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CASE REPORT

Kumarasamy Thangaraj,[1] *Ph.D.; Alla G. Reddy,*[1] *M.Sc.; and Lalji Singh*[1] *Ph.D.*

Mutation in the STR Locus D21S11 of Father Causing Allele Mismatch in the Child*

ABSTRACT: We analyzed a case of paternity dispute with 15 autosomal STR loci and found a mismatch in one of the alleles of the locus D21S11 in the child. The composition of the alleles of this locus in the mother, suspicious father, and child were 29/32, 29/29, and 29/30, respectively. The combined paternity index (2.4×10^{10}) and paternity probability (0.9999) suggest that the suspicious father is the biological father of the child. Further analysis of 6 Y chromosome STR loci revealed matching of all the Y chromosomal alleles of the child with that of the suspicious father. Since there was a perfect match of all the paternal alleles inherited (15 autosomal and 6 Y chromosomal) in the child with that of the suspicious father except the allele D21S11, it is suggested that this might be a case of mutation. Cloning and sequencing of all the alleles of the locus D21S11 of the suspicious father, mother, and the child helped in determining that the suspicious father contributed the mutated allele.

KEYWORDS: forensic science, DNA typing, short tandem repeats, mutation, Y chromosome, paternity test, paternity index, plasmid cloning, DNA sequencing, Penta E, D18S51, D21S11, THO1, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S317, D5S818, DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS393

Short tandem repeats (STRs), which are commonly known as microsatellites, consist of tandemly repeated sequences of 2 to 7 bp. They are highly polymorphic in the repeat sequences and length. This property has been exploited for various case work, including parentage determinations and forensic applications (1). Based on the composition of repeat motifs, STRs are classified into three types: simple repeats, compound repeats, and complex repeats (2). A limitation of STRs is that the mutation rates in microsatellites are very high compared to other polymorphic markers. Although most of the STR mutations are neutral, some of the STRs, particularly triplet repeats, are associated with genetic disorders (3,4). Single-step mutations (gain or loss of one repeat) account for about 90% of the STR mutations. Expansion of a repeat motif involves either unequal crossing over or an error in DNA replication (5,6). Here we report a mutation in the father's autosomal STR locus D21S11, during spermatogenesis, giving rise to an allele mismatch in the child.

Materials and Methods

Samples

A suspicious father wanted to confirm the paternity of his four-year-old child by DNA profiling. Blood samples were collected from the suspicious father, mother, and child. Samples were sent to our Centre to establish the paternity of the child.

STR Profiling and Genotyping

DNA isolation from the blood samples was carried out as per the protocol described elsewhere (7). Samples were amplified using AmpF*l* STR Profiler Plus (Perkin Elmer, Foster City, USA) and PowerPlex 16 (Promega Corporation, Wisconsin) kits as per the manufacturers' instructions. Six Y chromosome STR loci—DYS19, DYS389I, DYS389II, DYS390, DYS391, and DYS393—were amplified in a multiplex reaction (8). All the PCR amplicons were analyzed on the ABI377 using GeneScan software. Allele sizes were obtained using Genotyper software.

PCR and Cloning of D21S11 Alleles

Primer sequences for the D21S11 locus were obtained from the website: www.cstl.nist.gov/biotech/strbase/ret_d21s.htm and synthesized using an ABI392 (Perkin Elmer, Foster City, USA) oligosynthesizer. DNA samples of the suspicious father, mother, and child were amplified under the following conditions: initial denaturation at 94°C for 1 min, 30 cycles at 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, and final extension at 60°C for 45 min. PCR amplicons were electrophoresed in 2% agarose gel to check the amplification and to quantify the amplicons. PCR products were ligated in pMOS*Blue* vector (Amersham Biosciences, Buckinghamshire, UK) as per the manufacturer's instruction. The recombinant plasmids were transformed into DH5α bacterial host cells. Transformed cells were plated on IPTG–X-gal and Ampicillin-treated agar plates for blue-white and antibiotic selections, respectively.

[1] Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India.

