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Loss of heterozygosity in tumour-adjacent normal tissue of breast and bladder cancer

A. Försti [a,*], J. Louhelainen [a], M. Söderberg [b], H. Wijkström [c], K. Hemminki [a]

[a] *Department of Biosciences at Novum, Karolinska Institute, S-14157 Huddinge, Sweden*
[b] *Department of Pathology, Huddinge Hospital, S-14186 Huddinge, Sweden*
[c] *Department of Urology, Huddinge Hospital, S-14186 Huddinge, Sweden*

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Abstract

Normal tumour-adjacent breast tissue samples from 12 breast cancer patients forming six monozygotic twin pairs were analysed for loss of heterozygosity (LOH) on chromosomes 1, 13 and 17. 7 patients showed LOH at one or more markers. Each of them had a different LOH pattern. Only one twin pair showed LOH at the same locus, but the twins had lost a different allele. Multiple ($n = 1$–13), histologically normal samples were collected from 6 bladder cancer patients and analysed for LOH on chromosomes 3 and 9. On chromosome 9, all 6 patients analysed showed LOH in at least one sample and one marker. Four of them also showed LOH on chromosome 3. Samples surrounding different tumours of a given patient resembled each other. More heterogeneity was seen between the patients, even though they shared some similarities in LOH clustering. The results demonstrate that tumour-adjacent normal tissues already harbour genetic changes typical for tumours. These alterations can reveal the earliest changes leading to tumorigenesis.

Keywords: Loss of heterozygosity; Normal tissue; Breast cancer; Bladder cancer

1. Introduction

Cancer is a multistage process where the first genetic alterations in humans may precede the clinical diagnosis of cancer by decades. For example, in colon carcinogenesis, loss of heterozygosity (LOH) at chromosome 5q is found already in the hyperproliferative epithelium and this is followed by a sequence of successive tumorigenic stages [1]. In contrast to colon cancer, the chronology of the tumorigenic events in breast and bladder cancer is not clear. Each breast cancer seems to have its own, unique pattern of genetic changes caused by a complicated interaction of accumulating genetic predisposing factors with somatic changes [2–4]. Allelic losses seen in intermediate and high nuclear grade *in situ* breast tumours already resemble the losses seen in invasive breast cancer, where the most frequent LOHs occur in chromosomal arms 1p, 1q, 3p, 6q, 11p, 11q, 13q, 16q, 17p, 17q and 22q [2]. Ductal carcinoma *in situ* (DCIS) is thought to be a precursor for breast cancer, but only 30–50% of women with DCIS will develop breast cancer [5]. However, only 50–60% of invasive ductal carcinomas contain an *in situ* component [6]. This suggests that some part of the invasive breast cancers may have developed directly from morphologically normal epithelium. Thus, at least some of the genetic changes found in the invasive cancers may be present in the tumour-adjacent normal tissue.

Molecular pathology of bladder cancers shows large heterogeneity. Chromosomal alterations have been found in many, if not most, chromosomes. Chromosome 9 appears to be the main target of alterations, other common findings are LOHs including 3p, 4p, 8p, 11p, 13q and 17p [7,8]. It has been suggested that multiple pathways, all including multistep genetic alteration, are involved in the development of bladder tumours [9,10]. Whether these pathways have a common point of origin is not known.

In this study, we have analysed microscopically normal surrounding tissues of breast and bladder tumours. To reduce the heterogeneity problems, we have chosen multiple normal tissue samples with identical genomes — normal breast tissues from breast cancer samples of monozygotic twins and surrounding tissues

* Corresponding author. Tel.: +46-8-6089244; fax: +46-8-6081501.
E-mail address: asta.forsti@cnt.ki.se (A. Försti).

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of multifocal bladder tumours. Multiple surrounding tissues of a given bladder cancer patient have initially identical genomes like breast cancer samples from monozygotic twins. Tumour-adjacent breast tissue samples of monozygotic twins were analysed with multiple markers on chromosomes 1, 13 and 17. Similarly, histologically normal samples surrounding tumours were collected from multifocal bladder cancer patients and analysed for LOH on chromosomes 3 and 9 using several microsatellite markers.

2. Materials and methods

2.1. Patients and tissue specimens

Monozygotic twins concordant for breast cancer were identified from the Swedish Twin Registry linked to the Swedish Cancer Registry [11]. The cases of breast cancer diagnosed from 1959 to 1992 in twins born in the period from 1886 to 1958 were included to this study. Paraffin-embedded tissue samples were collected from the hospitals of diagnosis. Paraffin material was cut in 10 μm sections, mounted on microscopic slides and stained with haematoxylin and eosin. A pathologist identified areas containing tumour tissue and those containing normal breast tissue, which were then microdissected. Histologically-verified normal breast tissue samples adjacent to the tumour were available from six monozygotic twin pairs, making in total 12 patients with breast cancer. No other normal tissue material from the patients was available. None of the twins was *BRCA1* or *BRCA2* mutation carriers [12].

Tumours, visually normal mucosal tissue and blood were collected from 6 Swedish patients with superficial, multifocal bladder cancer. All the tumours were recurrent and of a transitional cell type. No treatment other than transurethral resections had been attempted before. All tumours were resected transurethrally and each tumour sample was separated into two halves. One half was sent for routine histopathological examination and the other half immediately frozen in liquid nitrogen. Biopsies (2 mm in size) from macroscopically normal bladder mucosa, adjacent to each tumour, were handled likewise. Altogether, 1–13 normal tissue samples per patient were collected.

2.2. DNA isolation

DNA was isolated from the paraffin-embedded tissue samples and from blood as described earlier [13,14].

