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Evaluation of gastrointestinal cancer tissues as a source of genetic information for forensic investigations by using STRs

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Abstract

Malignant tissue samples may sometimes be the only source of biological material for forensic investigations, including identification of individuals or paternity testing. However, in use of such samples, uncertainties due to microsatellite instability (MSI) and loss of heterozygosity (LOH) often associated with neoplasias may be encountered. In this study, we have analysed the applicability of autosomal tetranucleotide short tandem repeat (STR) markers, which are routinely used in forensic analysis, to gain genetic information. MSI and LOH were analysed in 41 surgically removed gastrointestinal cancer specimens and the adjascent non-cancerous tissue marginals. The cancer specimens showed great variability in their genetic phenotypes due to MSI or LOH, with only 32% being microsatellite-stable. Of the 15 autosomal STR loci analysed, only TH01 had no MSI-type alteration in these samples. The loci most frequently affected by MSI were D8S1179, D21S11, D18S51 and D19S433 (MSI in 15–17% of cases). LOH-type alterations were observed at all of the loci, including the amelogenin locus used for sex determination. The highest LOH frequency was found at locus D18S51 (27%). The genetic alterations at the marker loci may indicate false homozygosity or heterozygosity, and false gender may result from erroneous deduction of DNA profiles. Therefore, typing of autosomal STRs from malignant tissues in forensic settings warrants careful interpretation of MSI and LOH results together with microscopic analysis of a tissue specimen. Results by two commercially available and widely used forensic DNA profiling kits used here were comparable.

Keywords: Neoplastic tissues; Forensic DNA; Forensic pathology; Short tandem repeat; PCR

1. Introduction

Short tandem repeats (STRs), or microsatellites, show high levels of polymorphism at a population level. This is due to a large number of repeat-structured alleles per locus, which are inherited in a Mendelian fashion. Modern gene technology based on DNA amplification using PCR enables simultaneous (multiplex) analysis of many STR loci in the genome. Analysis of STRs offers an efficient and reliable way of discriminating between sample source and individuals, e.g. a mislabelled histological tissue block could be matched to its source.

STR analysis is also widely used in mapping genetic diseases, in population genetic studies and in studies on the genetic instability of tumours [1]. Neoplastic tissues manifest a great variety of genetic alterations, such as chromosomal instability, allelic deletions (loss of heterozygosity, LOH) and allelic insertions (microsatellite instability, MSI) [2]. In clinical settings, MSI has been divided into two groups, MSI-low (MSI-L) and MSI-high (MSI-H), according to the incidence of instable loci. The latter has been best characterized in colon carcinoma in which 12–16% of

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sporadic neoplasias show high MSI frequency resulting from deficient mismatch repair of errors upon DNA replication [3]. In hereditary non-polyposis colon cancer (HNPCC), almost all of the tumours possess MSI-H, hence, detection of instable loci is one of the diagnostic methods used. In MSI-L, where less than one-third of the loci analysed are affected, the molecular basis is still unclear [4]. The National Cancer Institute of USA (NCI) has suggested the use of a reference set of STR markers for discrimination between MSI-H and MSI-L [5]. Alternatively, the MSI-H status can be detected with a single mononucleotide marker, BAT-26, which reveals the MSI-H phenotype with a 99.4–100% efficiency in colorectal and gastric cancers [6,7].

Loss of heterozygosity, representing the other type of genetic instability affecting STR loci, is also a frequent finding in cancerous tissues [1,8]. Chromosomal instability, aneuploidy and chromosomal rearrangements, are usually expressed in the DNA profile as LOH [2]. LOH has been used in studies of cancer pathways and tumour progression and in the search for tumour suppressor genes near the deleted STR loci [2,8]. However, the chromosomal areas affected by LOH differ according to type of tumour [1]. For instance, in colon carcinomas the tumour suppressor genes APC, p53 and DCC (located in chromosomes 5q, 17p and 18q) are known to be deleted by LOH [14,17]. A decision on whether a locus represents LOH is based on allelic ratios between the tumour and the corresponding control sample, the cut-off value usually being <0.5 [8]. The condition of allelic imbalance, i.e. when one allele has a significantly lower but still detectable intensity, may be due to the presence of a benign tissue in the tumour sample, to heterogeneity of the tumour or to technical problems [8].

