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Typing of XY (male) Genotype from Malignant Neoplastic Tissue by the Amelogenin-based Sex Test

ABSTRACT: DNA profiling of a cancer tissue can be problematic because of genomic instability. Here we have analyzed gastrointestinal cancer specimens from 46 males, of which seven (15%) showed aberrations in determination of gender by the widely used amelogenin test. The X-type amelogenin allele in all cases remained intact. All male tumor samples showing frequent autosomal loss of heterozygosity had a decreased signal of the Y allele from the amelogenin marker. When tested with an alternate set of primers for the amelogenin locus, the Y-type allele showed loss of heterozygosity in the same seven cases. However, when amplified with 15 Y-specific STR primers, all the cancerous tissue Y chromosomes seemed to be intact. These results indicate when malignant neoplastic tissue specimens are used, that amelogenin-based gender determination should be carefully interpreted.

KEYWORDS: forensic science, forensic pathology, amelogenin, Y-chromosome, gender determination, short tandem repeat

Short tandem repeat (STR) or microsatellite markers are highly polymorphic loci in the human genome, where they show great individual variation. STR genotyping is therefore widely used in forensic identity testing and paternity analysis. When the identity or putative paternity of a deceased person is to be determined, various tissue samples from the archives of a pathology unit are readily available. Often these tissues originally are sampled because they represent abnormal tissues, e.g., malignant neoplasia. However, using neoplastic tissue as one source for genetic investigation can be problematic because neoplasias tend to show a variety of genetic alterations, some of which can affect STR analysis. Two main types of alterations have been described: microsatellite instability (MSI) and loss of heterozygosity (LOH), which usually occur mutually exclusively (1). MSI, in which new alleles emerge because of deficient repair of errors in DNA replication, is well characterized in colon cancer, occurring in 12 to 16% of all cases (2,3). LOH, in which one of the ancestral alleles of a heterozygote is lost, is also a frequently reported alteration in cancerous tissues (4,5). In malignant tumors, total or partial deletion of the Y chromosome has been reported in, for instace, pancreatic (6,7), prostate (8,9), and gastric cancers (10,11).

In our work on neoplastic samples, we have noticed aberrations in gender determination using amelogenin test included in commercially available forensic DNA kits (unpublished data). The focus of the present work was to characterize those Y chromosomes of the DNA samples with a diminished amelogenin signal. A total of 46 tumor cases were screened by commercial forensic protocols for the loss of the Y-allele of the amelogenin gene. For those cases showing LOH, an alternate primer set was used for the amelogenin gene. In addition, a set of 15 Y-STR markers were analyzed. The aim of the work was also to determine whether the diminished amelogenin signal was due to a point mutation in the target sequence(s) for the primer or was due to larger Y-chromosomal deletions affecting amelogenin locus and typing.

Materials and Methods

Tissue Specimens

Surgically resected tissue specimens from primary gastrointestinal cancers and from cancer-free adjacent areas were collected during the period 1995 to 2002 and stored at the Department of Pathology of Jorvi Hospital, Espoo, Finland. After excision, these tissues were snap-frozen in liquid nitrogen, overlaid with Tissue-Tek® O.C.T. Compound (Sakura Finetek, Zoetervoude, NL) and stored at –70°C. A total of 46 sporadic gastrointestinal primary tumors were analyzed, including 22 gastric, 22 colorectal, and 2 gastrointestinal stromal tumors.

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 column (Qiagen, Hilden, Germany). The DNA fraction was recovered in 50 μL of water and quantitated either by spotting on ethidium bromide gels or or by UV-absorbance with the GeneQuant DNA/RNA Calculator

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(Pharmacia Biotech, Uppsala, Sweden). The DNA was in 1-μL aliquots (0.5–5 ng DNA) in the subsequent PCR amplifications.

