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Biochemical characterization of Molecular Markers for Human Genetic Identification in Paternity testing by DNA profiling

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Abstract
Human Genome is made of 3 billion nucleotide bases Adenine A, Cytosine C, Thymine T and Guanine G. The DNA sequencing infers the order of nucleotides in the genome. The regions of genome having variation in the sequence are crucial for human genetic identification. The molecular markers of 15 different genomic regions of human with repeat nucleotides sequence of 4 bases in the non-coding regions of genome exhibit a high degree of polymorphism. The biochemical characterization of molecular markers elucidates the chromosomal location of the marker, sequence of repeat units and variation among the individuals for human identification in forensic investigation and genetic inheritance. The 15 molecular markers were analysed for human identification in a mother, child and father trio. The STR loci were amplified by PCR using AmpFlSTR Identifiler Plus PCR amplification kit. The amplified markers were separated through capillary electrophoresis on Applied Biosystems Genetic Analyzer ABI 3100 as per the manufactures instructions. The data was analysed by GeneMapper v3.5 software. The statistical analysis was performed using Bayesian mathematics using in house allele frequency data. The paternity index and probability of paternity were 206759811 and 0.999999999. The DNA test conducted on the samples of mother, child and father trio convincingly established the paternity of the child by perfect match of sequence variation short tandem repeats STR genotypes of the genome with the biological parents. The paternity index of molecular markers and probability of paternity in the human identification are very high. The results of examination of 15 molecular markers with their biochemical characterization conclusively establish the genetic identification.

Keywords: DNA, STR, PCR, molecular markers

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INTRODUCTION

The repeat DNA sequence variation among individuals was identified almost thirty years back[1]. The repeat sequence form short tandem repeat STR molecular markers. These markers have different density than rest of DNA in the genome and therefore termed as satellite DNA. The 2–7 base pair repeats are termed as microsatellite and thereafter upto 60 base pairs are as minisatellite and more than that are macrosatellite molecular markers. The STRs of 4 base pair unit in the non-coding region of genome are mostly used for elucidating the polymorphism among individuals. The 15 tetra repeat STR loci and gender specific amelogenin locus are analysed in the human identification. The markers are amplified through PCR by fluorescent dye labelled primers hybridizing to the conserved flanking regions of the repeat sequence. The STR makers generate an array of allele with a site specific range from 6 to 27. The alleles of 15 STR loci with diploid genetic architecture of human create possibility of generating 10^{19} different DNA profiles and rule out the probability of similar DNA profiles in the entire global human population. The genetic identification by molecular markers is based on the principle of Mendelian inheritance. All the alleles of the tested markers in an individual must be unambiguously inherited from its biological parents. The markers are validated and commercially available for human genetic identification[2]. The selected markers in the panel are from unlinked, interior to the ends of chromosomes and least involved in crossing over in the fertilization process. The genetic identification is dependent on the allele frequency of the STR loci and Bayesian mathematics infers the paternity index and probability of paternity. This investigation will be a reference resource for paternity test and family inheritance in forensic and disease diagnostics.

MATERIALS AND METHODS

The 2 ml blood samples of mother, child and father were collected in sterile EDTA coated vials. The consent forms of donors were filled as per ethical guidelines of laboratory. The genomic DNA was extracted by standard proteinase K digestion, phenol-chloroform extraction and ethanol precipitation using molecular biology grade chemicals[3-4]. The quality and quantity of extracted DNA were checked by gel electrophoresis on 0.8% agarose. The STR loci were amplified by PCR using AmpF*l*STR Identifiler Plus PCR amplification kit[2]. The PCR was carried out in reaction volume of 12.5 µl containing 5µl reaction mix, 2.5 µl primer mix and 5 µl template DNA at thermal cycling conditions of 95°C for 11min, followed by 28 cycles of 94°C for 20sec and 59°C for 3min and final extension of 60°C for 10min on Gene Amp 9700 thermal cycler. The amplified markers were separated through capillary electrophoresis on Applied Biosystems Genetic Analyzer ABI 3100 as per the manufactures instructions. The data was analysed by GeneMapper v3.5 software. The statistical analysis was performed using Bayesian mathematics using in house allele frequency data.