In this study, we analysed 41 gastrointestinal carcinomas in parallel with control samples from adjacent non-cancerous tissues. The DNA profiles were achieved by established markers and genotyping protocols used in forensic studies. The aim was to detect possible STR alterations and thus false genotypes in the tumour samples to estimate the reliability of the results when neoplastic tissues are used as a source of genetic information. The accuracy of the commercial STR-kits to detect a MSI genotype in a tumour was compared with that of the mononucleotide marker BAT-26.

2. Materials and methods

2.1. Samples

Surgically resected tissue specimens from sporadic primary gastrointestinal cancers and adjacent cancer-free areas were collected from 41 individuals during 1995–2002 at the Department of Pathology, Jorvi Hospital, Espoo, Finland. After excision, a portion of the tumours and the non-cancerous margins were separated and snap-frozen in liquid nitrogen, overlayed with Tissue-Tek® O.C.T. Compound (Sakura Finetek, Zoetervoude, the Netherlands) and stored at −70 °C until further studies. Eighteen of the neoplasias were gastric and 23 colorectal carcinomas. Twenty-four (58.5%) of the tissue specimens were from males and 17 (41.5%) from females, with a mean age of 69.6 years and 62.9 years (range 55–85 and 49–83), respectively.

2.2. DNA extraction

DNA extraction from tissue samples (200–500 mg) was carried out by washing with lysis buffer (0.32 M sucrose, 10 mM Tris–HCl pH 7.5, 5 mM $MgCl_2$, 1% Triton X-100) and digestion with 2 mg/ml proteinase K in 10 mM Tris–HCl pH 8.0, 10 mM EDTA, 100 mM NaCl, 2% SDS and 100 mM DTT at 55 °C overnight, followed by purification on Qiaquick DNA columns (Qiagen, Hilden, Germany). The DNA fraction was recovered in 50 μl of water and quantitated by spotting on ethidium bromide agarose gels. The DNA was used in 1–3 ng aliquots in subsequent PCR amplifications.

2.3. DNA amplification

DNA amplification was performed using either the AmpF*l*STR® SGM Plus™ or the AmpF*l*STR® Profiler™ PCR amplification kits (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations in conjuction with the PTC-225 DNA Engine Tetrad (MJ Research, Boston, MA, USA). AmpF*l*STR® Profiler™ contains primers for tetranucleotide STR loci D3S1358, vWA, FGA, TH01, D13S357, TPOX, CSF1PO, D5S818, D7S820 and amelogenin. AmpF*l*STR® SGM Plus™ contains primers for loci D3S1358, vWA, FGA, TH01, D16S539, D2S1338, D8S1179, D18S51, D21S11, D19S433 and amelogenin. Both kits were used in parallel for the STR analysis, and the first four markers shared by both kits also served as a control. The amplified alleles were separated by ABI PRISM CE 310 capillary electrophoresis (Applied Biosystems) using ROX 500 as an internal standard and allelic ladders to evaluate the sizes of the PCR products. The electrophoresis results were analysed using Genotyper version 2.0 software (Applied Biosystems) with a cut-off filter Kazam 20, which is generally used to exclude background noise when working with forensic samples of single origin.