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Plasmid DNA Preparation

Because of the presence of IPTG and X-gal, colonies with recombinant plasmid (with insert DNA) appear in white, and the nonrecombinant plasmid appears in blue colors. Twenty isolated white colonies from each transformation (from mother, child, and the suspicious father) were picked up and inoculated in LB broth medium along with one blue colony as a control. Plasmid DNA preparation was carried as follows. About 3.0 mL of overnight culture was spun down for 1 min at 12,000 *g*, the pellet was resuspended in 100 μL of double distilled water, and the content was transferred to a 1.5 mL Eppendorf tube. To this 100 μL of lysis buffer (1 mL of lysis buffer consists of: 100 μL 10% of SDS, 20 μL of 0.5 *M* EDTA, and 10 μL of 10 *N* NaOH) was added and kept in boiling water bath for 2 min. Fifty microlitres of 1 *M* $MgCl_2$ was added to the tube and kept on ice for 2 min. After mixed by tapping, the tube was centrifuged at 12,000 *g* for 2 min, and 50 μL of 5 *M* potassium acetate was added to the same tube and kept on ice for 2 min after mixing. The tube was centrifuged at 12,000 *g* for 2 min, and the supernatant was collected in a fresh Eppendorf tube. To precipitate DNA, 600 μL of Isopropanol was added to the tube and kept on ice. After 2 min, the tube was centrifuged at 12,000 *g* for 2 min and the DNA pellet was washed twice with 70% alcohol, air-dried, and resuspended in 50 μL of TE.

Sequencing of D21S11 Alleles

About 150 ng of DNA from each clone was sequenced (both forward and reverse strands) using the BigDye terminator ready reaction kit (Perkin Elmer, Foster City, USA) and U19 and M13 primers. Since the father had homozygous allele, about 50 ng of the PCR amplicon was directly sequenced. Sequencing was performed in an ABI3700 automated DNA analyzer (9).

Statistical Analysis

Paternity index (PI) determines whether the suspicious father is the biological father of the child and was calculated by the following method (10). Paternity index = (X/Y) = (probability of likelihood ratio of a child's alleles resulting from a mating of the mother and suspicious father)/(allele frequency in the population). Probability of paternity was calculated using the following formula: probability of paternity = [0.5 × PRODUCT (all paternity indices)]/[{0.5 × PRODUCT (all paternity indices)} + 0.5] (http://www.medal.org/adocs/docs_ch43/doc_ch43.12.html). The allele frequency of the Indian population was used to calculate the paternity index (11).

Results and Discussion

Our initial analysis of a family included in a paternity dispute using Profiler Plus kit (nine loci) showed mismatching of one of the alleles of the locus D21S11 of the child (Fig. 1*A*). Further analysis of the samples with PowerPlex 16 system, which has six additional autosomal STR loci, confirmed the mismatch only in the locus D21S11. The remaining 14 loci showed perfect matching (Fig. 1*B*; Table 1). For the locus D21S11, the mother's lane (Lane 1 of Figs. 1*A* and 1*B*) contained two (heterozygous) alleles—29 and 32, and the suspicious father's lane (Lane 3 of Figs. 1*A* and 1*B*) had one (apparently homozygous) allele—29/29. This was further confirmed by the peak height of the homozygous allele (29), which was double the other allele of the father, mother, and the child (as evident in the vertical scale of Fig. 2). If both the tested adults were the true biological parents of the child, the expected genotype of the child would have been either 29/29 or 29/32, but the alleles observed in the child's lane (Lane 2 of Figs. 1*A* and 1*B*) were 29 and 30 (Fig. 2). This may be explained either by assuming that the suspicious father is not the biological father or there is a mutation. Therefore, we calculated the paternity index separately for every locus (see Table 1). The combined paternity index estimated for 15 loci was 2.4×10^{10}, which is very high compared to other populations studied for the same STR loci (12). Probability of paternity calculated using the paternity indices in this case was 0.9999, suggesting that there was a high probability of the suspicious father being a biological father. No more testing was needed in this case since the paternity index was so high. However, we further analyzed the child and suspicious father's samples with Y chromosome-specific STR markers, which are inherited paternally. In fact, the American Association of Blood Bank (AABB) has strongly encouraged laboratories to perform additional tests whenever a single locus exclusion is observed in paternity testing (http://ww.aabb.org).