RESULTS

The molecular markers of different locations distributed in the chromosomes of genome were examined. The locus wise possible number of 104 alleles and size of Amelogenin X and Amelogenin Y are shown in table 1. The alleles of all examined loci in the child were matched completely with the biological parents on the principle of Mendelian inheritance. The paternity index and probability of paternity were 206759811 and 0.999999999. The allele frequencies of in-house generated database were used for calculation. The results of paternity examination with chromosomal position, STR unit sequence and GenBank accession numbers are shown in table 2. The electropherograms of DNA profiles of mother, child and father are shown in figures 1, 2 and 3 respectively. The results confirmed the genetic identity and inheritance of child from the biological parents.

Locus	Possible Alleles										Total Alleles
D8S1179	10	11	12	13	14	15	16	17			8
D21S11	28	29	30	31.2	32.2	33.2					6
D7S820	8	9	10	11	12						5
CSF1PO	9	10	11	12	13						5
D3S1358	14	15	16	17	18	19					6
THO1	6	7	8	9	9.3	10					6
D13S317	8	9	10	11	12	13	14				7

Table 1: continued……

D16S539	8	9	10	11	12	13	1 4				7
D2S1338	17	18	19	20	21	22	23	24	25	26	10
D19S433	9	12	13	13.2	14	14.2	15	16	16.2		9
vWA	14	15	16	17	18	19					6
TPOX	8	9	10	11	12						5
D18S51	11	12	13	14	15	16	17	18	19	20	10
D5S818	9	10	11	12	13						5
FGA	20	21	22	23	23.2	24	25	26	27		9
AMELOGENIN	Amelogenin X: 106 base pairs and Amelogenin Y: 112 base pairs										
											104

Table 1: The locus wise possible number of alleles

Locus	ChP	STR sequence	GenBank Acession	Mother		Child		Father		PA	LOP	PI
D8S1179	8q	TCTA	G08710	15	15	10	15	10	12	10	0.5	2.07
D21S11	21q	TCTA	M84567	27	29	27	28	28	28	28	1	5.6
D7S820	7q	GATA	G08616	11	11	9	11	9	12	9	0.5	18.66
CSF1PO	5q, 6 intron	AGAT	X14720	11	11	11	12	11	12	12	0.5	1.22
D3S1358	3p	TCTA	AC099539	17	17	14	17	14	16	14	0.5	7
THO1	11p, 1 intron	AATG	D00269	6	9.3	7	9.3	6	7	7	0.5	2.33
D13S317	13q	TATC	G09017	9	9	9	9	9	9	9	1	11.2
D16S539	16q	GATA	G07925	9	11	9	11	11	11	9, 11	1	2.03
D2S1338	2q	TGCC	G08202	17	20	20	20	17	20	20	0.5	8
D19S433	19q	AAGG	G08036	12	13.2	12	14	14	14.2	14	0.5	2.33
vWA	12p, 40 intron	TCTA	M25858	18	18	18	18	17	18	18	0.5	3.11
TPOX	2p, 10 intron	AATG	M68651	11	12	11	11	11	12	11	0.5	1.1
D18S51	18q	AGAA	X91254	14	14	12	14	12	16	14	0.5	2.15
D5S818	5q	AGAT	G08446	12	12	12	12	12	12	12	1	3.3
FGA	4q, 3 intron	TTTC	M64982	20	23	20	20	20	23	20	0.5	4.67
AMELOGENIN	Xp22.1-22.3 and Yp11.2,` 1 intron	106, 112 base pairs	M55418, M55419	X	X	X	X	X	Y			
										CPI2067 59811.1 POP: 0.999999995		

Table 2: The paternity examination of child
ChP: Chromosomal Position, PA: Paternal Allele, LOP: Likelihood of Paternity
PI: Paternity Index, CPI: Combined Paternity Index, POP: Probability of Paternity