Amplification of the mononucleotide tract BAT-26 was performed with previously reported primers [6], with the forward primer FAM-labelled. A 20-μl reaction volume was used containing 1 U AmpliTaqGold (Applied Biosystems), 1× AmpliTaqGold PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTP and 0.5 pmol of each of the primers. The samples were amplified for 30 cycles comprising 1 min at 94 °C, 2 min at 58 °C and 2 min at 72 °C, which was preceded by initial denaturation at 95 °C for 4 min and followed by final incubation at 72 °C for 60 min. The replication error phenotype detected as shortened alleles compared with the control sample was evaluated with ABI PRISM instrumentation as described above using GeneScan version 3.1 for analysis.

2.4. Evaluation of MSI

Microsatellite instability in a carcinoma sample was identified by detection of abnormal alleles (allelic insertion) at the STR loci compared with the control tissue. Carcinomas showing extra alleles at $\geq$5 of the 15 STR loci analysed were designated as high frequency MSI (MSI-H). Cancer tissues with 1–4 loci showing extra alleles were designated as low frequency MSI (MSI-L), as suggested by the NCI panel for colorectal cancer [4].

2.5. Evaluation of LOH

Loss of heterozygosity was identified by allelic loss (allelic deletion) in the tumour tissue as compared with the heterozygotic control sample. A sample was also considered to be LOH when a decreased fluorescence signal was observed in relation to the other allele. As commonly used in clinical settings, a peak intensity ratio (At/Bt: An/Bn, where At/Bt is the peak ratio in the tumour tissue, and An/Bn the corresponding peak ratio in the normal tissue), of <0.5 was regarded as LOH [8]. Similarly to the definition of MSI-H and MSI-L, tumour samples showing LOH at $\geq$33% of the heterozygote STR loci were designated as LOH-high (LOH-H) [9]. Tumour samples with <33% of the informative loci missing heterozygosity or having allelic imbalance were designated as LOH-low (LOH-L). The tumours showing both MSI and LOH phenotype were categorized separately.

2.6. Evaluation of microsatellite stability

We determined the microsatellite-stable (MSS) type to represent both MSI-negative and LOH-negative DNA profiles. However, the NCI panel suggests that all non-MSI tumours, including LOH, should be designated as MSS [4].

2.7. Evaluation of gender

Gender determination was performed in the multiplex procedure by amplification of the amelogenin locus at the X and Y chromosomes. The amplification product of the Y-chromosomal amelogenin gene has an extra 6-bp insertion compared with that of the X chromosome, thus revealing gender in the genotyping assay [10].

2.8. Ethics

The study protocol was evaluated to meet the principles of the Helsinki Declaration and was approved by the local ethics committee.

2.9. Statistics

Pearson's correlation test was used to compare mutation rates at STR loci between cancers and paternal meiosis and to determine differences between the two commercial test kits. A P-value of <0.05 was considered to be statistically significant.

3. Results and discussion

In forensic medicine and pathology, sometimes the only source of DNA from a deceased person is a paraffin block of tumour tissue from a pathology laboratory. This DNA sample then represents the individual in paternity testing or identification assays. However, paraffin blocks may lack labels or be mislabelled in the laboratory. In these cases, DNA typing between the tissue block and the patient is the only reliable way to find the source of the archival tissue sample [11]. The unstable nature of a tumour genome can be problematic when using neoplastic tissue samples in medico-legal analyses, as reported by Rubocki et al. [12], who described an LOH case in bladder cancer. The problem could be solved with histopathological analysis and by resecting only the non-affected tissue for analysis, but as shown before, histologically normal tissues may also possess STR alterations [13]. We chose gastrointestinal tumours for the present work because their genetic aberrations for both MSI and LOH have been well characterized. However, cancers other than gastrointestinal cancers may also harbour similar STR alterations. Moreover, the occurrence of the MSI phenotype in sporadic cancers varies widely, as indicated in a recent thorough review by Lawes et al. [14], whose data are shown in Table 1. Because the frequency for observation of LOH depends on the STRs used, consistent data on the incidence of LOH in various cancers are lacking.