Tumor Genotyping

The tumors and their adjacent healthy tissues were genotyped with the AmpFℓSTR® SGM Plus™ and AmpFℓSTR® Profiler™ kits (Applied Biosystems, Foster City, CA) and analyzed with ABI Prism CE310 capillary electrophoresis (Applied Biosystems) with ROX500 as the internal standard. The kit AmpFℓSTR® SGM Plus™ contained 10 autosomal STR loci and AMEL (D3S1358, vWA, FGA, TH01, D16S539, D2S1338, D8S1179, D21S11, D18S51 and D19S433) and the AmpFℓSTR® Profiler™ 9 autosomal STR loci and AMEL (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820). Crude data were analyzed with Genescan 3.1, and the genotyping was carried out with Genotyper 2.1 software (Applied Biosystems) at a threshold of 50 rfu (relative fluorescence units) throughout the study. The resulting DNA profile from each tumor and its adjacent normal tissue were compared and the tumor samples categorized based on type of autosomal allelic alteration. The MSI group showed emergence of novel alleles. The samples showing frequent MSI were designated as MSI-high (MSI-H, ≥5 of loci with new alleles). Cases showing alterations at 1 to 5 loci were designated as MSI-low (MSI-L). In microsatellite-stable tumors (MSS), no alterations were visible. As described by D'Adda and colleagues (12), a tumor sample was considered to have LOH at a locus when the LOH ratio between the tumor sample (Yt/Xt) and the respective normal tissue (Yn/Xn) fell below 0.5 ($Yt/Xt : Yn/Xn < 0.5$, t = tumor tissue, n = normal tissue). In this work, neoplasias expressing the LOH ratio <0.5 were also classified as of the LOH phenotype. Samples with frequent LOH were designated as LOH-high (LOH-H, ≥33% of heterozygote alleles showing LOH) and LOH-low (LOH-L) with <33% of altered heterozygote alleles.

Amelogenin Typing

These commercial multiplex sets of STRs include gender determination by amplification of the amelogenin locus in the X and Y chromosomes (13). In the genotyping assay, the amplification products of the Y- and X-alleles of the amelogenin gene are of different sizes, allowing unequivocal typing of the gender. The Genotyper 2.1 software routinely used for the SGM Plus™ and Profiler™ DNA genotyping kits recognizes the XY genotype at a cutoff value of 20% (Kazam 20). Genotyper 2.1 software, however, allows selection of lower cutoff values of 1 to 3% for amelogenin, as for analysis of a mixed sample. The mean amelogenin Y:X ratio of 0.91(range 0.58–1.42) was calculated for the 46 adjacent healthy tissue samples.

Alternate PCR primers for amplification of the amelogenin gene were synthesized as previously reported (14), with the forward primer labelled with FAM. The binding sites for the new primers were in the region flanking the DNA amplified by the AmpFℓSTR® SGM Plus™ and AmpFℓSTR® Profiler™ kits, thus revealing the same 6-base size difference for the X- and Y-chromosomal products. The sizes for the amelogenin PCR products in the commercial procedures are 103 bp and 109 bp for the X and the Y chromosome, while the corresponding sizes achieved with the alternate primers are 218 bp and 224 bp. PCR reactions were performed by use of a 20-μL reaction volume containing 1 U AmpliTaqGold (PE, Applied Biosystems), 1x AmpliTaqGold PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 0.5 pmol each of the primers. The samples were amplified through 30 cycles comprising 1 min at 94°C, 45 s at 60°C, and 1 min at 72°C, preceded by initial denaturation at 95°C for 5 min and followed by final incubation at 72°C for 60 min, and were analyzed by ABI PRISM® 310 capillary electrophoresis using GeneScan 3.1 software and ROX-500 as its internal standard.

Multiplex Y-STR Analysis

Y-STR amplification was perfomed in two multiplexed PCR reactions for 15 different Y-chromosomal STRs. The loci used for the 9-plex reactions were DYS19, DYS389, DYS390, DYS391, DYS392, DYS393 and DYS385 ("minimal haplotype", www.ystr.org), and those for the10-plex reaction DYS19, DYS391, DYS392, DYS435, DYS436, DYS437, DYS438, DYS439, GATA A 7.1 and GATA H4. The primers were synthesized and labelled as previously described (15,16). The Y-STR amplification products were analyzed as described above.

Multiplexed PCR for the nine Y-chromosomal markers (9-plex) was performed with a 20-μL reaction volume containing 1 U AmpliTaqGold (PE, Applied Biosystems), 1.6x AmpliTaqGold PCR buffer (without $MgCl_2$), 0.2 mM dNTP, BSA 0.325 mg/mL, 1.5 mM $MgCl_2$, and each of the primers: 10 pmol of DYS19, DYS389, DYS390, DYS392, and DYS385, and 5 pmol of DYS391 and DYS393. All samples were amplified in 30 cycles comprising 1 min at 94°C, 2 min at 56°C, and 2 min at 72°C preceded by initial denaturation at 95°C for 7 min and followed by final incubation at 60°C for 45 min.