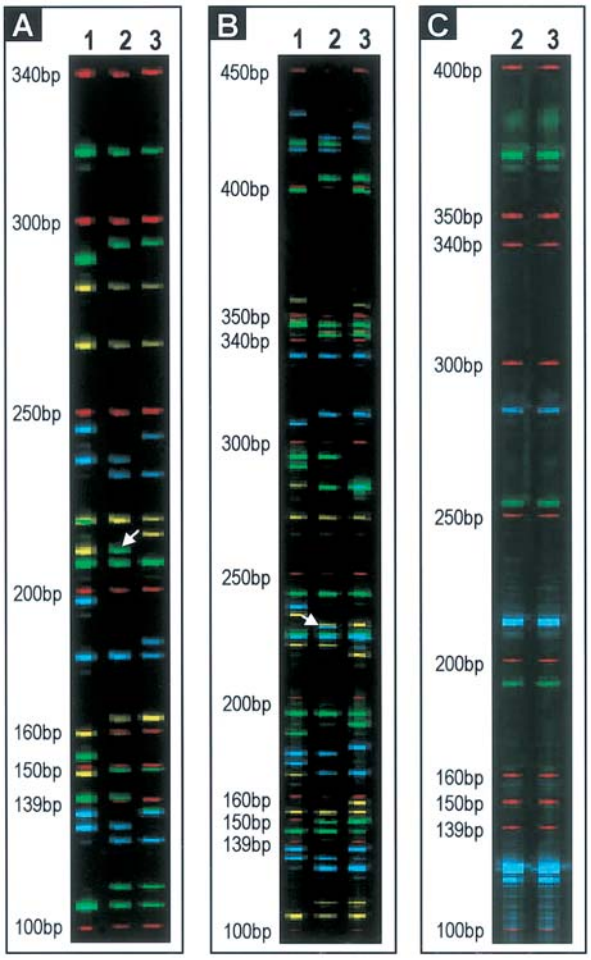

FIG. 1—*GeneScan analysis of (A) profiler Plus kit, (B) PowerPlex 16 system, and (C) Y chromosome STRs. Samples were amplified and analyzed in 4% denaturing acrylamide gel in the presence of GS ROX500 and formamide. Red color bands, which are common in all the lanes, are size standard, whereas the other bands are various STR alleles. Lane 1 = mother, Lane 2 = child, and Lane 3 = father.*

Y chromosome specific STR profiles revealed that all the loci of the child matched the suspicious father (Fig. 1*C*; Table 2). Since the Y chromosome is inherited paternally without recombination,

TABLE 1—*Genotype/alleles of the parents and the child for autosomal STR loci and paternity index.*

S. No	Locus	Genotypes/Allele(s)			Paternity Index $P = (X/Y)$
		Mother	Child	Father	
1	Penta E	12/15	12/13	13/14	9.43
2	D18S51	12/19	13/19	13/19	3.16
3	D21S11	29/32	29/30*	29/29	1.0
4	THO1	8/9	7/9	7/10	5.0
5	D3S1358	16/17	15/16	15/17	1.35
6	FGA	24/26	23/24	23/25.2	2.92
7	TPOX	8/11	8/8	8/11	1.21
8	D8S1179	12/15	12/14	11/14	3.35
9	vWA	18/21	18/19	19/19	15.15
10	Penta D	9/12	10/12	9/10	2.11
11	CSF1PO	12/12	11/12	11/12	2.31
12	D16S539	12/13	10/13	10/10	18.86
13	D7S820	9/13	9/13	9/13	8.47
14	D13S317	11/13	13/13	12/13	7.81
15	D5S818	10/13	13/14	14/14	111.11

* No match between a nonmaternal allele in the child and the suspicious father.

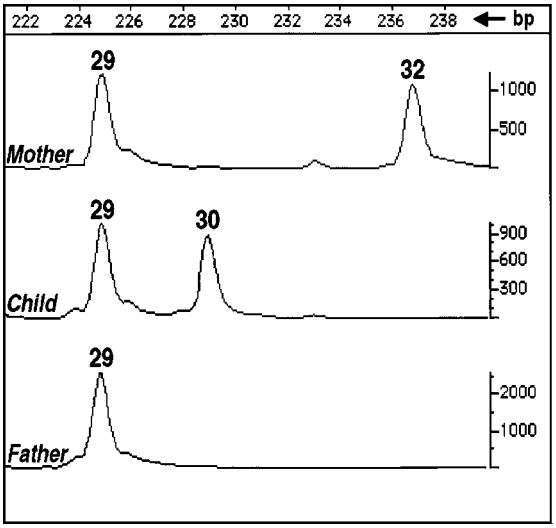

FIG. 2—*Genotype of the locus D21S11 of the mother, child, and father. Amplicons of the multiplex PCR with PowerPlex were analyzed in ABI377 using GeneScan software. Electropherograms of alleles were obtained using Genotyper software. Note the presence of allele 30 in the child and the peak height of all the allels of the mother, the suspicious father and the child (shown in the vertical scale).*