2.3. LOH analysis

The LOH analysis was performed mainly as described in Refs. [12,14]. LOH was analysed from the normal tissue of the breast cancer samples by using 13 microsatellite markers distributed over the whole chromosome 1, 16 microsatellite markers in the long arm of chromosome 13 and 14 markers distributed over the whole chromosome 17. For bladder samples, 20 markers on chromosome 3 and 29 markers on chromosome 9 were used. Primer sequences and the order of the markers were obtained from the Genome Data Base (http://www.gdb.org at Johns Hopkins University). Primers labelled with a fluorescent Cy5 dye were obtained from Amersham Pharmacia Biotech. Unlabelled primers were from Ransom Hill Bioscience, Inc. Polymerase chain reaction (PCR) was performed by using Perkin-Elmer thermal cycler model 480 or MJ Research PTC-200, followed by fluorescent quantitation using automated fluorescent ALFexpress (Amersham Pharmacia Biotech) or the Applied Biosystems 377 sequencer (PE Biosystems), respectively. Because the allele ratio between the shorter and longer allele in the heterozygous samples varied between 0.7 and 1.43, LOH was defined as a decrease of the signal of either allele of at least 30% in the case of bladder cancer [14]. In the case of breast cancer, a decrease of at least 50% was required.

3. Results

3.1. Breast cancer

Microscopically normal tumour-adjacent tissues from three monozygotic twin pairs with breast cancer were analysed for LOH on each of the chromosomes 1, 13 and 17. Three additional pairs were analysed for LOH only on chromosomes 13 and 17. In total, 43 markers were used. The allelic patterns of the two normal tissues within a twin pair were compared with each other. Examples of the electropherograms of the samples showing LOH in the normal tissue are shown in Fig. 1. Seventeen LOHs were observed, 14 of which were repeated in the corresponding tumour tissue (Table 1; see Ref. [12]). On chromosome 1, 33% of the samples with LOH in the tumour tissue showed LOH in the tumour-adjacent normal tissue, while only 11 and 13% of the samples with LOH in the tumour tissue on chromosomes 13 and 17, respectively, showed LOH in the tumour-adjacent normal tissue (Table 1). As seen in Fig. 2, none of the twin pairs showed an identical LOH pattern. Only one twin pair (pair 3) showed LOH at the same locus (D1S2141), but the twins (patients 3599 and 1930) had lost a different allele (data not shown).

LOH at least at one marker was found in seven patients (Fig. 2). Each of the patients showed a different LOH pattern. They showed LOH either in each of the chromosomes (patient 11 031), in one of the chromosomes (patient 3599 on chromosome 1, patients 1206

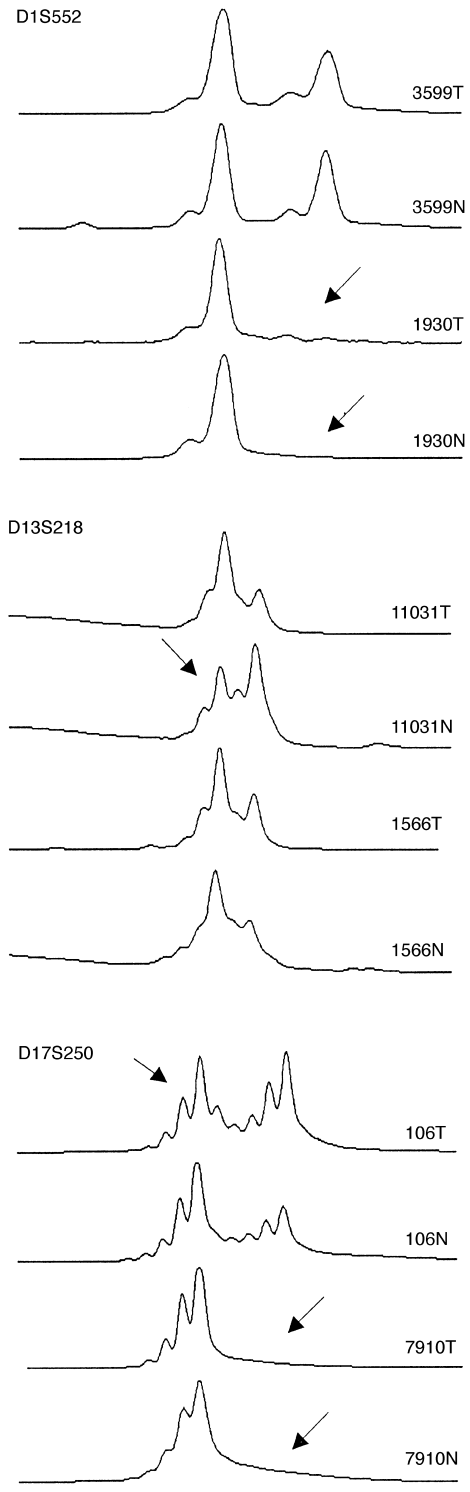

Fig. 1. Examples of the analysis of loss of heterozygosity (LOH) using automated fluorescent ALF Express sequencer. Comparison of the tumour (T) and normal tumour-adjacent (N) tissues within a monozygotic twin pair concordant for breast cancer. The arrows point to the lost allele. At marker D1S552, the longer allele is lost both in the tumour and normal tissue of twin 1930. At marker D13S218, twin 11031 has lost the shorter allele only in the normal tissue. At marker D17S250, twin 106 has lost the shorter allele in the tumour tissue and twin 7910 the longer allele both in the tumour and normal tissues.

and 3836 on chromosome 13, patient 767 on chromosome 17) or in two of the chromosomes (patient 1930 on chromosomes 1 and 17, patient 7910 on chromosomes 13 and 17).