Multiplexed PCR for the 10 Y-chromosomal STR markers (10-plex) was performed in a 20-μL reaction volume containing 1 U AmpliTaqGold (PE, Applied Biosystems), 1x AmpliTaq Gold PCR buffer with 1.5 mM $MgCl_2$, and 0.2 mM dNTP, and 20 pmol of the primers for DYS19, DYS392, and H4 loci, 10 pmol of DYS391, DYS436, DYS437, DYS438, DYS439, and A 7.1 loci, and 5 pmol of the primers for the DYS435 locus. All samples were amplified in 25 cycles comprising 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, preceded by initial denaturation at 95°C for 7 min and final incubation at 60°C for 45 min.

To compare the relative number of Y chromosomes in different tumors versus normal tissue, a stable X-chromosomal marker DXS7423 (17) was included (5 pmol of each primer) in the 9-plex reaction. The sets of nine and ten Y-STRs have four markers in common, which enables the comparison of both sets with respect to the DXS7423 signal.

Ethics

This study protocol was evaluated and approved by the local ethics comittee.

Results and Discussion

A crucial aspect of identity testing and anthropological analysis is determination of gender. Erroneous results in gender testing can lead to false identity, and therefore have juridical consequences, or in comparison of anatomical and molecular analyses, they can lead to biased results. Problems in determination of sex have also occurred in healthy individuals (reviewed in 18), due to supposed spontaneous deletion of the amelogenin Y-chromosomal allele by some unknown mechanism. Santos and colleagues (19) have found two Sri Lankan males out of 24 tested having a deletion of the AMELY gene. Similarly, Roffey and colleagues (20) reported one case in Australia, Tharangaj and colleagues (21) five cases out of

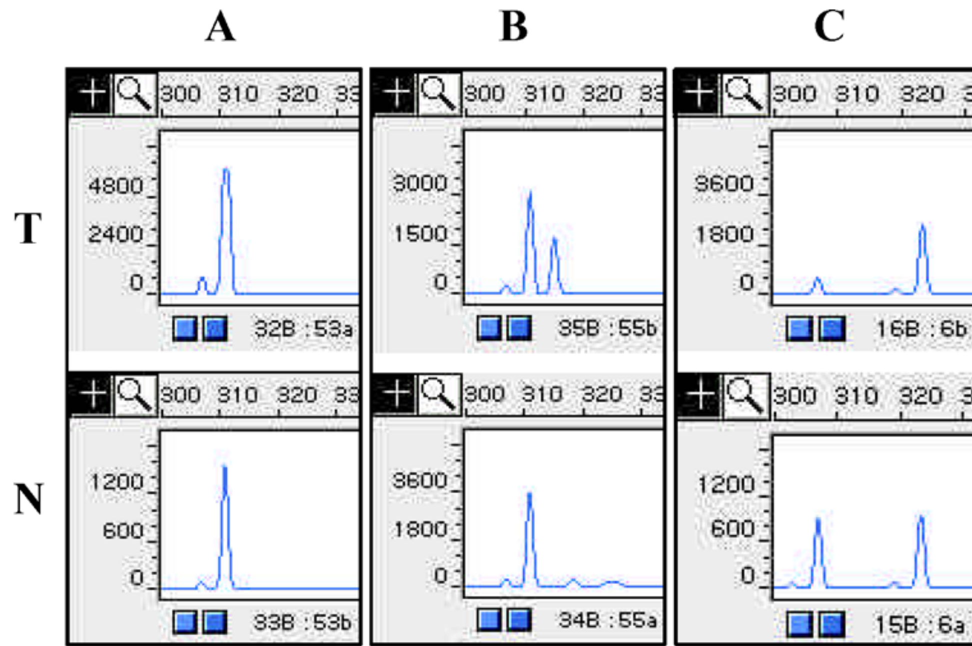

FIG. 1—*Genetic phenotypes of tumors. Panels show results from the autosomal FGA locus included in both AmpFlSTR® SGM Plus™ and AmpFlSTR® Profiler™ kits. Upper electrophoretogram of each panel comes from tumor (T) tissue and lower from normal (N) tissue. Panel A for a MSS cancer shows no changes at the FGA locus. Panel B shows an extra allele at the FGA locus in a MSI tumor, resulting in a heterozygous instead of homozygous genotype. Panel C indicates LOH at the FGA locus in the tumor genome, producing a homozygous not heterozygous genotype.*

270 males tested in India, and Steinlechner and colleagues (14) six cases in Austria. Based on the data available, this deletion seems to be more prevalent among South Asian populations than among Caucasians. Thus far, no case of failure in the amelogenin gender test has been reported in the Finnish population.

