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Case Report

Using DNA in Paternity Testing by Identifying 16 STR Loci with Chromosomal Positions

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Abstract

Paternity cases arise as a result of major issues between married couples, as women may have a sexual partner in their lives, resulting in a child who is not her husband's son. This case is being investigated using Short Tandem Repeat (STR) analysis, which is commonly utilized in paternity cases to identify the biological father. STR analysis is based on 16 STR loci that are discovered on chromosomes, as well as the length of each locus.

Introduction

The International Society of Blood Transfusion has a list of 33 blood groupings that represent over 300 antigens. The majority of them have cloned and sequenced their genes. Red blood cells are part of the blood group system. The most prevalent methods utilised in paternity cases to identify the biological parents are the ABO, Rh, Kell, and Duffy systems [1,2].The ABO system is the most important in paternity case detection. It's used to assess whether a case should be included or excluded. It's also necessary for transfusions and transplants. Anti-A and/or anti-B antibodies are clinically significant in everyone above the age of six months. Antibodies to blood groups A and B can be found in each other. There is no A or B antigens in blood group O. Both of their antibodies, however, are present in serum. [3] The ABO blood group can be encoded by a single genetic locus. In blood grouping, there are three alternative (allelic) forms: A, B, and O. A child inherits one of the three alleles from each parent, resulting in six different genotypes and four distinct phenotypes [4]. Eukaryotic genomes contain a large number of repeated DNA sequences, which exist in a variety of sizes and are usually identified by the length of the core repeat unit. Long repeat units can have hundreds to thousands of bases in the core repetition depending on the number of contiguous repeat units or the total length of the repeat region. Microsatellites are short tandem repeats that can also be referred to as Simple Sequence Repeats (SSRs). Microsatellites and simple sequence repeats are DNA segments that resemble accordions and contain core repeat units ranging in length from two to seven nucleotides that are tandem repeated from a half dozen to several dozen times. The length of the repeat unit, for example, is used to name short tandem repeat sequences. Two nucleotides are repeated next to each other over and over again in dinucleotide repeats. Trinucleotides have three nucleotides in the repeat unit, tetranucleotides have four, pentanucleotides have five, and hexanucleotides have six nucleotides in the core repeat. For mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats, there are 4, 16, 64, 256, 1024, and 4096 potential motifs, respectively. The length of the repeat unit and the number of repeats differ in the STR sequence. The severity with which they adhere to an incremental repeat pattern differs as well. [5] STR is divided into many categories based on repetitive patterns. These categories are simple repetition, compound repetition, complex repetition and complex hypervariable repetition. Simple repeats have sequence units and the same length. Compound repetitions include two or more adjacent simple repetitions. [6] Complex repeating sequences may contain multiple repeating units of variable unit length and variable insertion sequences [7]. There are also many non-uniform alleles in complex hypervariable repeats. These alleles are different in size and sequence, so it is difficult to repeat genotyping. [8,9] Because these DNA markers show the highest possible variation or less polymorphic markers that can be combined to gain the ability to distinguish sample STR projects that began in April 1996 and ended in November 1997. DNA markers aimed at identifying humans are essential. Evaluation of 22 DNA typing laboratories and 16 candidate STR loci: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amel, D5S818 and FGA. These STR loci are on the chromosome [9-11].