In our set of 41 gastric and colorectal tumours, the non-cancerous control tissues showed normal genotypes in 98% of cases. One control tissue had a locus with three alleles (as did the corresponding cancerous tissue) that was concluded to result from a sporadic mutation in the epithelium. While contamination or invasion of tumour tissue cannot be excluded, in histopathologically normal-appearing tissue genetic alterations can also occur [13].

A previous investigation of the applicability of oral tumour tissues for forensic analysis using the AmpFlSTR® Profiler™ kit observed both LOH and MSI alterations at the nine marker loci [15]. The DNA profiles from these cancer tissues showed alterations in 33% of cases when compared with the DNA profile obtained from blood cells. In our study on gastrointestinal cancer with the same set of markers, 68% of the DNA profiles in the neoplasias were altered (Table 2). The different origin of the tumour may account for the dissimilar rate of alterations.

3.1. Tumour phenotypes

One-third of tumour and control tissues had identical genotypes of the MSS phenotype (Table 2). The rest of the tumours had at least one altered locus, with either MSI or LOH, as illustrated in Fig. 1. The MSI phenotype is

Table 1
Incidence of MSI in different sporadic cancers types[a]

Malignancy	Incidence of MSI in sporadic cancer (%)[b]
Oesophageal	
Squamous cell	2–50
Adenocarcinoma	22
Stomach	2–15
Small bowel	0
Colorectal	15
Biliary tract	0
Pancreatic/biliary	<4
Brain	0
Head and neck	2–30
Thyroid	8–10
Lung	
Non-small cell	4–68
Small cell	0
Breast	<10
Ovarian	11–17
Endometrial	9–45
Prostatic	2–65
Urinary tract	0–8
Sarcoma	13–19
Melanoma	5

[a] Data adapted from Lawes et al. [8].
[b] Figures quoted are from MSI-H tumours when possible.

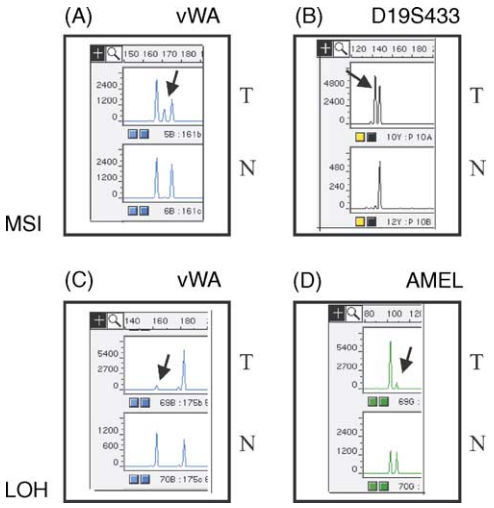

Fig. 1. Examples of MSI (A and B) and LOH (C and D) at STR loci vWA, D19S433 and AMEL obtained by the AmpFlSTR® SGM Plus™ procedure. The markers (vWA and AMEL) shared by AmpFlSTR® Profiler™ yielded similar results (data not shown). The MSI data (A and B) indicate emergence of extra alleles (arrow). In panel B, an extra allele on a homozygote indicates an apparent heterozygote genotype. The LOH data (C and D) indicate deletion (arrow) of the other allele on a heterozygote, resulting in apparent homozygosity (C). In panel D, LOH at the Y-chromosomal amelogenin locus gives female gender for a male. T denotes tumour and N control tissue.

characterized by extra alleles at a locus. The locus may show multiple alleles with unequal or almost equal intensities (Fig. 1A), which can result in a homozygote being falsely classified as a heterozygote (Fig. 1B). The LOH phenotype is characterized by loss of an allele. A tumour can falsely show homozygosity if one of the alleles of a heterozygote individual is lost by LOH (Fig. 1C). LOH classification cannot be made with loci possessing homozygosity. No samples showed simultaneous loss of both loci.

