Usefulness of SNPs as Supplementary Markers in a Paternity Case with 3 Genetic Incompatibilities at Autosomal and Y Chromosomal Loci

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Summary

Background: In kinship testing, investigation of 15 short tandem repeats (STRs) usually provides decisive genetic information for resolving relationship cases. However, in complex deficiency cases, in cases with more than 2 mutations at different STR loci or when close (untested) relatives of the alleged father are suggested to be the biological father of the child, STR typing alone may not be sufficient. In these cases, the application of supplementary markers such as single nucleotide polymorphisms (SNPs) is recommended. **Methods:** We describe a paternity case with 3 genetic incompatibilities (Penta D, VWA, and DYS385) between the alleged father and the child after analyzing 23 autosomal and 16 Y chromosomal STR loci. The question arose as to whether the alleged father could be excluded and a related person could be the biological father of the child, or whether the observed genetic incompatibilities were mutations. Interestingly, the 2 excluded full brothers of the alleged father possessed identical genetic incompatibilities at locus VWA and DYS385 as the alleged father. **Results and Conclusions:** Additional performance of a 50-plex SNP assay demonstrated that the observed mismatches were indeed mutations and the alleged father was the biological father of the child. The results show the usefulness of SNPs as supplementary markers in relationship testing when STR analyses show ambiguous results.

Introduction

Short tandem repeat (STR) typing is widely used for the genetic identification of individuals, in paternity testing and in forensic trace analysis [1, 2]. Investigation of a minimal set of 12 STR loci located at 10 different chromosomes is usually performed in paternity testing [3]. Recently, the German commission for paternity testing (GEKO) increased the number of STR markers to at least 15 autosomal loci [4]. Additionally, an alleged father can now only be excluded when 4 or more exclusions on different chromosomes are found [4]. When investigating deficiency cases or when 1 or 2 genetic incompatibilities are found between child and putative father, additional autosomal STR loci are usually investigated to obtain a clear result for the assumed fatherhood. In the case of 1 or 2 mismatches, the existence of mutational events and the possibility that a close relative of the alleged father is the biological father should be considered. In paternity cases with male children, Y-STR typing can be very valuable especially when the alleged father is already deceased or simply not available for investigation and male relatives of this man are available [5–8]. The use of additional autosomal or Y chromosomal loci can lead to further problems since such mutations in primer binding sites or additional mutations may again lead to ambiguous results. Therefore, the usefulness of single nucleotide polymorphisms (SNPs) as supplementary markers has been widely discussed and reported [9–11]. In a number of studies, the advantage of supplement SNP analysis in paternity cases with genetic incompatibilities at different STR loci has been convincingly demonstrated [11–14]. In the study of Schwark et al. [11] in all trio cases (mother, child and putative father) with 1 or 2 mutations, SNP typing gave higher paternity indices (PIs) than analysis of 17 STRs. A major advan-

Table 1. Genetic incompatibilities after analysis of 23 autosomal STR loci and 16 Y chromosomal STR loci

Locus	Child	Mother	Alleged father	Brother 1 of the alleged father	Brother 2 of the alleged father
Penta D	11*–12	12	10*	10–11	10–11
VWA	15–17*	14–15	16–18*	15–18*	18*
DYS385	16–19*	–	16–18*	16–18*	16–18*
Three mutations at loci Penta D, VWA and DYS385 (*) between the alleged father and the child were observed. Additionally, 2 of these loci were also inconsistent between the child and the 2 brothers of the alleged father (*).					

tage of SNP analysis in kinship investigation is the low mutation rate of approximately 10^{-8} compared to 10^{-3} to 10^{-4} for STRs [10–13]. In addition, the short amplicon sizes (< 100 bp) of SNPs offer greater success when typing highly degraded DNA, e.g. in forensic identification cases [12]. However, the usually biallelic nature of SNPs results in a poor biostatistical power per locus, which has to be compensated by using a large number of these markers. Therefore, more than 50 SNPs are required to gain the discriminatory power of 15 STRs [10, 11, 14].

Here, we report a paternity case with 3 genetic inconsistencies between child and putative father after investigating 23 autosomal and 16 Y-specific STRs. The supplementary investigation of 50 SNPs supported the assumption that these mismatches were indeed mutations and made it possible to exclude the possibility that a close relative of the alleged father was the biological father.