Case Report

A 35-year-old man, his wife has 26 year's old and her female child has 6 year's old visited our clinical lab to detect biological father. In this case, we have to take buccal swab from each one in order to extract the DNA Bucaal swab was placed in a 2 ml micro centrifuge tube. 400 µl of cotton and DACRON swab was added to the sample. 20 µl of QIAGEN protease stock solution and 400 µl cotton DACRON swab buffer AL were added to the sample and then mixed immediately by vortexing for 15 sec. The sample was incubated at 56 °C for 10 min. 400 µl of cotton or DACRON swab was added to the sample, mixed again by vortexing and then centrifuged briefly to remove drops from inside the lid. 700 µl of the mixture was applied to the spin column in a 2 ml collection tube without wetting the rim, the clap was cap and centrifuge at 6000 x g (8000 rpm) for 1 min The spin column was placed in a clean 2 ml collection tube and the tube containing the filrate was discarded. Previous step was repeated by applying up to 700 µl of the remaining mixture from step four to the spin column. The spin column was opened carefully and 500 µl buffer AW1 was added without wetting the rim. The cap was closed and centrifuged at 6000 x g (8000 rpm) for 1 min. the spin column was placed in a clean 2 ml collection tube. The spin column was opened and 500 µl of buffer AW2 was added without witting the rim. The cap was closed and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. The spin column was placed in a new 2 ml collection tube and the collection tube with the filrate was discarded and centrifuged at 20,000 x g(14,000 rpm) for 1 min. The spin column was placed in a clean 1.5 microcentrifuge tube and the collection tube containing the filrate was discarded. The spin column was opened and 150 µl buffr AE was added. The sample was incubated at room temperature for 1 min and then centrifuge at 6000 x g(8000 rpm) for 1 min.. after the DNA of each one was extracted, Each sample was amplified by DNA amplification using AmpFISHER® Identifiler® Plus Kit and then prepared for electrophoresis on the 3500 genetic analyzer.