Using tumor tissue as a source of genetic reference may lead to problems in genotyping if the data analysis is not carried out carefully. For instance, LOH tumors may be problematic, depending on the different loci tested. If the identity of the donor of the sample is in question, such as cases in which malpractise or a pathology laboratory error is at issue, then mistyping at the amelogenin locus may be relevant. Malignant tissue samples having frequent allelic deletions (LOH-H) at their autosomal STR loci may be mistyped as homozygotes. These cancer specimens may also harbour a similar deletion in the X- or Y-chromosomal amelogenin allele. However, since the tumors are sampled from known individuals of a known gender, the loss of the Y amelogenin locus would have little effect when these samples serve as a reference in identifying unknown samples (e.g., tissue from a mass disaster) or in determining paternity.

Studies on cancers in male patients have observed partial or total loss of the Y chromosome. In pancreatic cancer, Wallrapp and colleagues (6), using fluorescence in situ hybridization (FISH), detected frequent fragments or deletion of the Y chromosome in tumors and cancer cell lines. Frequent (36%) loss of the Y chromosome in male pancreatic endocrine tumors was observed through FISH and STR analysis by Missiaglia et al. (7). Their group noticed that the X chromosome was never deleted, suggesting that for cellular function X-chromosomal genes are essential. In prostatic cancer, Alers and colleagues (8) detected, by comparative genomic hybridization, losses of the Y chromosome in 16% of the cases, but they also found gains in the X chromosome q region, and a specific amplification at the androgen receptor locus (Xq12-q13) area; the latter was associated with metastatic cancers. Perinchery and colleagues (9) analyzed microdissected prostate cancer specimens for six different Y-specific genes (SRY, ZFY, BPY1, SMCY, RBM1, and BPY2). They observed in 90% of the cases loss of at least one of these genes. Furthermore, also in cultured gastric adenocarcinoma cell lines, loss of the Y chromosome is found (10). Van Dekken and colleagues (11) reported similar observations in gastric cancer, using comparative genomic hybridization and xenografting. In short, studies on malignant neoplastic samples show frequent aberrations in the Y chromosome. To the best of our knowledge, however, no studies exist on the Y-chromosomal amelogenin region in cancers.

TABLE 1—*Origin and genetic phenotypes of the neoplasias analyzed.*

		Genetic phenotype†, n (%)				
Tumor*	(n)	MSS	MSI-L	MSI-H	LOH-L	LOH-H
GCA	22	13 (59.3)	3 (13.6)	2 (9.0)	1 (4.5)	3 (13.6)
CRC	22	6 (27.2)	9 (41.2)	2 (9.0)	3 (13.6)	2 (9.0)
GIST	2	1 (50.0)	1 (50.0)	0	0	0

* GCA, gastric cancer; CRC, colorectal cancer; GIST, gastrointestinal stromal tumor.

† Genetic phenotypes are: MSS, microsatellite stable; MSI-L, microsatellite instable low frequency; MSI-H, microsatellite instable high frequency; LOH-L, loss of heterozygosity low frequency; LOH-H, loss of heterozygosity high frequency (for further details for determination of genetic phenotype see Materials and Methods).