On chromosome 1, 13 microsatellite markers were used for the analysis of LOH in 6 breast cancer patients. Three of them showed LOH at least at one marker (Fig. 2). In total, 7 LOHs were seen, which were divided between five different markers. Three of the allelic losses were seen on the p arm, two at two separate markers at 1p36 (D1S1597 and D1S552) and one at 1p31 (D1S1665). LOH at marker D1S1597 (patient 3599) was not repeated in the corresponding tumour (unpublished data). The three other losses were seen at two markers on the q arm, one at 1q31 (D1S518) and the other at 1q32 (D1S2141).

Sixteen microsatellite markers were analysed for LOH in 12 patients on chromosome 13. Four of the samples showed LOH at five different markers (Fig. 2). Two of these allelic losses were seen in markers located between the *BRCA2* and *RB-1* loci at 13q12.3-14.1 (D13S267 and D13S218). However, the tumour sample of patient 11031 had retained heterozygosity at marker D13S218 (see Ref. [12]). One LOH was observed distal to the *RB-1* locus (D13S279), and the two other LOHs at the most centromeric (D13S221) and the most telomeric markers (D13S173) in our study, respectively.

On chromosome 17, fourteen microsatellite markers were analysed for LOH in 12 patients. Four of them showed LOH at four different markers, including one patient with LOH at two different markers (Fig. 2). Two of the allelic losses were seen in markers distal to the *TP53* locus (D17S926 and D17S786). Two other LOHs in our study were seen at marker D17S250, which is located at 17q11.2-12, centromeric to the *BRCA1* locus (Fig. 2). LOH at marker D17S921 in the normal tissue of patient 11031 was not repeated in the corresponding tumour tissue (see Ref. [12]).

3.2. Bladder cancer

Normal mucosal tissue and blood samples from 6 bladder cancer patients, 5 of them having multiple normal tissues ($n = 2$, 6, 11, 13 and 4), were analysed for LOH on chromosomes 3 and 9. The total number of microsatellite markers was 49. The majority of the informative samples had retained heterozygosity. LOH in the normal tissue could be found on both chromosomes. Of the 112 LOHs observed in the normal tissue samples, 24 were not repeated in the corresponding tumour tissue [15,16]. At most markers, however, at least one normal–tumour tissue pair in a given patient showed LOH. Only two markers (D3S2465 and D3S1744) showed LOH only in the normal tissue [16]. Table 2 summarises the data of the LOH frequencies in the tumour-adjacent normal tissues and the corre-

sponding tumour tissues at the patient level (see Fig. 4, the column on the right side of the picture). Forty-five and 40% of the samples with LOH in the tumour tissues on chromosomes 3 and 9, respectively, showed LOH also in the tumour-adjacent normal tissue. Samples from each patient usually resembled each other, forming LOH clusters around certain markers. In addition, the LOH patterns of different patients shared some similarities. Fig. 3 shows examples of the electropherograms of the samples with LOH.

Chromosome 9 had frequent LOH (Fig. 4). All 6 patients analysed showed LOH in at least one sample and one marker. Patients 1, 3, 4 and 5 had multiple allelic losses with breakpoints in between, whereas in patients 2 and 6 only one sample showed LOH at one marker. The first cluster of LOHs was found at and telomeric to the *p16* tumour suppressor gene at 9p21-22 (D9S741-D9S942) (Fig. 4). Two main regions of LOH were found in 9q (Fig. 4). The first region was centromeric to the *PTCH* tumour suppressor gene at 9q13-22 (D9S301-1AJL). 1AJL is an intragenic *PTCH* marker [17]. The second candidate region on 9q in our study was located telomeric to the *PTCH* locus and maps to 9q31-34.2 (D9S302-D9S290).

Chromosome 3 had several loci, which showed LOH in the normal tissue (Fig. 4). Four patients out of the six analysed showed LOH in at least one sample and one marker. We found LOH from multiple samples of 2 patients at marker D3S3050, which is located at 3p25-26, near the Von Hippel-Lindau (*VHL*) tumour suppressor gene locus. One of these patients had LOH also in several samples at marker D3S3038. This LOH pattern resembled the LOH pattern at marker D3S3050. Interestingly, there was one heterozygous marker between these two loci. Centromeric to this region, three markers showed LOH in the normal tissues at region 3p21-14 (D3S2432-D3S1300). Near the telomere of chromosome 3 at 3q27, 3 out of 4 informative patients showed LOH at D3S2418. One of the patients displayed allelic loss in all of the normal tissue samples.

4. Discussion

According to the multistage models, cancer develops through age-dependent stages. The examples from colon cancer show that genetic changes are present in early adenomas years before the clinical diagnosis of cancer [1]. For breast and bladder cancer, the chronology of the tumorigenic events is not clear. Both cancer types are known to be very heterogenic, presenting various genetic abnormalities in different chromosomes [2,18]. Even clinically early tumours possess numerous genetic changes, which can sometimes also be found from the normal epithelium adjacent to tumours [19–23]. In our study, we have analysed LOH in normal tumour-adjacent tissue samples from monozygotic twins with breast cancer and from multifocal bladder cancer patients. These normal tissue samples have initially identical genomes, which reduce the heterogeneity and phenotype problems.

In our study, most of the LOHs observed in the tumour-adjacent normal tissues were repeated in the corresponding tumour tissue. However, in the breast cancer study, one marker in each of the studied chromosomes (D1S1597, D13S218, D17S921) showed LOH only in the normal tissue (see Ref. [12]). Similarly, in the bladder cancer study, LOH at two markers on chromosome 3 (D3S2465, D3S1744) was present only in the normal tissue [16]. The samples showing these LOHs may represent a subpopulation of breast and urothelial tissue cells, where the genetic changes are transient and not enough to change the phenotype, reflecting the heterogeneity in the development of breast and bladder cancers.