Material and Methods

DNA Extraction and STR Amplification

DNA was extracted from buccal swabs from the child using the DNA IQ system (Promega, Madison, WI, USA) following the manufacturers' instructions. DNA from EDTA blood samples of the mother, the putative father and his 2 brothers was extracted using the NucleoMag 96 Blood Kit (Macherey-Nagel, Düren, Germany). DNA quantification was performed using the Quantifiler Human DNA quantification kit according to the manual (Applied Biosystems, Foster City, CA, USA). STR typing was done using the following multiplex kits: PowerPlex®16 HS system, PowerPlex®ESI 17 system (Promega), AmpFISTR® Identifier® & AmpFISTR® NGM Select™ (Applied Biosystems), according to the manufacturers' recommendations. Thus, altogether 23 STR loci could be investigated and 21 loci were analyzed independently in a minimum of 2 different assays. Amplification of Y-specific STRs was carried out using the AmpFISTR® Yfiler™ PCR amplification kit (Applied Biosystems) in an Eppendorf thermal cycler using the conditions recommended by the manufacturer. Capillary electrophoresis of PCR amplicons were conducted in an ABI Prism 3100-Avant genetic analyzer (Applied Biosystems) according to the manufacturer's instructions, and data were analyzed using the GeneMapper ID software v.3.2.1 (Applied Biosystems).

SNP Typing

The amplification of 50 SNPs (selected by the SNPforID consortium [11]) was done in 2 multiplex PCRs containing 21 or 29 primer pairs, respectively. The Qiagen® multiplex PCR kit (Qiagen, Hilden, Germany) was used in a total volume of 25 µl for each PCR containing 1 µl DNA extract (approximately 500 pg total DNA). The SNPs were amplified in the following PCR program: denaturation at 95 °C for 15 min followed by 35 cycles of (1) 94 °C for 30 s, (2) 60 °C for 90 s and (3) 72 °C for 60 s

and concluding with 60 °C for 30 min in a GeneAmp® PCR system 2700 (Applied Biosystems). PCR-products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and SBE products were cleaned using FastApp thermosensitive alkaline phosphatase (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocols. The detection of the SNPs was done using the ABI PRISM® SNaP-shot™ kit (Life technologies) with 1 µl of the PCR product in 2 separate reactions analogous to the mentioned PCRs in a total volume of 8 µl. The fragments were amplified in the following SBE program: a total of 30 cycles of (1) 96 °C for 10 s, (2) 50 °C for 5 s and (3) 60 °C for 30 s on the same PCR machine. Fragment analysis was performed on the ABI PRISM™ 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) using 2 µl of the SNaPshot product. The data were processed using the GeneMapper™ ID Software version 3.2.

Statistics

The probabilities for paternity were calculated according to Essen-Möller using the commercially available computer program VAT (M. B. Baur, R. Fimmers, W. Spitz, Version 2.0.2.4, Bonn, Germany). Calculation of probability that the grandfather could be the alleged father was performed under consideration of linkage between the loci VWA/D12S391 ($\theta = 0.1172$), Penta D/ D21S11 ($\theta = 0.3568$) and D5S818/CSF1PO ($\theta = 0.2522$, [15]) using LINKAGE 5.1 plus manual adaptation to include the mutational events.

Results and Discussion

In the first approach, 17 autosomal STR loci were examined using the PowerPlex®16 HS System and AmpFISTR® Identifier®, and resulted in 2 mismatches at loci Penta D and VWA between the child and the alleged father (table 1). Paternity probability including these 2 mutations led to a value of 98.53% (table 2), which is not sufficient for the verbal predicate 'paternity practically proven' [4]. To obtain the required probability of at least 99.9%, 6 additional STR loci (D10S1248, D22S1045, D2S441, D1S1656, D12S391, and SE33) were amplified using AmpFISTR® NGM Select™ and PowerPlex®ESI 17 System, revealing no additional mismatches. This resulted in 21 matching STRs plus the 2 mismatches and led to the sufficient paternity probability of 99.99983% (table 2). Since the child investigated was male, we included 16 Y-STRs in our analysis and compared the child's Y chromosomal-specific pattern with that from the alleged father. As a result, 1 more genetic incompatibility between the alleged father and the child was noticed at locus DYS385 (table 1) adding up to 3 mismatches. Calculation of the probability of paternity regarding these 3 mismatches as mutations in a regular trio case resulted in 99.999999%, combining pa-

Table 2. Paternity probability (W) / paternity index (PI) values after analysis of STRs, Y-STRs and SNPs for different postulated hypotheses