The tumour samples with STR alterations were further subdivided into four categories, with criteria used in clinical studies, depending on the type and number of loci altered (Table 2). The observations are in agreement with previous data on, for example, colorectal carcinoma, in which MSI and LOH represent about one- and two-thirds of the genetic alterations (reviewed in [16]). Nine of the twelve MSI-L cases, however, also exhibited an LOH phenotype (Table 2). Three of these nine cases represented LOH-H and six cases LOH-L. The finding of an LOH phenotype showing also an MSI-L phenotype is in agreement with the results of Goel et al. on colorectal cancers [17] and Choi et al. on gastric cancers [9].

Table 2
Genetic profiles in the gastrointestinal cancer specimens

Alteration[a]	*n*	Proportion (%)
MSI-H	5	12
MSI-L	3	7
LOH-H	6	15
LOH-H with MSI-L	3	7
LOH-L	5	12
LOH-L with MSI-L	6	15
MSS	13	32
Total	41	100

[a] MSI-H: ≥33% of loci altered, MSI-L: <33% of loci altered, LOH-H: ≥33% of heterozygous loci altered, LOH-L: <33% of heterozygous loci altered, MSS: no locus alterations.

The occurrence of an LOH-H phenotype has been shown to correlate inversely with that of a MSI-H phenotype [2,9,17], a finding we confirm. A particular LOH has also been reported to result from allelic drop-out (due to a point mutation in either of the primer sequences) [18], sample degradation or chemical modification of DNA strands by environmental factors [8]. However, rather than the possibility of many point mutations, the LOH-H phenotype in our study is more likely to reflect the chromosomal instability associated with cancers [9,11].

The two commercial tetranucleotide kits and the BAT-26 marker used detected MSI-H type cancers with a correlation of 100%. For gastric cancer specimens, the incidence of 5% for MSI-H is in reasonable agreement with the results of previous studies (4–15% for MSI-H) using the BAT-26 marker [7]. The ability of different forensic markers to

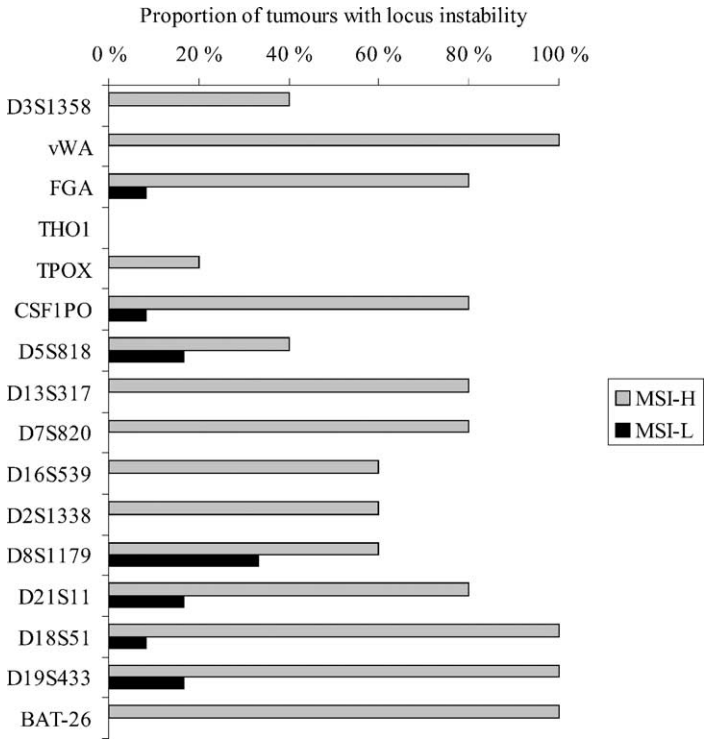

Fig. 2. The sensitivity of different STRs to detect MSI-H and MSI-L phenotypes. The bars indicate the proportion (%) of tumours with a locus alteration. AmpFlSTR® SGM Plus™ and AmpFlSTR® Profiler™ procedures together detected five MSI-H and twelve MSI-L cancers.

detect the MSI-H and MSI-L phenotypes, in comparison with BAT-26, is presented in Fig. 2. The loci vWA, D18S51 and D19S433 detected MSI-H cases with a 100% accuracy, i.e. they showed new alleles in every MSI-H tumour. The markers D18S51 and D19S433 were also altered in some MSI-L tumours, while vWA behaved identically to BAT-26 (Fig. 2).