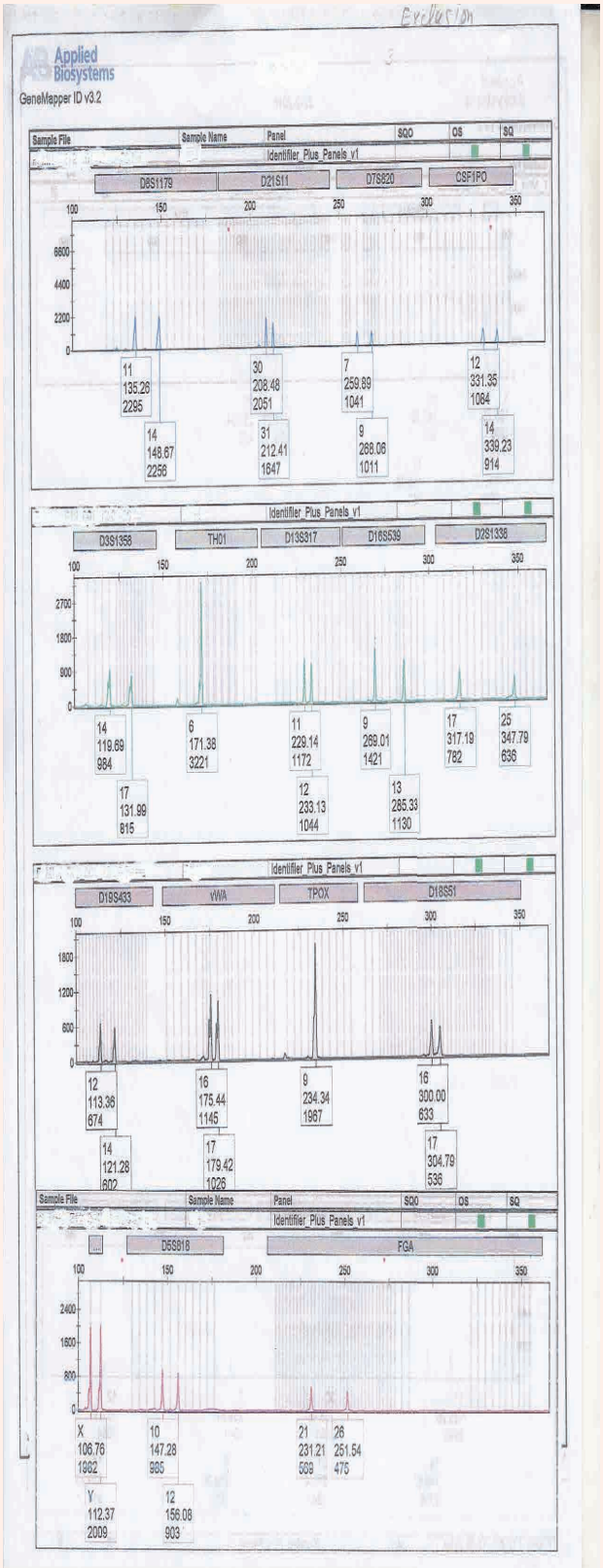

Figure 1: DNA profile of the father in paternity case

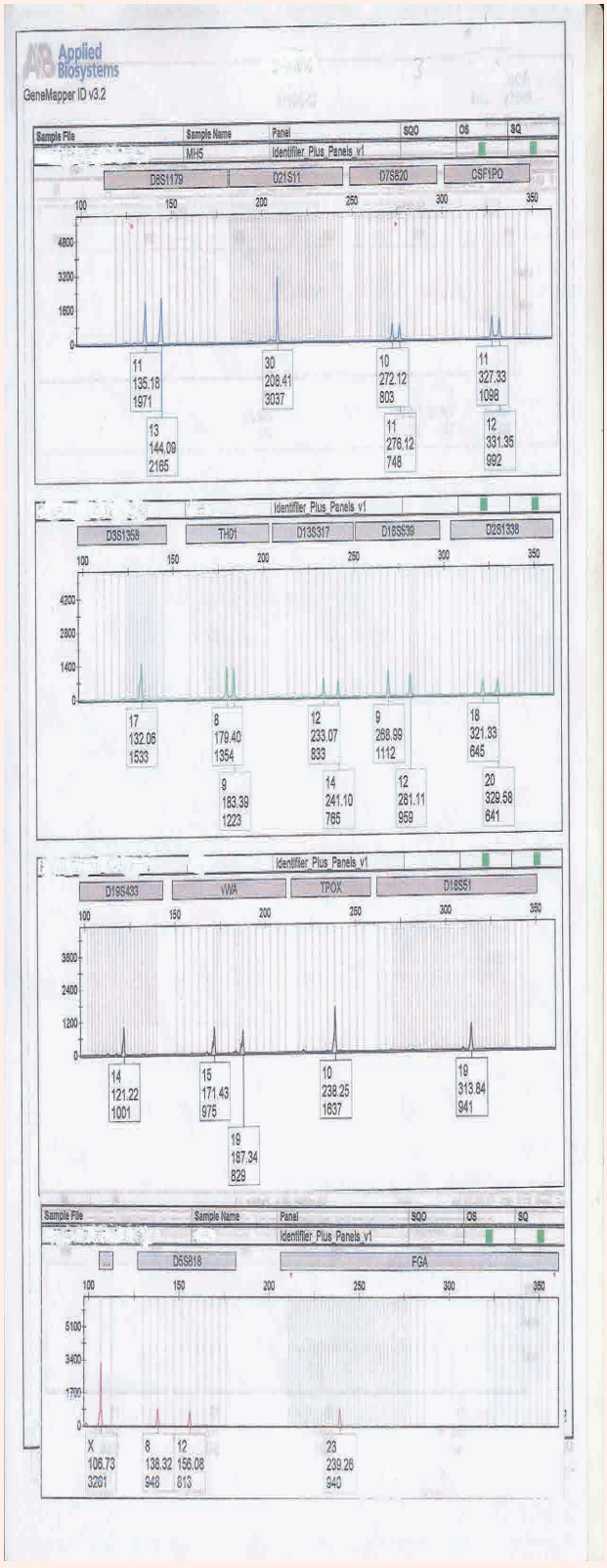

Figure 2: DNA profile of the mother in paternity case

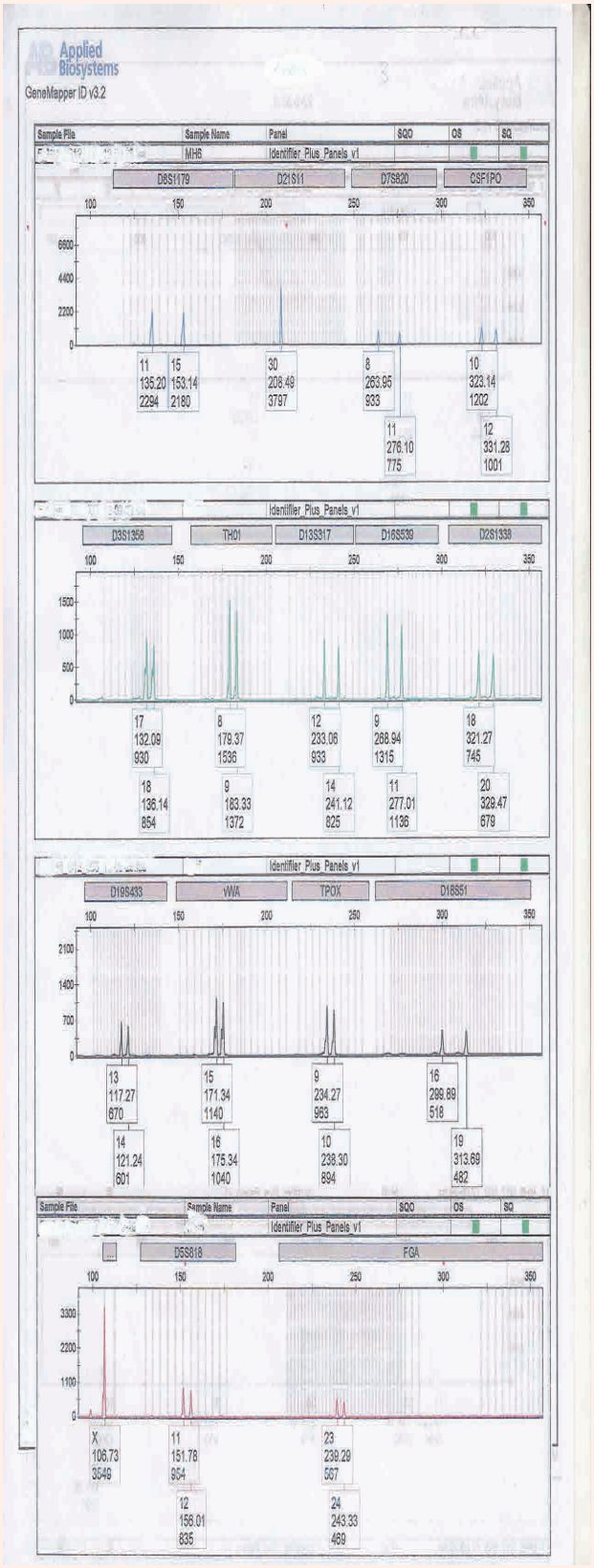

Figure 3: DNA profile of the female child in paternity case 3

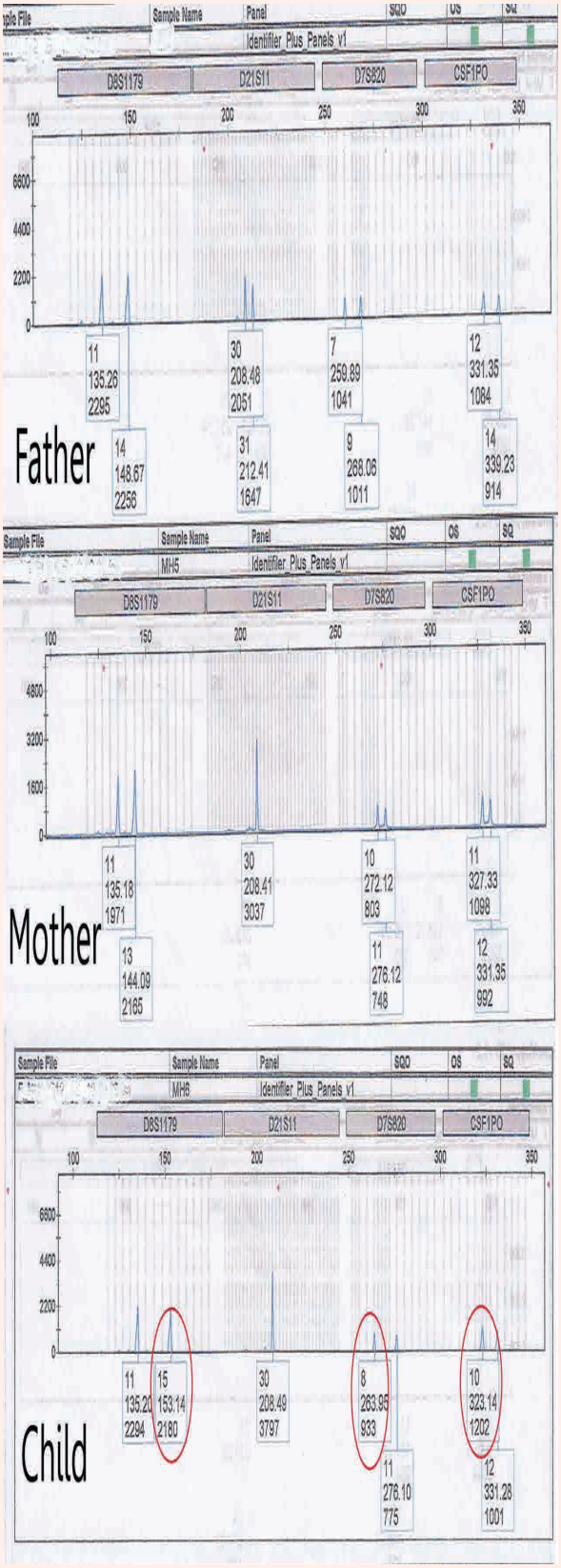

Figure 4: Differencing loci between child and father

Table 1: Patenity case 3

Identifier	locus	D8S1179		D21S11		D7S820		CSF1PO		D3S1358		THO1		D13S317		D16S539		D2S1338		D19S433		vWA		TPOX		D18S51		Amel.		D5S818		FGA	
Father	Alleles	11	14	30	31	7	9	12	14	14	17	6	6	11	12	9	13	17	25	12	14	16	17	9	9	16	17	X	Y	10	12	21	26
Mother	Alleles	11	13	30	30	10	11	11	12	17	17	8	9	12	14	9	12	18	20	14	14	15	19	10	10	19	19	X	X	8	12	23	12
Child (female)	Alleles	11	15	30	30	8	11	10	12	17	18	8	9	12	14	9	11	18	20	13	14	15	16	9	10	16	19	X	X	11	12	23	24