Table 1
LOH frequencies in the tumour-adjacent normal tissues and the corresponding tumour tissues in breast cancer patients[a]

Chromosome	No. of samples analysed	No. of informative	No. of normal tissues with LOH (% of informative samples)	No. of tumour tissues with LOH (% of informative samples)	No. of samples with LOH both in normal and tumour tissues (% of tumours with LOH)
1p	53	39	4 (10)	10 (26)[b]	3 (30)
1q	23	17	3 (18)	8 (47)[b]	3 (38)
1	76	56	7 (13)	18 (32)[b]	6 (33)
13q	133	78	5 (6)	38 (49)[c]	4 (11)
17p	61	36	3 (8)	12 (33)[c]	2 (17)
17q	86	34	2 (6)	18 (53)[c]	2 (11)
17	147	70	5 (7)	30 (43)[c]	4 (13)
1 + 13 + 17	356	204	17 (8)	86 (42)	14 (16)

LOH, loss of heterozygosity.
[a] See Fig. 2 for the number of patients and markers analysed on each chromosome.
[b] Unpublished data.
[c] See Ref. [12].

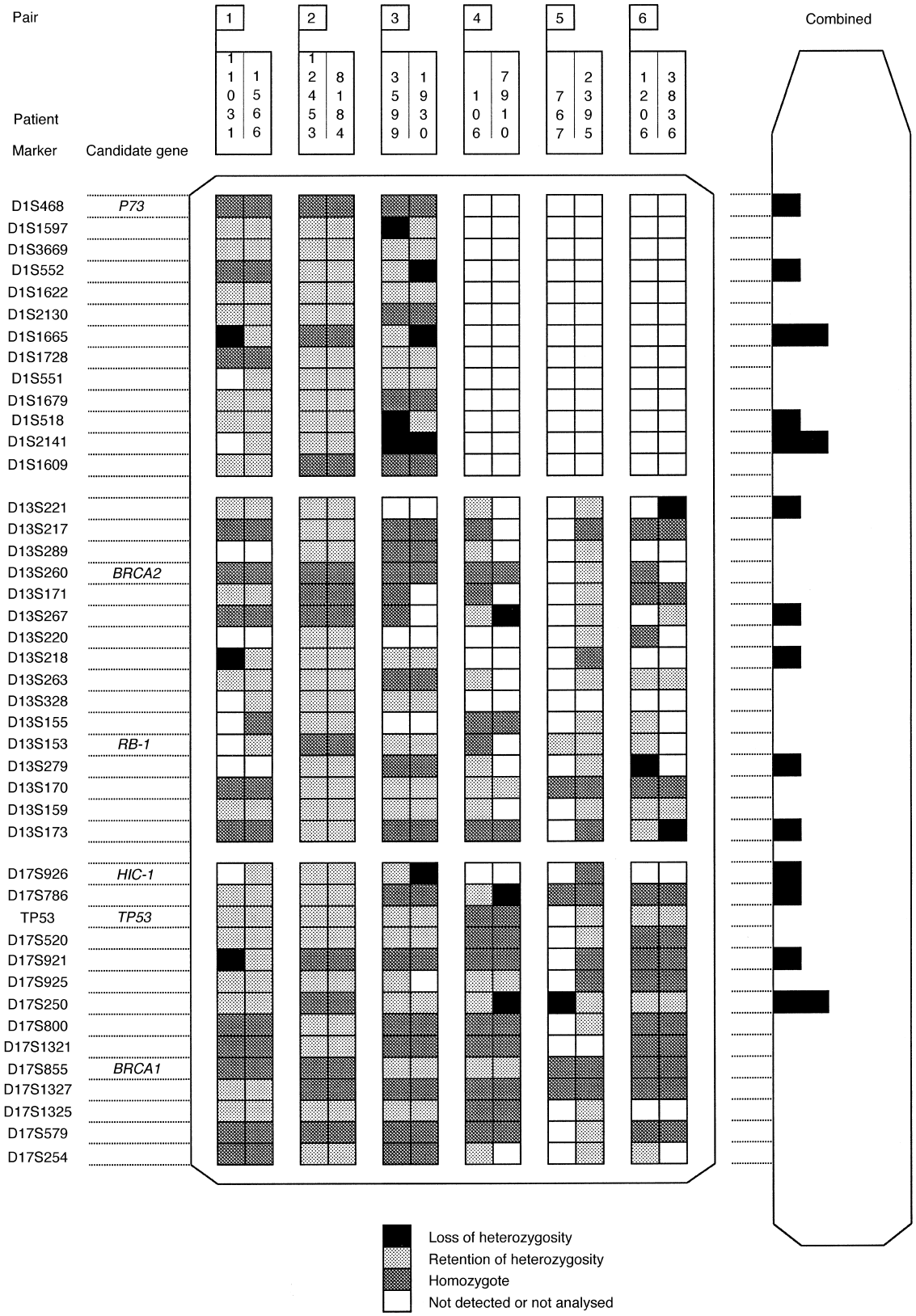

Fig. 2. LOH pattern on chromosomes 1, 13 and 17 in the normal tumour-adjacent tissues from six twin pairs. Twin pair and patient identification numbers are indicated above each column. The markers analysed are shown on the left side of the picture. Approximate locations of the known tumour suppressor genes are also indicated. The columns on the right side of the picture show the number of different patients with LOH at each marker.

Many of the observed LOHs in the normal tumour-adjacent tissues were relatively small, often involving a single microsatellite marker. Even though the mechanisms of LOH, like mitotic recombination and chromosomal nondisjunction, often lead to loss of large regions of chromosomes, our and other studies [15,20,24] have shown an increase in the size of LOH during progression from the normal tissue to invasive cancer.