3.2. Characteristics of different STR loci

The differences in the extent of alterations at the 15 STR loci and the amelogenin gene are shown in Table 3. The number of LOH slightly exceeded the number of MSI. The lowest alteration frequencies were found at TH01 and TPOX. The most frequently altered loci were D18S51 and FGA, with twice as many LOH as MSI alterations (Table 3). The new alleles resulting from MSI were mainly contractions or expansions of one repeat unit, as can be seen in Table 4. MSI-H cases, however, more often showed mutations on both loci and multiple new alleles than MSI-L cases (Table 4). The size of the new alleles ranged from -4 to $+4$ repeat units, and samples with the largest contractions or expansions also showed intermediary alleles, suggestive of single-step mutational events. We also observed four loci with simultaneous MSI and LOH (deletion of one allele and emergence of a new allele), which was concluded to result from a clonal expansion of the neoplastic cells, with the new allele covering the original one. These loci were thus classified as MSI.

Sobrido et al. [20] have reported formalin-fixed brain tumour samples to show STR artifacts, probably due to degradation of samples. We did not notice such signs of degradation or artifacts, most probably because we used fresh snap-frozen samples. In general, STR analysis of a sample was carried out twice with both SGM and Profiler sets, which share four autososomal markers. In cases of MSI the new alleles represented expansions or contractions of one to four repeat units, thus reliably excluding artifactual peaks.

Our set of tumour tissues also revealed aberrations in determination of gender. Tumours with frequent allelic deletions (LOH-H) were also found to possess LOH at the Y-chromosomal amelogenin locus (Fig. 1D) in 20.8% of all males (Table 3). A male individual may be falsely genotyped as XX (female) if the peak height of the Y-chromosomal allele falls below 20% of that of the X-chromosomal allele and no precautions are taken when interpreting the filtered genotyping data. In a normal population, a spontaneous deletion of the amelogenin locus in the Y chromosome has been reported, which might also raise problems in determination of gender [19].

Comparing the two commercial forensic DNA profiling kits in genotyping of neoplastic tissues, the AmpF/STR® Profiler™ kit seemed to reveal less alterations (79/410

Table 3
STR alterations per locus in cancer tissues of 41 patients in relation to normally observed mutation rates

Locus	STR altered[a] (*n*)	MSI	LOH	Het (%)[b]	MSI (%) in tumours	Paternal meiosis mutation (%)[c]
D3S1358	3	2	1	61.0	4.9	0.11
vWA	11	5	6	87.8	12.2	0.34
FGA	14	5	9	92.7	12.2	0.29
AMEL	5	–	5	61.0	0	n.d.[d]
TH01	3	0	3	73.2	0	0.02
TPOX	3	1	2	46.3	2.4	0.02
CSF1PO	13	4	9	70.7	9.7	0.13
D5S818	7	3	4	78.0	7.3	0.15
D13S317	11	5	6	80.5	12.2	0.15
D7S820	9	4	5	78.0	9.7	0.15
D16S539	7	3	4	73.2	7.3	0.08
D2S1338	9	3	4	87.8	7.3	n.d.
D8S1179	9	7	2	82.9	17.0	0.26
D21S11	9	6	3	78.0	14.6	0.24
D18S51	17	6	11	87.8	14.6	0.30
D19S433	8	7	1	82.9	17.0	n.d.
Total	138	61	77			

[a] Total number of alterations observed at each locus (combined from AmpFlSTR® SGM Plus™ and AmpFlSTR® Profiler™ data) is indicated and categorized into LOH and MSI types. The amelogenin gene is not a STR marker, and therefore, no mutations of the MSI type at this allele are detected.