Here, we have analyzed 46 male gastrointestinal tumors to gain deeper insight into alterations at the Y-amelogenin locus. Figure 1 indicates the characteristic electropherograms for MSS, MSI, and LOH, which form the basis for phenotypic classification of neoplasias by autosomal STRs. Table 1 shows that, as stratified by the information provided by the autosomal SGM and Profiler STRs, the proportions of the different genetic phenotypes are in line with the proportions found in gastrointestinal neoplasias obtained with other sets of markers (3,22). In our samples the two commercial

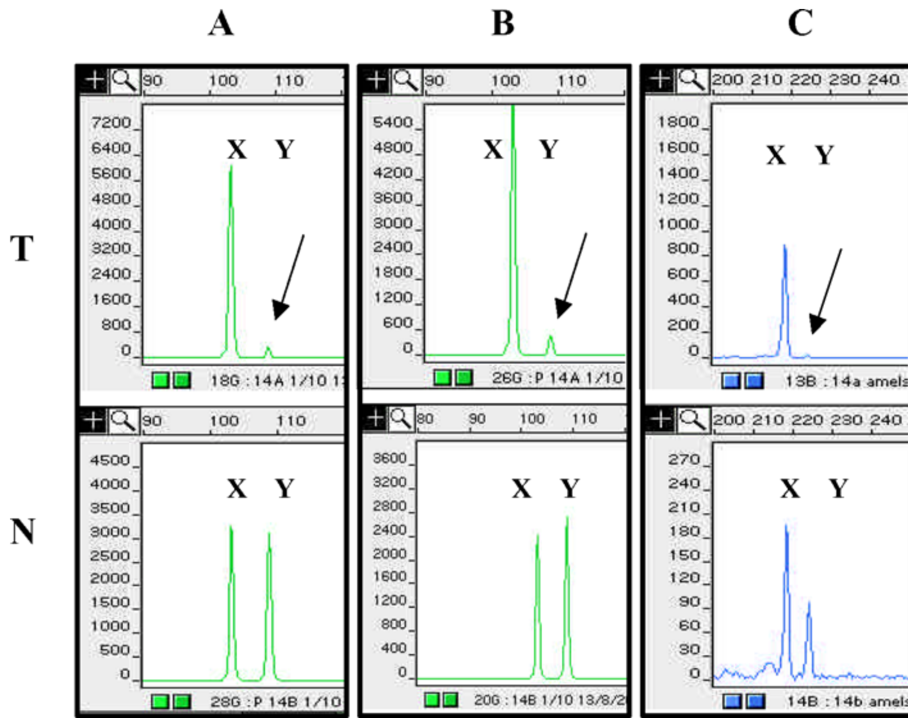

FIG. 2—*Y allele in LOH-H type colorectal carcinoma (case #14, Table 2). Panels A and B show electropherograms obtained with AmpFlSTR® SGM Plus™ and with AmpFlSTR® Profiler™ kits, with LOH ratios of 0.05 and 0.08. When A and B samples were analyzed by Genotyper 2.1 software using the Kazam 20 cutoff filter, both were genotyped as XX. LOH (arrow) of Y allele detectable in tumor (T) in contrast to normal (N) tissue. Panel C findings obtained with the alternate set of flanking primers for amelogenin with an LOH ratio of 0.02 for amelogenin. X- and Y-amelogenin alleles indicated.*

TABLE 2—*Summary of analysis of seven of the neoplastic tissue samples with aberrant Y-amelogenin signal by autosomal and Y-chromosomal STRs and by amelogenin-based gender test in comparison to their adjacent healthy (control) tissues. No control tissues showed any genetic aberrations.*

Case (#)	Tumor	Genetic phenotype	Alterations at the 15 Y-STRs	Genotyper 2.1 SGM		Genotyper 2.1 Profiler		Alternate AMEL STRs LOH ratio
				LOH† ratio	Gender by Kazam 20	LOH ratio	Gender by Kazam 20	
6	GCA	LOH-H	n.d.*	0.40	XY	0.36	XY	0.29
13	CRC	LOH-H	n.d.	0.22	XY	0.22	XY	0.35
14	CRC	LOH-H	n.d.	0.05	XX	0.08	XX	0.02
53	GIST	MSS	n.d.	0.27	XY	0.08	XX	0.25
55	GCA	MSI-H	n.d.	0.20	XY	0.35	XY	0.38
99	GCA	LOH-H	n.d.	0.31	XY	0.19	XY	0.18
175	GCA	LOH-H	n.d.	0.35	XY	0.11	XX	0.06

* n.d.: no alterations detected compared to adjacent healthy tissue.
† LOH ratio: ratio of Y/X of peak heights in cancer vs. normal (Yt/Xt : Yn/Xn).

kits revealed similarly MSI and LOH phenotypes. No tumor samples originally heterozygous or homozygous represented total loss of a locus.