having a Y-STR profile similar to the child is possible in the case of the grandfather, paternal uncles, and their male children. Hence, the possibility of paternal side males being a father cannot be ruled out. However, as per the statement of the suspicious father, he does not have any brothers and his father expired long before the birth of the child. He has one paternal uncle and his family separated long ago and does not have any contact. Therefore, a illicit relationship has been ruled out. Considering the high probability of the matching of autosomal STR and Y chromosome haplotypes, it is obvious that the single allele mismatch of the alleles of the locus D21S11 of the suspicious father is due to mutation and that the suspected father under investigation is the true biological father.

For further characterization of the mutant allele, all the alleles (mother, child, and the suspicious father) of the locus D21S11 were cloned and sequenced. Direct sequencing of PCR amplicons and sequencing of 20 clones of the suspicious father revealed only one

TABLE 2—*Y chromosome specific STR profiling of the child and the suspected father shows the matching of all the alleles.*

S. No.	Locus	Alleles	
		Child	Father
1	DYS19	14	14
2	DYS389I	11	11
3	DYS389II	29	29
4	DYS390	24	24
5	DYS391	10	10
6	DYS393	14	14

allele (29) with the structure $[(TCTA)_4\ (TCTG)_6\ (TCTA)_{11}]$. The remaining eight repeat units were not polymorphic and are highlighted by yellow in Fig. 3. Sequencing of the mother's alleles showed variation at every repeat motif of both the alleles. Alleles 29 and 32 consist of the following polymorphic repeat structures: $[(TCTA)_6\ (TCTG)_5\ (TCTA)_{10}]$ and $[(TCTA)_5\ (TCTG)_6\ TATA\ (TCTA)_{12}]$, respectively. Other than the variation in the repeat units in allele 32 of the mother, we have observed a C → A transversion in the last repeat motif (indicated by a star in Fig. 3). Sequencing of the child's alleles revealed that allele 29 consists of polymorphic repeat structure $[(TCTA)_6\ (TCTG)_5\ (TCTA)_{10}]$ and the allele 30 consists of $[(TCTA)_4\ (TCTG)_6\ (TCTA)_{12}]$ (repeat structure). Comparison of both the alleles of the child with the parents' alleles revealed that the repeat structure of the child's allele 29 matched with the allele 29 from the mother. From allele 30 of the child, two out of three polymorphic repeats showed a match with the father. The third repeat motif (TCTA) consists of one extra repeat unit (Fig. 3). Results demonstrate a mutation in repeat motif (TCTA), which might have occurred in the paternal germ cells.

STRs, in general, are prone to mutations secondary to recombination and replication slippage (5,6). The average mutation rate of the autosomal alleles is estimated to be 1.2×10^{-3}/locus/gamete/generation. The average mutation rate in tetra-nucleotide repeats is estimated to be about four times more than the dinucleotide repeats (13). Brinkmann et al. (14) looked at 10,844 parents-child comparisons with nine STR loci from random populations and observed 23 STR mismatches, including one in D21S11. However, they could not trace the origin of the mutated allele in the case of D21S11 because the father was not available for the study. In another study, sequence variability at the locus D21S11 was observed in Ovambo and Papuan populations (15). Cumulative data from 52 paternity testing laboratories have shown that the frequency of mutation in locus D21S11 is 0.18% in maternal meiosis and 0.24% in paternal meiosis. The same study has also shown a null allele in 0.49% of the cases. These are very high frequencies, compared to 12 other loci (http://www.cstl.nist.gov/biotech/strbase/mutation.htm). In the present case, we were successful in tracing the origin of the mutated allele to the biological father. Identification of the mutated allele was possible only because of different repeat structures at this locus. If the locus consisted of an identical repeat structure, such analyses would not have been possible.

