a step that applies PrePCR product to add HiDiFormamid and size standard. Then samples were placed for 5 minutes in 950°C temperature that makes DNA single-stranded. Then the temperature was lowered to 0 °C so that HiDi could fix single-stranded DNA. The next step was placing samples to specific plate of genetic analysis machine.

Sample's profiles

This step was done with 3130xl genetic analyzer machine from Applied Biosystems Company. That machine can apply Argon laser with maximum stimulation of 488 and 514 nanometers to specify STR locus.

Data analysis

Initially, samples were analyzed using GeneMapper ID software [14,15]. After obtaining genetic information for individuals and to further analyze the data, in addition to genetic software and genetic information banks, specific native software with ability to search data and compare genetic and individual information is necessary [16,17]. For the mentioned event with high amount of genetic data, NoorGIS software was used for genetic analysis and genetic computation specially Paternity Index (PI) and Combined Paternity Index (CPI) [18,19]. Also Familias software was used for likelihood ratio analysis in cases when the amount of genetic comparison was specified [20].

Results

Genetic profiles of samples from victims and their family members were prepared in the laboratory using AmpF ℓ STR Identifiler. Then the results for unknown victims and 16 STR loci from family members were compared. Table 1 shows the number of victims and their families' samples and their relatives.

Samples of parents, children and siblings were used for genetic analysis and among them there were a parent with lost child and six children as family's samples.

Number of victims	Parents as victims' families	Single parent	Child	Other relationships
11	1	2	6	1

Table 1: Number of victims and their families' samples

DNA matching has done according Table 2. In two cases the comparisons were completely and other cases had an accurate DNA matching.

	Mother		Child1		Child2		Father		Mother		Child		Father		Mother		Child2	
Loci	113		11002725		11002726		227		11000293		11000294		117		11002737		11002726	
Csflpo	12	11	11	11	11	11	11	12	11	12	11	12	13	10	11	10	11	10
D135317	13	11	13	13	13	11	11	11	11	12	11	11	11	11	11	8	8	8
D165539	11	11	12	11	11	13	11	12	9	9	9	12	13	9	10	9	13	9
FGA	-	-	23	23	23	-	-	27	22	23	22	27	23	19	25	18	19	18
D75820	11	9	12	11	11	9	8	11	8	12	8	11	8	7	11	8	8	7
D18551	17	12	19	17	17	19	12	22	18	20	14	20	13	13	16	14	16	13
D21511	32.2	32.2	32.2	29	29	-	-	30.2	29	32.2	29	30.2	30.2	30.2	-	-	30.2	30
D251338	19	18	25	18	18	25	18	24	20	20	20	24	21	19	23	20	20	19
D195433	14	13	14	13	13	13	12	16	13	13	13	14.2	15.2	15	13.2	13	15	13
D3511358	16	15	15	14	14	16	16	17	17	18	17	17	16	15	18	16	16	15
D55818	13	13	13	12	12	13	12	13	11	11	11	12	11	10	12	11	11	11
D851179	14	13	13	13	13	14	13	13	10	12	10	13	13	12	14	10	14	12
TH01	9	9	9	9	9	9	7	9	7	8	8	9	9.3	9.3	9	6	9.3	9
TPOX	8	8	11	8	8	8	8	8	8	8	8	8	9	8	8	8	9	8
VWA	17	17	17	16	16	17	17	17	16	18	14	16	17	17	17	16	17	16