Normal breast tissue samples from 12 breast cancer patients forming six monozygotic twin pairs were analysed for LOH on chromosomes 1, 13 and 17. Because only tumour-adjacent normal tissues were available, making the analysis conservative, we may have missed some partial allelic losses. Seven of the patients showed LOH in their normal breast tissues at one or more locus. Each of the patients and pairs showed a different pattern of LOH. In total, 17 LOHs were observed. Seven of them were located on chromosome 1 associated with three different patients and five different markers. Two of these allelic losses were seen at two different markers at 1p36 proximal to the *p73* gene locus. Data from both breast cancer [25] and neuroblastoma [26] suggest the presence of a tumour suppressor gene in this region. The three other regions showing LOH on chromosome 1 in our study are frequently lost both in *in situ* and in invasive breast cancers [25,27,28,29].

On chromosome 13, 4 patients showed LOH at five different markers. Two of these allelic losses were seen in markers located between the *BRCA2* and *RB-1* loci at 13q12.3-14.1. This is a common region for LOH in invasive breast cancer [30,31]. In another study, no LOHs at 13q13 were found in morphologically normal lobules adjacent to breast cancer [19]. However, this discrepancy may be due to the small number of samples used in each study. It could also reflect the heterogeneity in breast tumorigenesis.

4 patients had LOH at four markers on chromosome 17. Two of these allelic losses were seen in markers distal to the *TP53* locus, where a recently identified tumour suppressor gene, hypermethylated in cancer 1 (*HIC-1*), is located [32]. One of the losses found in our study was restricted to this telomeric region of 17p. In the other case, the marker at the *TP53* locus was non-informative and we cannot exclude *TP53* as the cause for this LOH. *HIC-1* is inactivated by hypermethylation followed by LOH. It has been shown that *HIC-1* is densely methylated in approximately one-half of the alleles in normal breast epithelium, which may predispose this tissue to inactivation of this gene by LOH [33]. Studies on *in situ* breast cancers have also shown frequent LOH in this region [3,4,34]. The study of normal lobules adjacent to breast cancer showed one LOH in 19 informative samples at *TP53* locus 17p13.1 [19]. Two other LOHs in our study were seen at marker D17S250, which is located at 17q11.2-12, centromeric to the *BRCA1* locus. Data from invasive breast cancer suggest that a tumour suppressor gene may be located in this region [35] and one study on DCIS locates LOH at this region to a later phase in DCIS evolution [3]. *ERBB2* oncogene is located 2 cM distal to D17S250 [36]. It is amplified in approximately 20% of invasive breast tumours and even more often in DCIS [2]. Amplification of *ERBB2* can be observed as an allelic imbalance similar to LOH.

Multiple ($n = 1$–13), visually normal samples were collected from 6 bladder cancer patients and analysed for LOH on chromosomes 3 and 9 using multiple microsatellite markers. For each tumour, a matched normal sample was prepared and used. All 6 patients showed LOH in at least one sample and one marker on chromosome 9. 4 of the patients showed LOH also on chromosome 3. The LOH patterns were similar in the samples of each patient. Even though heterogeneity between the LOH pattern of the different patients

Table 2
LOH frequencies in the tumour-adjacent normal tissues and the corresponding tumour tissues in bladder cancer patients[a]

Chromosome	No. of samples analysed	No. of informative samples	No. of normal tissues with LOH (% of informative samples)	No. of tumour tissues with LOH (% of informative samples)	No of samples with LOH both in normal and tumour tissues (% of tumours with LOH)
3p	48	39	7 (18)	16 (41)[b]	6 (38)
3q	21	14	5 (36)	6 (43)[b]	4 (67)
3	69	53	12 (23)	22 (42)[b]	10 (45)
9p	51	37	9 (24)	23 (62)[c]	9 (39)
9q	96	63	18 (29)	45 (71)[c]	18 (40)
9	147	100	27 (27)	68 (68)[c]	27 (40)
3+9	216	153	39 (25)	90 (59)	37 (41)

LOH, loss of heterozygosity.

[a] See Fig. 4 for the number of patients and markers analysed on each chromosome.

[b] See Ref. [16].

[c] See Ref. [15].

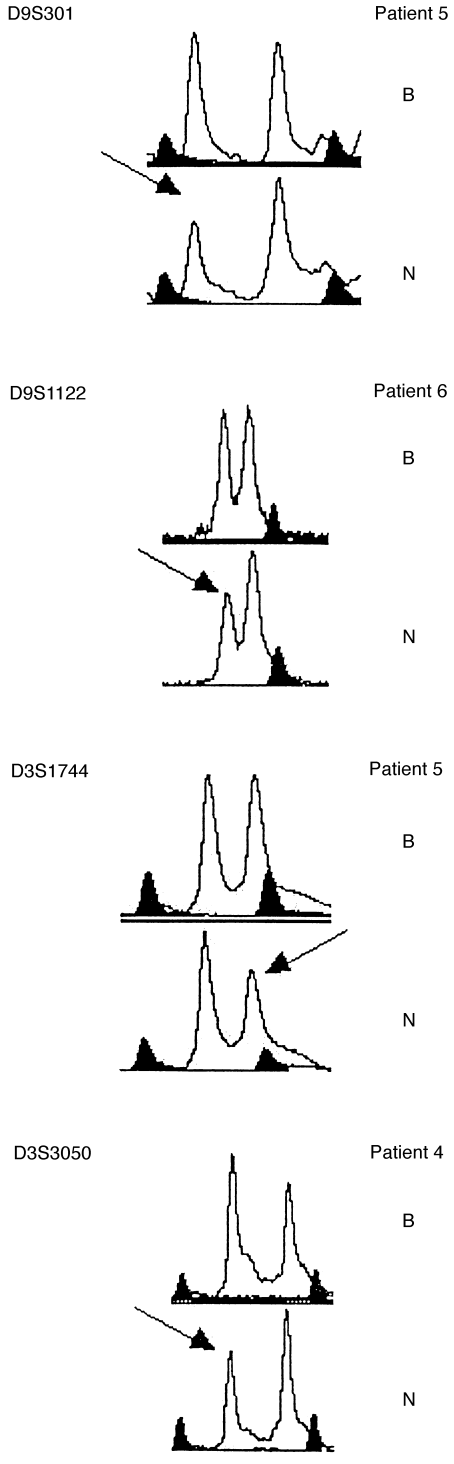

Fig. 3. Examples of the analysis of LOH using fluorescent quantitation on ABI 377 sequencer. Comparison of the normal tumour-adjacent tissue (N) with the blood sample (B) in one bladder cancer patient. The arrows point to the lost allele.

was observed, they shared some similarities in LOH clustering.