At D8S1179, D7S820 and CSF1PO locus, the alleles of female child are 11: 15, 8:11and 10:12 respectively while the alleles on the father of the same locus are 11:14, 7:9 and 12:14 respectively and the mother 11:13, 10:11 and 11:12. This indicates that the alleles 15, 8 and 24 are not shared from this father

Discussion

In order to identify that the case is whether inclusion or exclusion, profiling DNA of father, mother and child have to be compared with each other according to the length of short tandem repeats of each locus. There are 16 candidate STR loci which are D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amel, D5S818 and FGA. In inclusion cases, the child shares the length of each STR loci with his parents because each biological parent shares 23 chromosomes for their child. However, in exclusion cases, the child's length of STR loci differs between the father and mother. In this case , At D8S1179, D7S820 and CSF1PO locus, the alleles of female child are 11: 15, 8:11and 10:12 respectively while the alleles on the father of the same locus are 11:14, 7:9 and 12:14 respectively and the mother 11:13, 10:11 and 11:12. This indicates that the alleles 15, 8 and 24 are not shared from this father.

Conclusion

From the introduced data it can be showed that this case is exclusion. In this case, The alleles of female children at the D8S1179, D7S820, and CSF1PO loci are 11:15, 8:11, and 10:12, respectively, while the father's alleles are 11:14, 7:9, and 12:14, and the mother's alleles are 11:13, 10:11, and 11:12. This indicates that this father does not have alleles 15, 8, or 24.

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