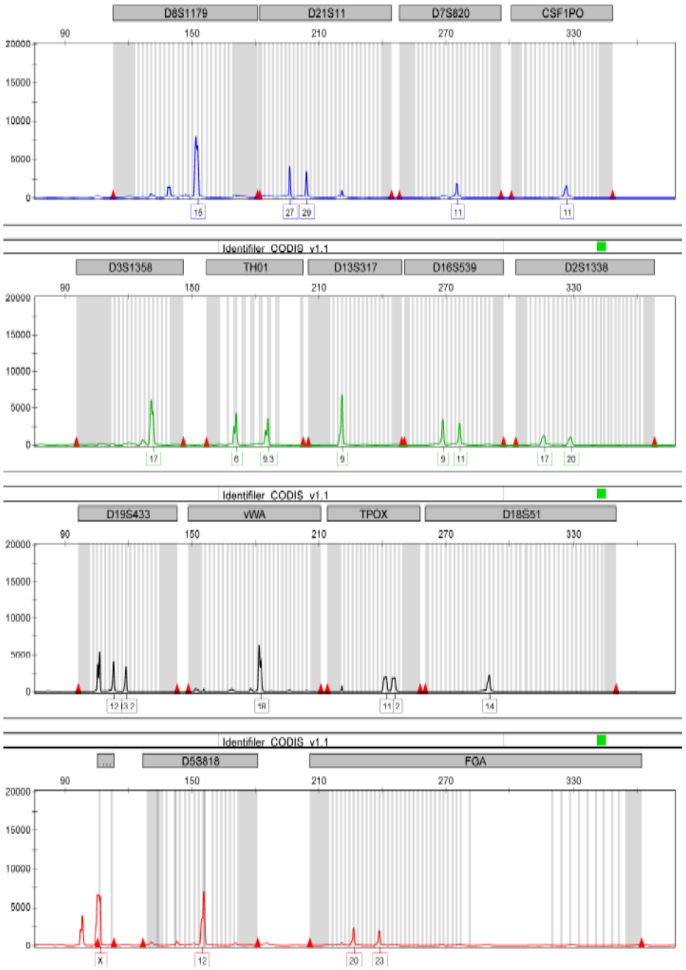

Figure 1: Elecropherogram of DNA profile of mother of child

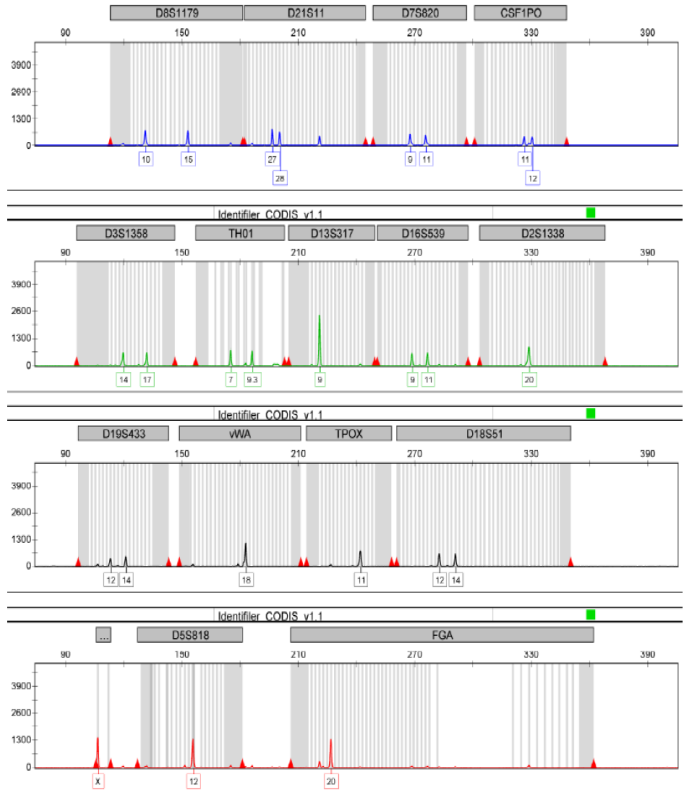

Figure 2: Elecropherogram of DNA profile of child

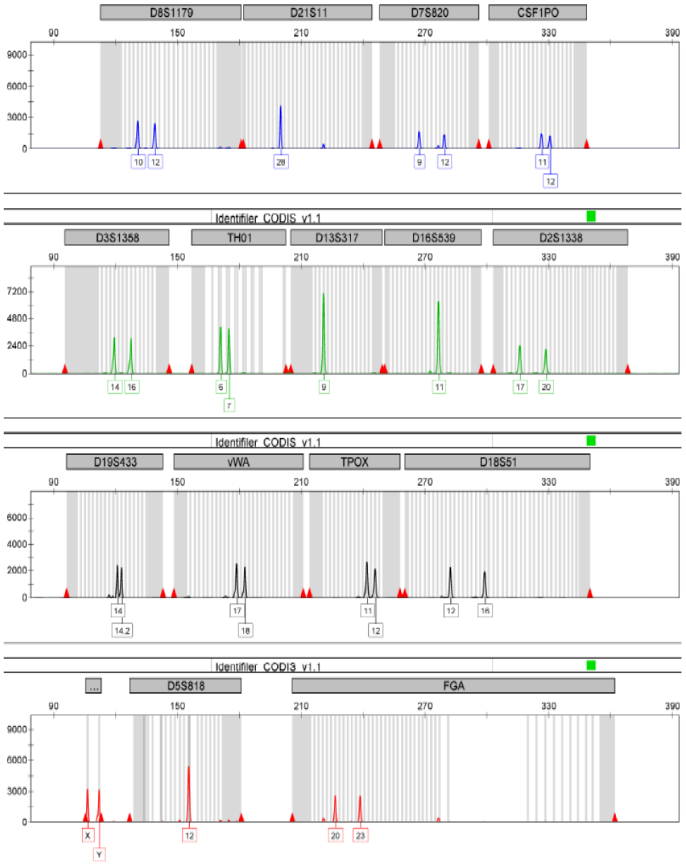

Figure 3: Elecropherogram of DNA profile of father of child

Discussion and Conclusion

The DNA profiling for human genetic identification is based on the principle of Mendelian inheritance. The child is equal ratio mix of genetic material from its biological parents in all its genetic architecture. The 15 short tandem repeat STR loci has the 104 possible alleles in diploid progeny and provide the power of discrimination of 10^{19}, making it impossible that two individual except monozygotic twins have the same DNA profile in the examined loci. The STR loci examined are in the non coding regions of genome and the variation in the repeat size does not encounter the genetic functioning of the individuals. The nomenclature of STR loci is DnSN, where D: DNA, n: no. of chromosome, S=single strand, N=no. of STR locus on the chromosome, as D8S1179 stands for 1179 STR locus on 8th chromosome single strand DNA. The CSF1PO, THO1, vWA, TPOX and FGA stands for c-fms Proto-Oncogene for CSF-1 receptor gene, Tyrosine Hydroxylase gene, von WillebrAnd factor gene, Thyroid Peroxidase gene, Fibrinogen Alpha chain gene respectively. The repeat units of intronic regions of the genes are examined for measuring the variation among individuals. The intronic region of 106 base pairs Amelogenin X and 112 base pairs of Amelogenin Y profiles the gender of the tested individual. The STR loci are from mutation free zone and selected from the

least disturbed regions during the meiotic divisions and not located on the age related depleting ends of chromosomes. The statistical test is performed to calculate the paternity index and probability of paternity. These two parameters provide the evidence to the test with a high cumulative paternity index. The paternity index is the measure of value that the transmitting alleles to child is from its father on concluding the paternity and inversely proportional to frequency of alleles in the population. The indexes of all examined loci are independent to each other and hence multiplied to provide high value. The probability of paternity is calculated to derive maximum possible value to the inclusion results using Bayesian mathematics[5-6]. The target regions of short tandem repeat sequences are amplified by nucleotides binding to the flanking conserved sites[7-8]. This study established the genetic identity of child unambiguously with elaborate experimental-statistical analysis in Indian population. The molecular markers with the described biochemical characteristics form a reference resource for human genetic identification in forensic and disease diagnostic investigations.

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