	Father		Child		Sibling		Father		Child		Child		Father		Mother	
Loci	115		114		11002727		118		112		116		11002740		11002739	
Csflpo	12	10	11	10	11	10	11	10	12	11	11	11	11	11	11	11
D135317	12	12	14	12	12	8	12	11	12	12	12	12	12	8	12	8
D165539	12	9	12	12	12	10	9	9	12	9	12	11	12	11	12	12
FGA	24	23	24	23	24	23	23	21	21	21	24	24	24	20	24	23
D75820	10	8	11	10	12	8	12	11	11	11	11	11	12	11	11	11
D18551	17	13	17	12	17	13	15	15	18	15	17	17	17	16	17	15
D21511	30	28	32.2	30	31.2	30	32.2	32.2	32.2	30	31	30	31	30	30	30
D251338	20	17	20	18	19	17	22	18	18	18	25	23	23	22	25	19
D195433	14	12	14	12	14	13	15.2	13	15.2	13	14	14	14	13	14	14
D3511358	17	17	17	16	19	17	17	14	15	14	17	15	17	15	17	15
D55818	12	12	13	12	14	12	11	9	11	9	12	12	12	11	13	12
D851179	13	10	14	13	14	13	14	12	12	12	15	14	14	14	15	12
TH01	9.3	6	9.3	9	9.3	6	9.3	9.3	9.3	9	8	7	7	6	9	8
TPOX	9	8	11	9	11	8	10	10	10	10	9	8	9	9	10	8
VWA	17	17	17	17	17	17	19	18	18	17	17	15	15	14	18	17

Table 2: DNA matching for different cases with STR markers of AmpFestR Identifier kit.

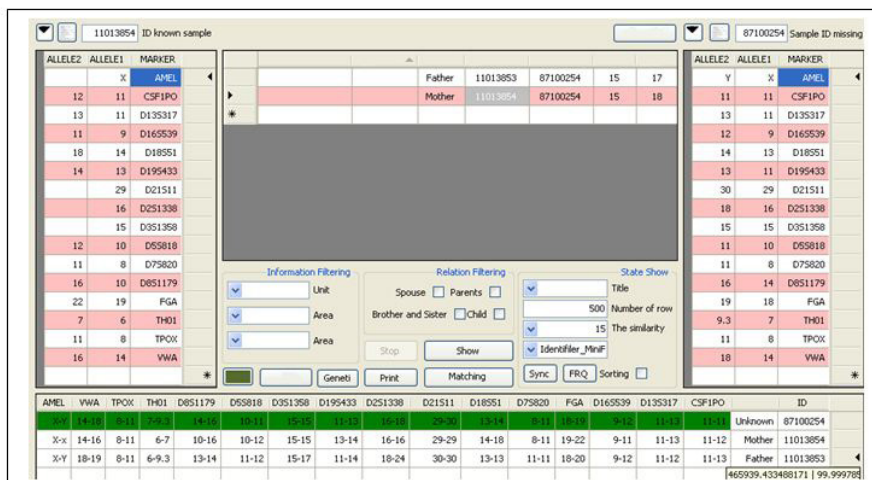


Figure 1: An example figure of profiles' genetic comparison by NoorGIS

Last step for genetic comparison was performed by calculating PI, as reported earlier [21]. For those individuals Paternity Index (PI) was calculated considering parent one child condition. All genetic profiles from corpses and relatives of victims were compared in a separate project using NoorGIS software. Figure 1 illustrates hypothetical genetic comparison in which marker and allele similarities were demonstrated. Amount of PI was changed between the lowest amount and the exact amount of PI (when genetic similarity is completed). For example the amount of LR for genetic comparison study is equal to 465939 and posterior probability is 0.9997. Those values could confirm the result of the genetic comparisons.

After saving the genetic profiles in NoorGIS software, amount of genetic comparisons between victims and their families have been estimated in two separate victims and families' bank. Figure 1 illustrates the amount and quality of that genetic similarity between an unknown victim and his parent.

Discussion

All genetic comparison process between victims and their families were performed in the minimum time and the results were reported. Some genetic information from individuals before death could be very useful in making genetic identification process faster however, for no genetic background was available from victims. Genetic analysis is exact scientific methods that can be important for genetic identification of victims in some events especially when ante mortem data are not present.

It may happen one or two STR from genetic profiles not similar which can be the result of mutation in that locus (22). For example, mutation rate in vWA locus is equal to 0.2%. For the result of genetic comparison from NoorGIS software, likelihood ratio (LR) test was performed using Familias software. The amount of LR for a population under study has been valuable. For example the amount of LR for genetic comparison study is equal to 46420760 and posterior probability is 0.9999999. Those values could confirm the result of the genetic comparisons.

By using NoorGIS software, it was possible to study and compare the genetic relationship between individuals and a given population, investigate personal genetic information, process obtained genetic information by employing genetic similarities and information. Also, processing capabilities was enhanced to achieve favorable outcomes in the genetic identification.

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