[b] Locus heterozygosity, the percentage of informative loci in the control tissues for the analysis of LOH.

[c] Data for apparent mutation rates observed in paternal meiosis at the 13 CODIS STR loci are from the internet STRbase (http://www.cstl.nist.gov/biotech/strbase) [25].

[d] n.d.: not determined.

loci analysed) than AmpF/RSTR® SGM Plus™ (94/451 loci analysed) (Table 5). However, because the latter kit includes one additional STR locus, the difference is statistically insignificant (Pearson's correlation $r = 0.19$, ns). The five markers shared by AmpF/STR® SGM Plus™ and AmpF/STR® Profiler™ kits gave identical results (Table 5).

Dietmaier et al. [21] compared the alteration frequency of different STRs in MSI-H and MSI-L cancers. They found mononucleotide and dinucleotide markers to be the most instable, of which BAT-26 was the most effective in determing the MSI-H phenotype. Tetranucleotide STRs, by contrast, were relatively stable. Sturzeneker et al. [22] studied the ability of different markers to detect MSI and found a positive correlation between MSI at a certain locus in cancer and its allelic variance in a population. The marker TH01, which showed little variance within a population, showed no MSI in their set of 26 MSI-H tumours. Alves et al. [23], in studying a pentanucleotide repeat at the GSTP1 gene in thyroid and gastric cancers, observed both MSI and LOH in gastric but not in thyroid tumours. In addition, they described the marker as being highly polymorphic at the population level. Hoff-Olsen et al. [24] investigated four STR markers in colorectal cancer. They reported a frequency of 17% for MSI-type alterations at vWA locus and frequencies of 2%, 3 and 7% for loci F13A1, TH01 and FES/FPS, respectively. Consistent with the above reports [22,24], the TH01 locus here had no MSI-type alterations, while vWA showed MSI in 12% of the cancer specimens (Table 3).

The forensic marker D18S51 in our study was located in the 18q chromosome, and consistent with a previous report [17], it showed LOH in 26.8% of cases, representing 13.9% of the total LOH observed (Table 3). The 18q area contains, for example, the gene DCC, which is often deleted in colorectal cancer. In a medico-legal setting, one case of LOH has been reported at D13S317 in bladder cancer [12]. No other data on allelic deletions observed with the markers included in this study are available. The two common forensic STR marker sets used here found LOH-H cases in gastrointestinal tumours equally well.

3.3. MSI and germ-line mutation

The mechanism for the emergence of new alleles at STR loci in MSI and germ-line mutations has been suggested to be similar, i.e. through the slippage of DNA polymerase during replication [22]. The improper function of DNA mismatch repair results in the accumulation of these slippage-associated mutations in the MSI-H phenotype [3,16]. No clear molecular explanation for the MSI-L phenotype has been found. It might originate from baseline mutations, the rate of which may be elevated in cancer tissue [5].

We compared the frequencies of MSI-type alterations at the different loci with previously reported mutational

Table 4
The new alleles found in MSI-H and MSI-L tumours. The mutating allele has been identified by assumption of the nearest possible allele [17]