Brinkman et al. (13) estimated the mutation rate of autosomal STR loci to be between zero and 7×10^{-3}/locus/gamete/generation. They also observed that the mutation rate in the male germ line was five to six times higher than in the female germ line. The present observation is in agreement with the above study. Xiao et al. (16) identified a deletion of 14 bp in the locus D21S11 giving rise to a new allele. They also found either a deletion of TG or an insertion of TC in the same study. In the present study, we found the addition

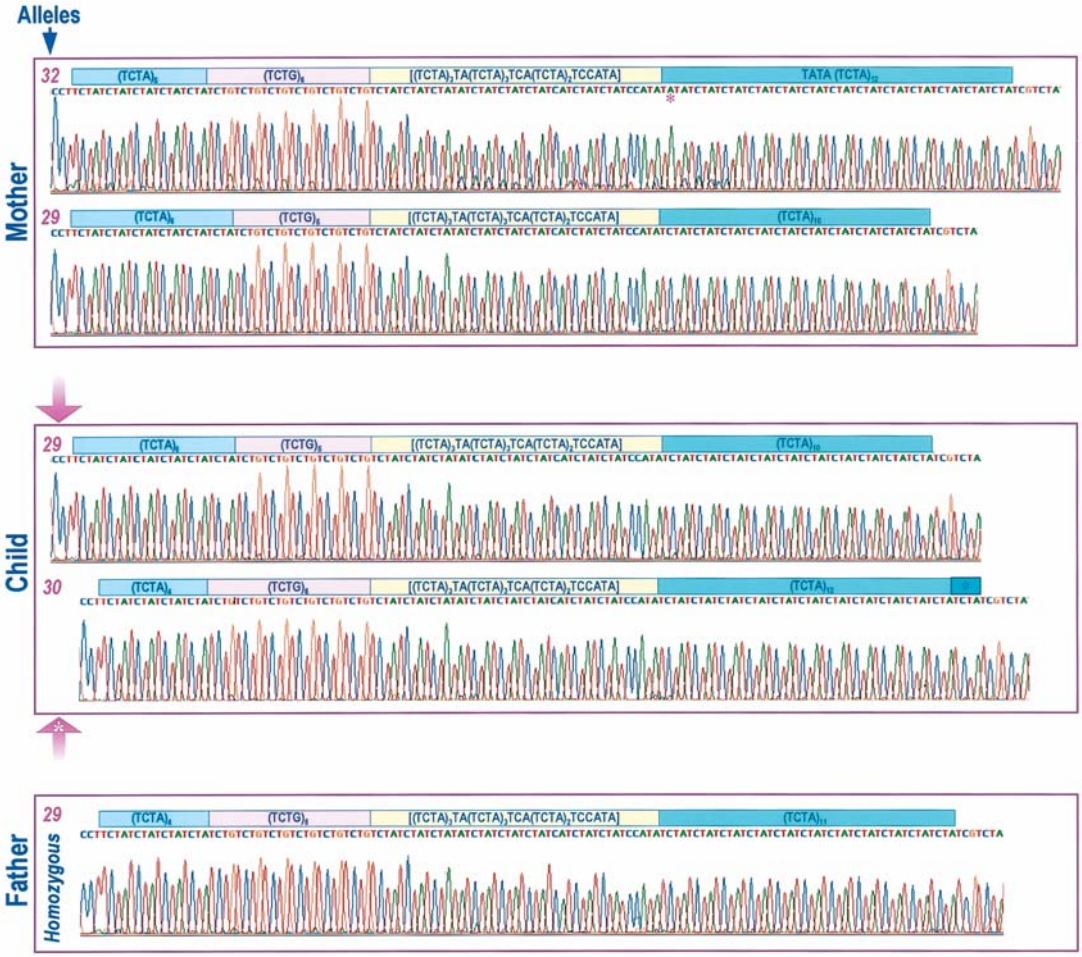

FIG. 3—*Sequence electropherogram of the locus D21S11. Sequences highlighted with yellow bars are nonpolymorphic regions, while the flanking repeat sequences (TCTA/TCTG/TCTA) are polymorphic. The upper panel shows sequences of the mother's alleles (32/29). C > A mutation in the mother's allele 32 is shown by a star. The middle panel shows sequences of the child's alleles (29/30). The lower panel shows a sequence of the father's homozygous allele (29). Although both mother and father possess allele 29, the polymorphic repeat structures are different. Allele 29 of the child perfectly matched allele 29 of the mother. The polymorphic repeat structure of allele 30 of the child did not match in one of the three polymorphic repeat motifs, which was due to single-step mutation (indicated by star).*

of one tetranucleotide repeat unit (TCTG) in the child and one transversion (C → A) in the mother.