	Exclusions STRs Y-STRs SNPs combined	W / combined PI 17 STRs	W / combined PI 23 STRs	W / combined PI 23 STRs + 16 Y-STRs	W / combined PI 50 SNP
Putative father*	STRs: 2 mismatches Y-STRs: 1 mismatch SNPs: 0 mismatch	W1: 98.526494% PI: 66.865	W1: 99.999833% PI: 5.98×10^5	W1: 99.999999% PI: 7.15×10^6	W1: 99.9999998% PI: 6.32×10^8
Unknown brother**	–	–	W1: 11.018791% W2: 88.981190% W3: 0.000018%	W1: 3.964131% W2: 96.035869% W3: 0.000001%	–
Brother 1	STRs: 8 mismatches Y-STRs: 1 mismatch SNPs: 3 mismatches	–	–	–	–
Brother 2	STRs: 9 mismatches Y-STRs: 1 mismatch SNPs: 4 mismatches	–	–	–	–
Grandfather *** linkage ignored	–	–	W1: 91.0441% W2: 8.9559% W3: 0%	W1: 91.0441% W2: 8.9559% W3: 0%	–
Grandfather *** linkage considered	–	–	W1: 88.4930% W2: 11.5069% W3: 0.0001%	W1: 88.4931% W2: 11.5069% W3: 0%	–

STR = short tandem repeat, Y-STR = Y chromosome-specific STR, SNP = single nucleotide polymorphism.
 * Calculation of W was performed under the hypotheses H1: 'The alleged father is the true father'; H2: 'A man unrelated to the alleged father is the true father'.
 ** Calculation of W was performed under the hypotheses H1: 'The alleged father is the true father'; H2: 'A brother of the alleged father is the true father'; H3: 'A man unrelated to the alleged father is the true father'.
 *** Calculation of W was performed under the hypotheses H1: 'The alleged father is the true father'; H2: 'The grandfather is the true father'; H3: 'A man unrelated to the alleged father is the true father'. Biostatistical analysis was performed under consideration or ignoring linkage between VWA/ D12S391, Penta D/D21S11 and D5S818/CSF1PO.

ternity indices from autosomal STRs and Y-STRs (table 2). In contrast, the calculation of the probability of paternity under the hypothesis that a brother of the alleged father could also be the true father was 96.03%, when combining the PI values from autosomal and Y chromosomal STR analysis. After contacting the judge, the existence of 2 full brothers of the alleged father was confirmed allowing the possibility that at least theoretically a brother of the alleged father could also be the true father. Therefore, blood samples from the 2 brothers were taken and STR analysis of the DNA samples was performed. Amplification of 23 autosomal STR loci resulted in 8 exclusions for the first brother and 9 exclusions for the second. Similar to the alleged father, 1 mismatch at locus VWA was noticed for both brothers (table 1). Typing of the 16 Y chromosomal STR loci of the 2 brothers resulted in the same mismatch at locus DYS385 that was observed in the alleged father. Single mutations of STRs have been observed and reported in paternity testing [16, 17]. However, non-exclusion trio paternity cases with 3 genetic incompatibilities are relatively rare. Recently, 1 case of a triple mutation in a paternity

case including the STR loci D8S1179, D18S51 and 1 RFLP marker between child and alleged father was reported [18]. Sun et al. [19] also found 3 1-step mutations at the autosomal loci D21S11, VWA, and D8S1179. In another case, inconsistencies at 2 autosomal loci (D21S11 and Penta D), due to maternal uniparental disomy 21, and 1 mismatch at Y chromosomal locus DYS389II were observed [20], showing the importance for the application of additional methods or markers. In our described paternity investigation, we observed 3 genetic incompatibilities at the loci Penta D, VWA and the Y-specific locus DYS385. All observed mutations were assumed to be the most frequent 1-step mutations as a result of the gain or the loss of an STR [21]. Those mutations can be explained by replication slippage, which is generally expected to be the major mechanism causing new STR mutations [22]. Mutations of autosomal STRs are reported to occur in approximately 0.2–0.4% of meiosis [23], and these observations are also similar to those of Y-STRs [24–26]. In contrast to the significant mutation rates of STR markers, a main advantage of SNPs is its negligible mutation rate. To overcome any