Case	STR														
	D3S1358	vWA	FGA	THO1	TPOX	CSF1PO	D5S818	D13S317	D7S820	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433
MSI-H#1	–	18*19,20§	23*24	–	–	12*11	–	10*9 10/12*11†	10*11	–	–	14*15 17*18	29*28 30*31	12*10 16*15	16.2*15.2
MSI-H#2	16*15 16/18*17†	14/16*15†	22*19	–	11*10	–	–	–	–	–	20*19	–	29*28	16*15	13*12
MSI-H#3	–	16*17 20*21	–	–	–	11*10	–	10/12*11†	12*13	12*10,13	–	–	–	15*14	16.2*15.2,14.2
MSI-H#4	–	17*19	19*18	–	–	12*13	12*14	11*12	10/12*11†	12*13	17*18 26*25	12*8,10,11 15*16,17,18	30*31	12*13 17*18,19	13*12 14*15
MSI-H#5	16*15	17/19*18†	21*18	–	–	10*11,12,13	11*10	14*15	12*11	11*9	17/19*18†	11*10 12*13	31*32,33,34,35	14*15	13*12,15,16
MSI-L ($n = 12$)	–	–	22*23‡	–	–	–	12*11	12*11 11*12	–	–	–	12/14*13† 11*9 13*12,14 11/13*12†	29*30 31*32‡	17*18	15.2*14.2,11.2 13*14

The MSI-H alterations are indicated by showing all of the five cases separately. If both alleles at a locus are affected, the tumour is scored for two different mutational events. (§) The mutating allele preceeds the new allele which is separated by an asterisk (*). Multiple new alleles are separated by commas. (†) If the mutating allele is not known, both possible alleles (separated by a slash) are shown. Alterations in the MSI-L tumours ($n = 12$) are summarized. Symbol (‡) indicates that two loci have mutated in one MSI-L tumour. In the rest of the MSI-L tumours, alteration at only one locus was found.

Table 5
Comparison of the AmpFlSTR® Profiler™ and AmpFlSTR® SGM Plus™ kits by the number of altered loci detected

PROFILER™				SGM Plus™			
Locus	No. of STR altered	MSI	LOH	Locus	No. of STR altered	MSI	LOH
D3S1358	3	2	1	D3S1358	3	2	1
vWA	11	5	6	vWA	11	5	6
FGA	14	5	9	FGA	14	5	9
AMEL	5	0	5	AMEL	5	0	5
TH01	3	0	3	TH01	3	0	3
TPOX	3	1	2	D16S539	7	3	4
CSF1PO	13	4	9	D2S1338	7	3	4
D5S818	7	3	4	D8S1179	9	7	2
D13S317	11	5	6	D21S11	9	6	3
D7S820	9	4	5	D18S51	17	6	11
				D19S433	8	7	1
Total	79	29	50		94	44	50

Total number of alterations at each locus was further categorized into MSI and LOH groups. The two kits share the first five loci.

frequencies in paternal meioses to estimate their susceptibility to genetic instability. The data indicate a high correlation between the mutations in germ-line and tumour DNA for a certain locus (Table 3). Pearson's correlation between the 13 observations of paternal mutation rate and proportion of MSI in tumours implies a nearly linear relationship ($n = 13$, $r = 0.86$, $P < 0.001$). The different susceptibility of STR loci to mutations may be related to their different structural properties which expose the loci to replication errors of varying degrees [22].

4. Conclusions

Conclusions about STR analysis of tumour tissue samples must be drawn with caution because of the genetic instability of neoplastic tissues and the possibility of genetic alterations also in histologically normal tissue. Tumours may have different alterations in their genotypes, as demonstrated in this study. LOH cases never show both alleles deleted, but in MSI, both alleles may be affected. Depending on the clonal expansion of the tumour, the original alleles may be lost and the tumour may display a new genotype. A male individual with a LOH-H phenotype can falsely be genotyped as XX (female). Using lower filtering may help in detecting alleles with LOH, but in MSI cases, it might reveal more alleles, which can complicate genotyping. The two forensic tetranucleotide STR kits used here detect the MSI-H phenotype with a high accuracy and also reveal other genetic phenotypes of gastrointestinal cancers. In cases of frequent allelic loss (LOH) or allelic insertions (MSI), a wide panel of markers should be used in parallel to obtain reliable results from the STR analysis. Moreover, a microscopically verified histopathological diagnosis should be obtained, and if possible, a marginal cut from non-malignant tissue should be used for forensic or paternity analysis.

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