This study clearly shows that there was single step mutation in the locus D21S11 in the germ cell of the father. Although no more testing was really needed in this case since paternity index was so high (2.4×10^{10}), we recommend that it is always better to use additional markers to have a high probability of paternity.

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References

1. Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, et al. Identification of the remains of the Romanov family by DNA analysis. Nat Genet 1994;6:130–5. [PubMed]
2. Urquhart A, Kimpton CP, Downes TJ, Gill P. Variation in short tandem repeat sequences—a survey of twelve microsatellite loci for use as forensic identification markers. Int J Leg Med 1994;107:13–20.
3. Patrizio P, Leonard DGB, Chen K-L, Ayup SH, Trounson AO. Larger trinucleotides repeat size in the androgen receptor gene of infertile men with extremely severe oligozoospermia. J Androl 2001;22:444–8. [PubMed]
4. Sinden RR. Biological implications of the DNA structures associated with disease causing triplet repeats. Am J Hum Genet 1999;31:346–53.
5. McMurray CT. Mechanisms of DNA expansion. Chromosoma 1995; 104:2–13. [PubMed]
6. Wells RD. Molecular basis of genetic instability of triplet repeats. J Biol Chem 1996;271:2875–8. [PubMed]
7. Thangaraj K, Joshi M, Reddy AG, Gupta NJ, Chakravarty B, Singh L. CAG repeat expansion in the androgen receptor gene is not associated with male infertility in Indian populations. J Androl 2002;23:815–8. [PubMed]
8. Thangaraj K, Ramana GV, Singh L. Y-chromosome and mitochondrial DNA polymorphisms in Indian populations. Electrophoresis 1999;20: 1743–7. [PubMed]
9. Thangaraj K, Joshi MB, Reddy AG, Rasalkar AA, Singh L. Sperm mitochondrial mutation as a cause of low sperm motility. J Androl 2003; 24:388–92. [PubMed]
10. Bridge PJ. The calculation of genetic risk. Worked examples in DNA diagnostics. 2nd ed. Maryland: The John Hopkins University Press, 1997;175–84.
11. Ashma R, Kashyap VK. Genetic polymorphism at 15 STR loci among three important subpopulations of Bihar, India. Forensic Sci Intl 2002; 13058–62.
12. Levedakou EN, Freeman DA, Budzynski MJ, Early BE, McElfresh KC, Schumm JW, et al. Allele frequencies for fourteen STR loci of the PowerPlex™ 1.1 and 2.1 Multiplex systems and Penta D locus in Caucasians, African-Americans, Hispanics, and other populations of

the United States of America and Brazil. J Forensic Sci 2001;64: 89–95.

13. Weber JL, Wong C. Mutation of human short tandem repeats. Hum Mol [PubMed] Genet 1993;2:1123–8.
14. Brinkmann B, Klintschar M, Neuhuber F, Huhne J, Rolf B. Mutation rate in human microsatellites: influence of the structure and length of the [PubMed] tandem repeat. Am J Hum Genet 1998;62:1408–15.
15. Brinkmann B, Meyer E, Junge A. Complex mutational events at the [PubMed] HumD21S11 locus. Hum Genet 1996;98:60–4.
16. Xiao F-X, Gilissen A, Cassiman J-J, Decorty R. Quadruplex fluorescent STR typing system (HUMVWA, HUMTHO1, D21S11 and HPRT) with sequence-defined allelic ladders identification of a new allele at D21S11. Forensic Sci Intl 1998; 94:39–46.

Additional information and reprint requests:
Dr. L. Singh
Centre for Cellular and Molecular Biology
Uppal Road, Hyderabad 500 007
India
Tel: +91-40-27160789
Fax: +91-40-27160591
E-mail: lalji@ccmb.res.in