Table 3. Results after typing 50 different SNPs of a 50-plex SNP assay

SNP	Child allele	Mother allele	AF allele	B1 of AF allele	B2 of AF allele
1	C/C	C/T	C/T	C/C	C/T
2	C/C	C/T	C/C	C/C	C/C
3	T/T	A/T	T/T	T/T	T/T
4	A/T	A/A	T/T	T/T	T/T
5	G/A	G/A	G/G	G/G	G/G
6	T/T	T/T	T/T	T/T	T/T
8	C/T	C/C	T/T	C/T	C/T
9	C/C	C/C	C/C	C/C	C/C
10	C/T	C/T	C/T	C/T	C/T
11	C/T	C/T	C/T	T/T	T/T
12	A/A	A/A	G/A	G/A	G/A
13	G/G	G/A	G/G	G/G	G/A
14	A/T	T/T	A/A	A/T	A/A
15	C/C	C/T	C/C	C/T	C/C
16	G/T	T/T	G/T	G/G	G/T
17	A/A	A/A	A/A	G/A	G/A
18	G/T	G/T	G/T	T/T	G/T
19	T/T	C/T	C/T	C/T	T/T
20	G/T	G/T	G/G	G/G	G/G
21	G/G	G/A	G/A	G/A	A/A
22	A/A	A/A	G/A	G/A	G/G
23	G/A	G/A	A/A	A/A	A/A
24	C/T	T/T	C/T	T/T	C/C
25	G/A	G/A	A/A	A/A	A/A
26	G/G	G/T	G/T	G/T	C/T
27	G/G	G/A	G/A	G/A	G/G
28	G/G	G/A	G/G	G/A	G/G
29	A/A	G/A	A/A	G/A	G/A
30	A/A	C/A	A/A	A/A	C/A
31	C/T	C/C	C/T	C/C	C/C
32	T/T	T/T	T/T	C/T	C/T
33	G/G	G/C	G/C	G/G	G/G
34	G/A	G/A	G/G	G/A	G/A
35	T/T	T/T	A/T	A/A	A/T
36	C/T	C/C	T/T	T/T	T/T
37	G/C	G/C	G/C	C/C	C/C
38	A/T	A/A	A/T	T/T	A/T
39	A/A	A/A	A/A	G/A	G/A
40	T/T	C/T	T/T	T/T	T/T
41	A/A	A/A	A/A	A/A	A/A
42	A/A	A/A	A/A	A/A	A/A
43	C/C	C/C	C/C	C/C	C/C
44	C/T	C/C	C/T	C/T	C/C
45	G/G	G/G	G/G	G/G	G/G
46	C/T	C/T	C/C	C/C	C/C
47	A/A	G/A	G/A	G/A	G/G
49	A/A	A/A	A/A	G/A	A/A
50	A/A	A/A	A/A	A/A	A/A
51	G/A	G/A	A/A	G/A	G/A
52	A/A	A/A	G/A	A/A	G/A

AF = alleged father, B = brother.

All primer sequences, alleles and frequencies were taken from [11].

In total, 50 different SNPs recommended from the SNPforID consortium were analyzed (SNP 7 (rs917118) and SNP 48 (rs8037429) were removed from the assay).

Exclusions are given in bold type.

doubt about the paternity of the alleged father, 50 autosomal SNPs were analyzed as supplementary markers. After typing of all SNP loci from the child, the mother and the alleged father, no mismatch was observed in any of the 50 SNPs (table 3). Both brothers were excluded by means of SNP analysis, while the putative father could be included in all 50 markers (table 3). SNP typing gave a higher combined PI than investigation of 17/23 autosomal and 16 Y chromosomal markers, resulting in a paternity probability of 99.999998% (table 2). Even though PIs with included mutation rates obtained after STR analysis still indicated a strong evidence for the fatherhood of the alleged father, a major effort for inclusion of additional markers was required. To conclude, results of the SNP analysis confirmed our previous STR data and indicated that the 3 genetic incompatibilities found at the autosomal and Y chromosomal STR loci are indeed mutations.