Chromosome 9 had frequent LOH. The first cluster of LOHs was found at and telomeric to the *p16* tumour suppressor gene at 9p21-22. At 9q, two main regions of LOH were found. The first region was centromeric to the *PTCH* tumour suppressor gene at 9q13-22. This region shows frequent LOH in bladder cancer [37–40]. Our results support the data that another tumour suppressor gene is located in this region. The second candidate region in 9q is located telomeric to the *PTCH* locus and maps to 9q31-34.2, a region frequently lost in bladder cancer [37,38,40]. This region contains recently identified *DBCCR1* and *TSC-1* genes, which are candidate tumour suppressor genes for bladder cancer [41,42].

Chromosome 3 also showed frequent LOH with several affected regions. We found LOH from multiple samples of two patients at marker D3S3050, which is located near the telomere of the short arm at 3p25-26. One of the patients showed a similar LOH pattern also at marker D3S3038. Interestingly, the samples were heterozygous at one marker between these two loci. This may be due to an error in the physical order of markers. Otherwise it may indicate two different loci, which are involved in bladder tumorigenesis. Centromeric to this region, three markers showed LOH in the normal tissues at region 3p21-p14. Chromosome 3 harbours a fragile histidine triad (*FHIT*) gene at 3p14.2. One patient showed LOH in several samples at the intragenic *FHIT* marker (D3S1300). Recently, a minimal deletion of 3p13->14.2 was associated with immortalisation of human uroepithelial cells [43]. In addition, allelic losses have been found at this region in bladder tumours [44]. Near the telomere of chromosome 3 at 3q27, we found a new region of LOH at marker D3S2418. Comparative Genomic Hybridisation (CGH) analysis has not detected any losses or gains at this region in non-invasive and invasive bladder tumours [45]. In our study, however, 3 out of 4 informative patients showed LOH at D3S2418. One of the patients showed LOH in all of the normal and tumour tissue samples [16].

In this study, we have analysed LOH in the normal tissue adjacent to breast cancer and bladder cancer. We found several LOHs on chromosomes 1, 13 and 17 in the normal tissues of the breast cancer samples. Normal tissues adjacent to low grade, low stage bladder tumours had frequent LOH on chromosomes 9 and 3 with several affected regions. Each sample contained also the invasive component of breast or bladder cancer, and most of the LOHs were repeated in the corresponding tumour tissues. The LOHs seen in the morphologically normal tissue may reflect early, critical events required for the progression to invasive breast and bladder cancers. Comparison of the tumour and the adjacent normal tissue may offer an alternative for the identification of early genetic changes in carcinogenesis.