Among the 46 tumors tested, we found diminished Y-amelogenin signals in one MSS, in one MSI-H, and in five LOH-H neoplasias (Fig. 2). A tumor sample was considered to have LOH at the amelogenin locus when the LOH ratio was <0.5 (14). The X and Y amelogenin peaks sized correctly. Table 2 shows the LOH ratios of the tumors with attenuated Y-amelogenin signals. Tumors with an altered signal from the amelogenin locus were then surveyed in more detail. Interestingly, the X-amelogenin allele showed no LOH in any of the 46 male tumors.

The possibility of point mutation(s) in the primer-binding sequences at the Y-amelogenin locus (as reported for example by Boutrand et al. (23) for the autosomal locus D13S317) was tested by using primers flanking the amplification region used in the commercial kits. In comparison to the SGM and Profiler kits, the alternate set of primers gave very much the same LOH ratios (Fig. 2, Table 2). The peaks with the alternate primers also sized correctly (218 and 224 bp).

Furthermore, to see the possible existence of the XY-genotype in cancers or to exclude extra deletions in the Y chromosome, we amplified 15 Y-specific STR markers. These 15 additional Y-STR loci gave similar haplotypes in the cancer and adjacent healthy specimens, suggesting conservation of the XY genotype (Table 2). None of the tumor samples showed loss of the 15 Y-STR loci, indicating that the allelic deletion was restricted only to regions at the Y-specific allele of the amelogenin gene.

When analyzing the Y-amelogenin locus in tumors, ambiguities may arise with regard to determination of gender if the locus shows an LOH. If routine settings in the Genotyper 2.1 software were used with Kazam 20 filtering, a false XX genotype might ensue. This was

evident also in our study with 1/7 and 3/7 XX genotypes obtained with the SGM and Profiler kits, respectively (Table 2).

In some tumors, the LOH ratios were 0.1 or less (Fig. 2 and Table 2). In a tumor sample, a signal from the Y-amelogenin allele may be deceptively higher perhaps because of the presence of contaminating normal tissue covering the attenuated signal, or from cellular subclones with deleted Y amelogenin. With Genotyper 2.1 software having a Kazam filtering cutoff of 1–3%, all LOH-H tumors would be genotyped as XY. However, in cases of tumors with a very low LOH ratio (<0.1), deletion of all of the Y-amelogenin loci in cancer cells is possible, in which case, typing of gender would result in a XX gender for the neoplastic tissue.

A tumor most probably represents a mixture of cell clones, some of which may have a deleted amelogenin locus or may lack the whole X or Y chromosome. The presence of the X-chromosomal amelogenin locus was observed in all of our tumors. To estimate the presence of the Y chromosome in the tumors, the stable X-chromosomal marker DXS7423 (17) was included in the 9-plex assays, which have four STRs in common with the 10-plex assay. Inferring from the comparable ratios between DXS7423 and the different Y-STR signals from normal and cancer samples, the relative numbers of X and Y chromosomes in these two tissues were approximately similar. This result contradicts the idea that loss of all or large portions of the Y chromosomes is a major cause for the attenuated Y-amelogenin signal in the cancer specimens.

In summary, the present study indicates that the Y-chromosomal amelogenin allele may frequently be altered resulting in an ambiguous result from a gender test. Use of additional Y-specific STR markers is thus suggested if determination of gender from neoplastic tissue samples is required. Furthermore, detection of an LOH phenotype or XX genotype or both in a tumor sample showing Y-STR haplotype warrants further analysis of the tissue specimen itself and probably use of alternative methods for gender typing. Our results also suggest the use of a lower filter setting when analyzing genotype data from any cancerous specimen. In general, LOH tumors may also be problematic if the identity of the tissue-sample donor is in question.