In this special case it should be kept in mind that the untested grandfather of the child can certainly also be considered as putative father. Both observed mutations (VWA and Penta D) are located at STR loci that are considered to be statistically dependent to other loci [27–29]. It has been suggested that a physical linkage between D12S391 and VWA could affect the likelihood ratio values in kinship testing [27, 30]. Additionally, D5S818 and CSF1PO are co-located on the same arm of chromosome 5. To determine if the untested grandfather of the child could be considered as putative father, the probabilities of 3 hypotheses ignoring or including linkage between the loci VWA/ D12S391, Penta D/ D21S11 and D5S818/ CSF1PO were calculated: H1: the alleged father is the true father; H2: the grandfather is the true father; and H3: a man unrelated to the alleged father is the true father. The STR results of 23 autosomal loci of the 3 full brothers, the mother and the child and the results of 16 Y chromosomal STRs of the male persons were always included. The combined probabilities are presented in table 2. Significant differences between the LR values when either ignoring or including linkage between the markers VWA/D12S391, Penta D/D21S11 and D5S818/CSF1PO were not observed. The probability for the hypothesis ‘grandfather’ was 11.5069% considering linkage and 8.96% ignoring linkage, indicating that the paternity of the untested grandfather is unlikely (table 2).

Conclusion

Application of SNP analysis gave at least a clear statement of the paternity of the alleged father and demonstrated the usefulness of SNPs in the reported paternity case. Especially in kinship analysis that shows ambiguous STR results, additional SNP analysis can provide decisive genetic information for elucidating the assumed relationship. To conclude, we recommend that additional supplementary markers should always be analyzed for a reliable investigation of kinship when more than 2 genetic incompatibilities are observed.

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Ethical Standards

Samples were obtained and used after ethical advice of the Medical Ethics Committee of the University of Rostock in accordance with the declaration of Helsinki. The anonymity of the individuals investigated was preserved corresponding to the rules of data protection of the Human Medical Faculty Rostock.

Disclosure Statement

The authors declare that they have no conflict of interest.