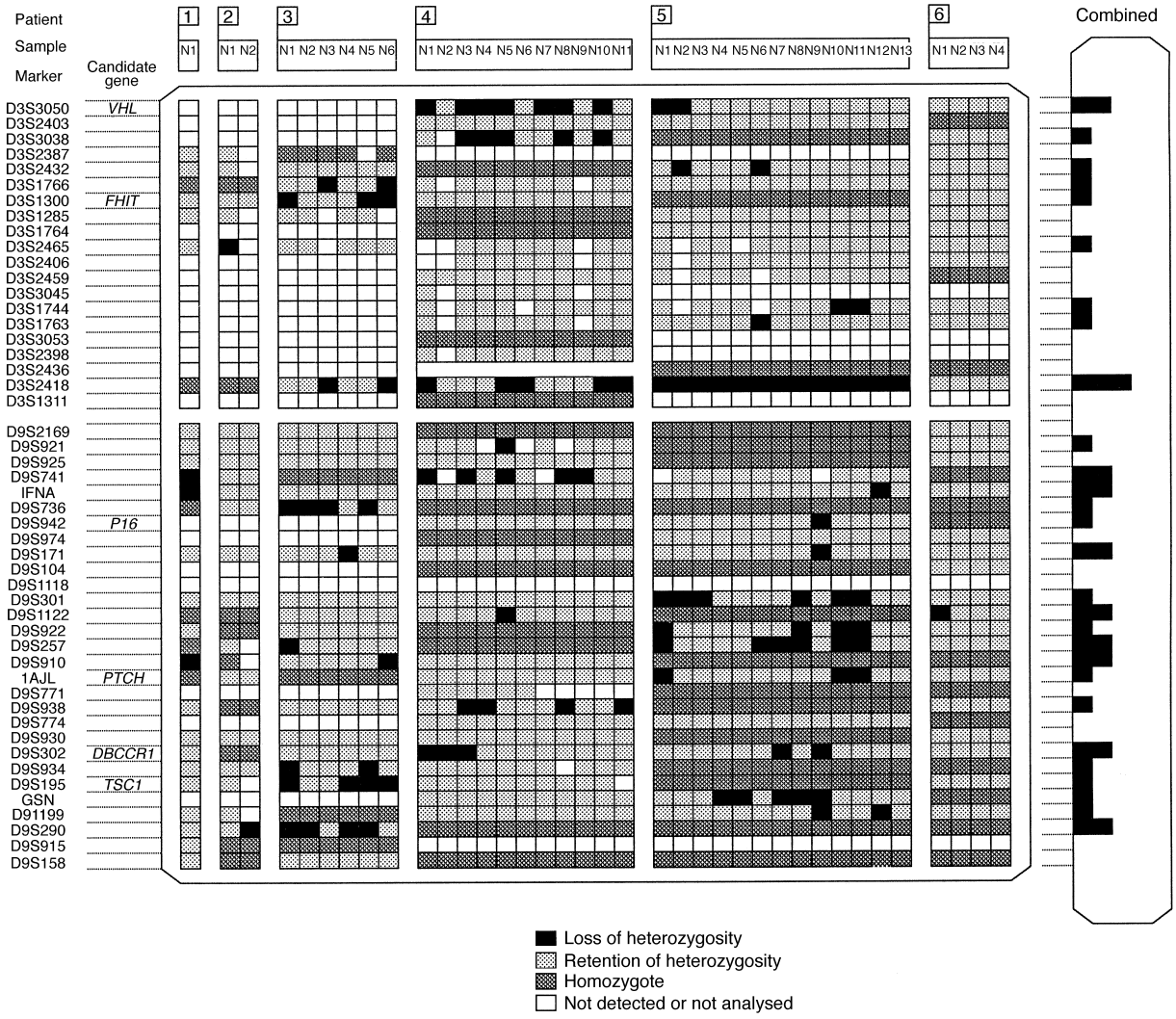

Fig. 4. LOH pattern on chromosomes 3 and 9 in multiple normal mucosal tissue samples ($n = 1$–13) from 6 bladder cancer patients. Patient identification numbers (1–6) are indicated on the top of the picture. N1–N13 indicate the multiple tumour-adjacent normal tissues collected from each patient. The markers analysed are shown on the left side of the picture. Approximate locations of the known tumour suppressor genes are also indicated. The columns on the right side of the picture show the number of different patients with LOH at each marker.

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References

1. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996, **87**, 159–170.
2. Beckmann MW, Niederacher D, Schnurch H-G, Gusterson BA, Bender HG. Multistep carcinogenesis of breast cancer and tumour heterogeneity. *J Mol Med* 1997, **75**, 429–439.
3. Fujii H, Szumel R, Marsh C, Zhou W, Gabrielson E. Genetic progression, histological grade, and allelic loss in ductal carcinoma in situ of the breast. *Cancer Res* 1996, **56**, 5260–5265.
4. James LA, Mitchell EL, Menasce L, Varley JM. Comparative genomic hybridisation of ductal carcinoma in situ of the breast: identification of regions of DNA amplification and deletion in common with invasive breast carcinoma. *Oncogene* 1997, **14**, 1059–1065.
5. Page DL, Steel CM, Dixon JM. ABC of breast diseases. Carcinoma in situ and patients at high risk of breast cancer. *BMJ* 1995, **310**, 39–42.
6. Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. Breast Cancer Linkage Consortium. *Lancet* 1997, **349**, 1505–1510.
7. Habuchi T, Ogawa O, Kakehi Y, Ogura K, *et al.* Accumulated allelic losses in the development of invasive urothelial cancer. *Int J Cancer* 1993, **53**, 579–584.
8. Knowles MA, Elder PA, Williamson M, Cairns JP, Shaw ME, Law MG. Allelotype of human bladder cancer. *Cancer Res* 1994, **54**, 531–538.
9. Spruck CH 3rd, Ohneseit PF, Gonzalez-Zulueta M, *et al.* Two molecular pathways to transitional cell carcinoma of the bladder. *Cancer Res* 1994, **54**, 784–788.