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References

1. Koreth J, O'Leary J, McGee J. Microsatellites and PCR genomic analysis. J Pathol 1996;178:239–48. [PubMed]
2. Loeb LA Microsatellite instability: marker of a mutator phenotype in cancer. Cancer Res 1994;54:5059–63. [PubMed]
3. Boland C, Thibodeau S, Hamilton S, Sidransky D, Herman J, Burt R, et al. A National Cancer Institute workshop on microsatellite instability for cancer detection on family predisposition; development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248–57. [PubMed]
4. Lasko D, Cavenee W, Nordenskjöld M. Loss of constitutional heterozygosity in human cancer. Annu Rev Genet 1991;25:281–314. [PubMed]
5. Shen C-Y, Yu J-C, Lo Y-L, Kuo C-H, Ye C-T, Jou Y-S, et al. Genome-wide search for loss of heterozygosity using laser capture microdissected tissue of breast carcinoma: an implication for mutator phenotype and breast cancer pathogenesis. Cancer Res 2000;60: 3384–892.
6. Wallrapp C, Hähnel S, Boeck W, Soder A, Mincheva A, Lichter P, et al. Loss of the Y chromosome is a frequent chromosomal imbalance in pancreatic cancer and allows differentiation to chronic pancreatitis. Int J Cancer 2001;91:340–4. [PubMed]
7. Missiaglia E, Moore P, Williamson J, Lemoine N, Falconi M, Za G, et al. Sex chromosome anomalies in pancreatic endocrine tumors. Int J Cancer 2002;98:532–8. [PubMed]
8. Alers J, Rochat J, Krijtenburg, Hop W, Kranse R, Rosenberg C, et al. Identification of genetic markers for prostatic cancer progression. Lab Invest 2000;80:931–42. [PubMed]
9. Perinchery G, Sasaki M, Angan A, Kumar V, Carroll, Dahiya R. Deletion of Y-chromosome specific genes in human prostate cancer. J Urol 2000;163:1339–42. [PubMed]
10. Castedo S, Correia C, Gomes P, Seruca R, Soares P, Carneiro F, et al. Loss of Y chromosome in gastric carcinoma. Cancer Genet Cytogenet 1992;61:39–41. [PubMed]
11. Van Dekken H, Alers J, Riegman P, Rosenberg C, Tilanus H, Vissers K. Molecular cytogenetic evaluation of gastric cardia adenocarcinoma and precursor lesions. Am J Pathol 2001;158:1961–7. [PubMed]
12. D'Adda T, Candidus S, Denk H, Bordi C, Höfler H. Gastric neuroendocrine neoplasms: tumour clonality and malignancy-associated large X-chromosomal deletions. J Pathol 1999;189:394–401. [PubMed]
13. Sullivan K, Mannucci A, Kimpton C, Gill P. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 1993;15:636–41. [PubMed]
14. Steinlechner M, Berger B, Niederstätter, Parson W. Rare failures in the amelogenin sex test. Int J Legal Med 2002;116:117–20. [PubMed]
15. Kayser M, Caglià A, Corach D, Fretwell N, Gehrig C, Graziosi G, et al. Evaluation of Y chromosomal STRs: a multicenter study. Int J Legal Med 1997;110:125–33,141–9.
16. Ruitberg C, Butler J. New primer sets for Y chromosome and CODIS STR loci. Poster presented at Int Sym Human Identification 2000, Biloxi, MS, US.
17. Zarrabetia M, Amigo T, Sañudo C, Zarrabetia A, Gonzáles-Lamuño D, Riancho J. A new pentaplex system to study short tandem repeat markers of forensic intrest on X chromosome. Forensic Sci Int 2002;129:85–9. [PubMed]
18. Brinkmann B. Editorial: Is the amelogenin sex test valid? Int J Legal Med 2002;116:63. [PubMed]
19. Santos F, Pandya A, Tyler-Smith C. Reliability of DNA-based sex tests. Nat Genet 1998; 118:103.
20. Roffey P, Eckhoff C, Kuhl J. A rare mutation in the amelogenin gene and its potential investigative ramifications. J Forensic Sci 2000;45:1016–9. [PubMed]
21. Thangaraj K, Reddy A, Singh L. Is the amelogenin gene reliable for gender identification in forensic cesework and prenatal diagnosis ? Int J Legal Med 2002;116:121–3. [PubMed]
22. Halling K, Harper J, Moskaluk C, Thibodeau S, Petroni G, Yustein A, et al. Origin of microsatellite instability in gastric cancer. Am J Pathol 1999;155:205–11. [PubMed]
23. Boutrand L, Egyed S, Furedi S, Mommers N, Mertens G, Vanderberghe A. Variations in primer sequence are the origin of allele drop-out at loci D13S317 and CD4. Int J Legal Med 2001;114:295–7. [PubMed]

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