References

- 1 Thomson JA, Pilotti V, Stevens P, Ayres KL, Debenham PG: Validation of short tandem repeat analysis for the investigation of cases of disputed paternity. *Forensic Sci Int* 1999;100:1–16.
- 2 Butler JM: Short tandem repeat typing technologies used in human identity testing. *BioTechniques* 2007;43:2–5.
- 3 Morling N, Allen R, Carracedo A, Geadia H, Guidet F, Hallenberg C, Martin W, Mayr WR, Olaisen B, Pascali V, Schneider PM: Paternity Testing Commission of the International Society of Forensic Genetic. Recommendations on genetic investigations in paternity cases. *Forensic Sci Int* 2002;129:148–157.
- 4 Richtlinie der Gendiagnostik-Kommission (GEKO) für die Anforderungen an die Durchführung genetischer Analysen zur Klärung der Abstammung und an die Qualifikation von ärztlichen und nicht-ärztlichen Sachverständigen gem. § 23 Abs. 2 Nr. 4 und Nr. 2b GenDG (in Kraft getreten 26.07.2012), www.rki.de.
- 5 Roewer L, Kayser M, de Knijff P, Anslinger K, Betz A, Caglia A: A new method for the evaluation of matches in non-recombining genomes: Application to Y-chromosomal short tandem repeat (STR) haplotypes in European males. *Forensic Sci Int* 2000;114:31–43.
- 6 Rolf B, Keil W, Brinkmann B, Roewer L, Fimmers R: Paternity testing using Y-STR haplotypes: assigning a probability for paternity in cases of mutations. *Int J Legal Med* 2001;115:12–15.
- 7 Gusmao L, Butler JM, Carracedo A, Gill P, Kayser M, Mayr WR: DNA commission of the International Society of Forensic Genetics (ISFG): An update of the recommendations on the use of Y-STRs in forensic analysis. *Int J Legal Med* 2006;120:191–200.
- 8 Ayadi I, Mahfoudh-Lahiani N, Makni H, Ammar-Keskes L, Rebai A: Combining autosomal and Y-chromosomal short tandem repeat data in paternity testing with male child: Methods and application. *J Forensic Sci* 2007;52:1068–1072.
- 9 Gill P: An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. *Int J Legal Med* 2001;114:204–210.
- 10 Schneider PM: Beyond STRs: The role of diallelic markers in forensic genetics. *Transfus Med Hemother* 2012;39:176–180.
- 11 Schwark T, Meyer P, Modrow JH, Harder M, von Wurmb-Schwark N: The SNPsforID assay as a supplementary method in kinship and trace analysis. *Transfus Med Hemother* 2012;39:187–193.
- 12 Phillips C, Garcia-Magarinos M, Salas A, Carracedo A, Lareu MV: SNPs as supplements in simple kinship analysis or as core markers in distant pairwise relationship tests: When do SNPs add value or replace well-established and powerful STR tests? *Transfus Med Hemother* 2012;39:202–210.
- 13 Borsting C, Morling N: Mutations and/or close relatives? Six case work examples where 49 autosomal SNPs were used as supplementary markers. *Forensic Sci Int* 2011;5:236–241.
- 14 Borsting C, Sanchez JJ, Hansen HE, Hansen AJ, Bruun HQ, Morling N: Performance of the SNPforID 52 SNP-plex assay in paternity testing. *Forensic Sci Int Genet* 2008;2:292–300.
- 15 Phillis C, Ballard D, Gill P, Snydercombe Court D, Carracedo A, Lareu MV: The recombinaton landscape around forensic STRs: Accurate measurement of genetic distances between syntenic STR pairs using HapMap high density SNP data. *Forensic Sci Int Genet* 2012;6:354–265.
- 16 Brinkmann B, Klitsch M, Neuhuber F, Hühne J, Rolf B: Mutation rate in human microsatellites: Influence of the structure and length of the tandem repeat. *Am J Hum Genet* 1998;62:1408–1415.
- 17 Kayser M, Sajantila A: Mutations at Y-STR loci: implications for paternity testing and forensic analysis. *Forensic Sci Int* 2001;118:116–121.
- 18 Jacewicz R, Berent R, Prośniak A, Dobosz T, Kowalczyk E, Szram S: Non-exclusion paternity case with three genetic incompatibilities between father and child due to maternal uniparental disomy 21 and a mutation at the Y chromosome. *Forensic Sci Int Genet* 2009;3:141–143.
- 19 Sun H, Li H, Zeng X, Ren Z, Chen W: A paternity case with mutations at three CODIS core STR loci. *Forensic Sci Int Genetic* 2012;6:e61–e62.
- 20 Mansuet-Lupo A, Henke J, Henke L, Blank C, Ernsting A, Kozłowski P, Rouger P, van Huffel V: A paternity case with three genetic incompatibilities between father and child due to maternal uniparental disomy 21 and a mutation at the Y chromosome. *Forensic Sci Int Genet* 2009;3:141–143.
- 21 Becker D, Bender K, Edelmann J, Götz F, Henke L, Hering S, Hohoff C, Hoppe K, Klitsch M, Muche M, Rolf B, Szibor R, Weirich V, Jung M, Brabetz W: New alleles and mutational events at 14 STR loci from different german populations. *Forensic Sci Int Genet* 2007;1:232–237.
- 22 Levinson G, Gutman GA: Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Mol Biol* 1987;E4:203–221.
- 23 Henke L, Henke J: Supplemented data on mutation rates in 33 autosomal STR polymorphisms. *J Forensic Sci* 2006;51:446–447.
- 24 Heyer E, Puymirat J, Dieltjes P, Bakker E, de Knijff P: Estimating Y chromosome specific microsatellite mutation frequencies using deep rooting pedigrees. *Hum Mol Genet* 1997;6:799–803.
- 25 Kayser M, Roewer L, Hedman M, Henke L, Henke J, Brauer S, Kruger C, Krawczak M, Nagy M, Dobosz T, Szibor R, de Knijff P, Stoneking M, Sajantila A: Characteristics and frequency of germline mutations at microsatellite loci from the human Y chromosome, as revealed by direct observation in father/son pairs. *Am J Hum Genet* 2000;66:1580–1588.
- 26 Hohoff C, Dewa K, Sibbing U, Hoppe K, Forster P, Brinkmann B: Y-chromosomal microsatellite mutation rates in a population sample from Northwestern Germany. *Int J Legal Med* 2006;121:359–363.
- 27 O'Connor KL, Hill, CR, Vallone PM, Butler JM: Linkage disequilibrium analysis of D12S391 and VWA in U.S. population and paternity samples. *Forensic Sci Int Genet* 2011;5:538–540.
- 28 Budowle B, Ge J, Chakraborty R, Eisenberg AJ, Green R, Mulero J, Lagace R, Hennessy, L: Population genetic analyses of the NGM loci. *Int J Legal Med* 2010;125:101–109.
- 29 Gill P, Phillips C, McGovern C, Bright J-A, Buckleton J: An evaluation of potential allelic association between the STRs vWA and D12S391: Implications in criminal casework and applications to short pedigrees. *Forensic Sci Int Genet* 2012;6:477–486.
- 30 O'Connor KL, Hill, CR, Vallone PM, Butler JM: Corrigendum to 'Linkage disequilibrium analysis of D12S391 and VWA in U.S. population and paternity samples'. *Forensic Sci Int Genet* 2011;5:541–542.