10. Steineck, G., Cordon-Cardo C, Scher HI. Bladder cancer. In Schrier RW, Gottschalk CW, eds. *Diseases of the Kidney*. New York, Little Brown & Co, 1996.
11. Ahlbom A, Lichtenstein P, Malmström H, Feychting M, Hemminki K, Pedersen NL. Cancer in twins: genetic and nongenetic familial risk factors. *J Natl Cancer Inst* 1997, **89**, 287–293.
12. Försti A, Luo L, Vorechovsky I, Söderberg M, Lichtenstein P, Hemminki K. Allelic imbalance on chromosomes 13 and 17 and mutation analysis of *BRCA1* and *BRCA2* genes in monozygotic twins concordant for breast cancer. *Carcinogenesis* 2001, **22**, 27–33.
13. Försti A, Söderberg M, Hemminki K. Use of twins in search for tumor suppressor genes. *Environ Mol Mutagen* 1997, **30**, 231–239.
14. Louhelainen J, Wijkstrom H, Hemminki K. Allelic losses demonstrate monoclonality of multifocal bladder tumors. *Int J Cancer* 2000, **87**, 522–527.
15. Louhelainen J, Wijkstrom H, Hemminki K. Initiation-development modelling of allelic losses on chromosome 9 in multifocal bladder cancer. *Eur J Cancer* 2000, **36**, 1441–1451.
16. Louhelainen J, Wijkstrom H, Hemminki K. Multiple regions with allelic loss at chromosome 3 in superficial multifocal bladder tumors. *Int J Oncol* 2001, **18**, 203–210.
17. Louhelainen J, Lindstrom E, Hemminki K, Toftgard R. Dinucleotide repeat polymorphism within the tumor suppressor gene PTCH at 9q22. *Clin Genet* 1998, **54**, 239–241.
18. Saran KK, Gould D, Godec CJ, Verma RS. Genetics of bladder cancer. *J Mol Med* 1996, **74**, 441–445.
19. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* 1996, **274**, 2057–2059.
20. Chaturvedi V, Li L, Hodges S, *et al*. Superimposed histologic and genetic mapping of chromosome 17 alterations in human urinary bladder neoplasia. *Oncogene* 1997, **14**, 2059–2070.
21. Baud E, Catilina P, Boiteux JP, Bignon YJ. Human bladder cancers and normal bladder mucosa present the same hot spot of heterozygous chromosome-9 deletion. *Int J Cancer* 1998, **77**, 821–824.
22. Chen X, Bonnefoi H, Diebold-Berger S, *et al*. Detecting tumor-related alterations in plasma or serum DNA of patients diagnosed with breast cancer. *Clin Cancer Res* 1999, **5**, 2297–2303.
23. Larson PS, de las Morenas A, Cupples LA, Huang K, Rosenberg CL. Genetically abnormal clones in histologically normal breast tissue. *Am J Pathol* 1998, **152**, 1591–1598.
24. Wistuba II, Behrens C, Milchgrub S, *et al*. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. *Oncogene* 1999, **18**, 643–650.
25. Nagai H, Negrini M, Carter SL, *et al*. Detection and cloning of a common region of loss of heterozygosity at chromosome 1p in breast cancer. *Cancer Res* 1995, **55**, 1752–1757.
26. Kaghad M, Bonnet H, Yang A, *et al*. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* 1997, **90**, 809–819.
27. Munn KE, Walker RA, Varley JM. Frequent alterations of chromosome 1 in ductal carcinoma in situ of the breast. *Oncogene* 1995, **10**, 1653–1657.
28. Hoggard N, Brintnell B, Howell A, Weissenbach J, Varley J. Allelic imbalance on chromosome 1 in human breast cancer. II. Microsatellite repeat analysis. *Genes Chromosomes Cancer* 1995, **12**, 24–31.
29. Benitez J, Osorio A, Barroso A, *et al*. A region of allelic imbalance in 1q31-32 in primary breast cancer coincides with a recombination hot spot. *Cancer Res* 1997, **57**, 4217–4220 (published erratum appears in *Cancer Res* 1998, **58**, 4480).
30. Cleton-Jansen AM, Collins N, Lakhani SR, *et al*. Loss of heterozygosity in sporadic breast tumours at the *BRCA2* locus on chromosome 13q12-q13. *Br J Cancer* 1995, **72**, 1241–1244.
31. Kerangueven F, Allione F, Noguchi T, *et al*. Patterns of loss of heterozygosity at loci from chromosome arm 13q suggests a possible involvement of *BRCA2* in sporadic breast tumors. *Genes Chromosomes Cancer* 1995, **13**, 291–294.
32. Wales MM, Biel MA, el Deiry W, *et al*. p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. *Nat Med* 1995, **1**, 570–577.
33. Fujii H, Biel MA, Zhou W, Weitzman SA, Baylin SB, Gabrielson E. Methylation of the HIC-1 candidate tumor suppressor gene in human breast cancer. *Oncogene* 1998, **16**, 2159–2164.
34. Radford DM, Fair KL, Phillips NJ, *et al*. Allelotyping of ductal carcinoma in situ of the breast: deletion of loci on 8p, 13q, 16q, 17p and 17q. *Cancer Res* 1995, **55**, 3399–3405.
35. Nagai MA, Yamamoto L, Salaorni S, *et al*. Detailed deletion mapping of chromosome segment 17q12-21 in sporadic breast tumours. *Genes Chromosomes Cancer* 1994, **11**, 58–62.
36. Hall JM, Friedman L, Guenther C, *et al*. Closing in on a breast cancer gene on chromosome 17q. *Am J Hum Genet* 1992, **50**, 1235–1242.
37. Simoneau AR, Spruck CH 3rd, Gonzalez-Zulueta M, *et al*. Evidence for two tumor suppressor loci associated with proximal chromosome 9p to q and distal chromosome 9q in bladder cancer and the initial screening for GAS1 and PTC mutations. *Cancer Res* 1996, **56**, 5039–5043.
38. Simoneau M, Aboulkassim TO, LaRue H, Rousseau F, Fradet Y. Four tumor suppressor loci on chromosome 9q in bladder cancer: evidence for two novel candidate regions at 9q22.3 and 9q31. *Oncogene* 1999, **18**, 157–163.
39. McGarvey TW, Maruta Y, Tomaszewski JE, Linnenbach AJ, Malkowicz SB. PTCH gene mutations in invasive transitional cell carcinoma of the bladder. *Oncogene* 1998, **17**, 1167–1172.
40. Wu Q, Possati L, Montesi M, Gualandi F, *et al*. Growth arrest and suppression of tumorigenicity of bladder-carcinoma cell lines induced by the P16/CDKN2 (p16INK4A, MTS1) gene and other loci on human chromosome 9. *Int J Cancer* 1996, **65**, 840–846.
41. Habuchi T, Luscombe M, Elder PA, Knowles MA. Structure and methylation-based silencing of a gene (DBCCR1) within a candidate bladder cancer tumor suppressor region at 9q32-q33. *Genomics* 1998, **48**, 277–288.
42. Hornigold N, Devlin J, Davies AM, Aveyard JS, Habuchi T, Knowles MA. Mutation of the 9q34 gene TSC1 in sporadic bladder cancer. *Oncogene* 1999, **18**, 2657–2661.
43. Vieten L, Belair CD, Savelieva L, *et al*. Minimal deletion of 3p13–>14.2 associated with immortalization of human uroepithelial cells. *Genes Chromosomes Cancer* 1998, **21**, 39–48.
44. Li M, Zhang ZF, Reuter VE, Cordon-Cardo C. Chromosome 3 allelic losses and microsatellite alterations in transitional cell carcinoma of the urinary bladder. *Am J Pathol* 1996, **149**, 229–235.
45. Richter J, Jiang F, Gorog JP, *et al*. Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. *Cancer Res* 1997, **57**, 2860–2864.