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ORIGINAL ARTICLE

Ductal carcinoma in situ of the breast with different histopathological grades and corresponding new breast tumour events: Analysis of loss of heterozygosity

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Abstract
To compare chromosomal alterations in ductal carcinoma *in situ* (DCIS) of different histopathological grades and to study aberrations between primary DCIS and corresponding ipsi- or contralateral new *in situ* or invasive tumours, a study was undertaken of the pattern of loss of heterozygosity (LOH) at chromosomal regions in which LOH has previously been described in invasive breast cancer. LOH was analysed using 19 microsatellite markers located on chromosomes 3p, 6q, 8p, 8q, 9p, 11p, 11q, 16q, 17p, and 17q in 30 women with a primary DCIS. Eleven women with DCIS of grade 1 and 19 with grade 3 according to the EORTC classification system were included. In six patients LOH was also analysed in a subsequent new breast cancer. Fractional allelic loss (FAL, the ratio of chromosomal arms where allelic loss was detected divided by the total number of chromosomal arms with informative markers) was statistically significantly higher in grade 1 DCIS compared with grade 3 ($p = 0.02$) for the 19 loci, indicating that the amount of allelic loss does not correlate with increasing aggressiveness of the studied tumours. Also observed was a complete heterogeneity of LOH in the primary DCIS and their corresponding new events, suggesting that these events probably developed from genetically divergent clones.

Ductal carcinoma *in situ* (DCIS) is a heterogeneous disease and a large number of classification systems have been proposed aiming at prognostic subgrouping [1,2]. There are some indications that different subgroups of DCIS are more likely to recur [3–5]. The biological events that make a ductal carcinoma *in situ* (DCIS) lesion progress into invasive breast cancer (IBC) are still unknown although different deletions of genomic segments may be significant. Several sites are suspected to be related to tumour progress but the biological importance of most deletions is still not clarified. Chromosomes that earlier have been associated with frequent loss of heterozygosity (LOH) in invasive breast cancer are 1, 3p, 6q, 7q, 8p, 9p, 11, 13q, 16q, 17, 18q, and 22q [6]. Recent genome-wide allelotyping of single nucleotide polymorphisms in invasive breast cancers has pointed out 17p, 17q, 16q, 11q, and 14q as the most common sites of LOH [7].

Various studies report involvement of numerous loci in different stages of breast cancer, and considerable evidence indicates that breast cancer is genetically heterogeneous [8–10]. Some reports suggest involvement of chromosomes 16q, 17p, and 17q in early stages, showing abnormalities in 25–30% of DCIS lesions [11–15]. Lesions on chromosome 3 have been found in benign breast epithelium at only a slightly lower frequency compared with subsequent breast cancers, suggesting involvement early in tumourigenesis of the breast [16–19]. LOH on 8p has been reported in both early lesions and in tumours of later stages, and has been associated with advanced clinical state and poor prognosis in breast cancer [11,13,15,20–23]. Clonal subpopulations from primary breast carcinomas and lymph node metastases have shown an increase in LOH events mainly on chromosomes 3p, 4q, 6p, and 18q upon aneuploidization, while LOH at 8q, 16p, 16q, 17p, and 17q was

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present in diploid clones and recurred in the aneuploid clones within the same tumour [24].

Although various studies have reported higher LOH frequencies in high-grade than in low- and intermediate-grade DCIS, conflicting data exist for some loci [14,25–27]. We analysed LOH at 19 loci in 11 EORTC grade 1 and 19 EORTC grade 3 tumours in order to assess a possible correlation with histopathological grade. In addition, six subsequent ipsi- and contralateral breast cancers were analysed to determine whether the LOH pattern was consistent with LOH in the primary DCIS.

Material and methods

Patients

The patients were selected from a population-based cohort of 195 women with DCIS from the catchment area of Uppsala University Hospital and Västerås Hospital between the years 1986 and 1994. We included all women with a DCIS grade 1 (n = 13), according to the EORTC classification system proposed by Holland and co-workers [1,5]. We selected 21 women of 83 with DCIS grade 3. In 11 of the DCIS grade 1 and in 19 of the DCIS grade 3 lesions we had enough tumour material to make new slides for microdissection. Follow-up was completed in December 1998. Material from ipsi- and contralateral new breast tumour events in the women included in the study, up to December 1998, were also collected for analysis.

Microdissection and DNA extraction

All histopathological specimens, routinely stained according to van Gieson, were classified according to the EORTC classification system (Holland grade 1 or 3) by two observers together (HN and FW). If there was a dominating histopathological pattern and only a small focus of another pattern we classified the lesion according to the dominating pattern. Otherwise we classified the lesion according to the most poorly differentiated pattern in the lesions with a mixed pattern. We defined a 'small' focus as being at maximum 25% of the total tumour area.

The samples were previously analysed by immunohistochemical staining of p53, c-erbB-2, Ki-67, Bcl-2, and the hormone-receptors for oestrogen and progesterone (results shown in Table I) [28]. New slides, 15–17 μm thick, were cut from the paraffin blocks and stained with toluidine blue. Two injection needles (1.2 mm) were used to sample tumour cells under a dissecting microscope. A minimum of 5–6 ducts with *in situ* carcinoma were collected and placed into 1.5 ml tubes with saline solution. The proportion of contamination with normal tissue cells was estimated to a maximum of 10–15%. A corresponding control with normal tissue was dissected from the same slide as the DCIS lesion and processed in a similar fashion. DNA was extracted from the slides using the QIAamp® DNA mini kit (VWR International AB, Stockholm, Sweden).

Table I. LOH frequencies for microsatellite markers analysed in 30 DCIS tumours.

Marker	Locus	Type of repeat	Informative	LOH
D3S1766	3p21.1-14.2	Tetra	22/27 (81%)	5/22 (23%)
D3S2432	3p24.2-22	Tetra	19/24 (79%)	1/19 (5%)
D3S2387	3p26.2-21.1	Tetra	22/26 (85%)	5/22 (23%)
D6S417	6q15	Di	10/23 (43%)	4/10 (40%)
D6S310	6q27-16.3	Di	16/24 (67%)	1/16 (6%)
D6S473	6q25.1	Di	19/23 (83%)	4/19 (21%)
D8S264	8p23	Di	15/26 (58%)	1/15 (7%)
D8S1110	8q11.21	Tetra	19/23 (83%)	3/19 (16%)
D9S171	9p21	Di	19/25 (76%)	5/19 (26%)
D11S904	11p14-13	Di	12/25 (48%)	1/12 (8%)
D11S1999	11p15.3	Tetra	20/24 (83%)	0/20 (0%)
D11S4175	11q21	Di	20/23 (87%)	4/20 (20%)
D11S1986	11q23.1	Tetra	18/24 (75%)	4/18 (22%)
D16S753	16q12.1	Tetra	16/24 (67%)	1/16 (6%)
D16S2622	16pter-qter	Tetra	19/26 (73%)	1/19 (5%)
D16S2624	16q22.1	Tetra	18/24 (75%)	0/18 (0%)
D16S539	16q23.1-22	Tetra	20/24 (83%)	3/20 (15%)
D17S921	17p12-11.2	Di	12/23 (52%)	1/12 (8%)
D17S787	17q22	Di	13/25 (52%)	3/13 (23%)

PCR amplification

Tumour and control DNA samples were amplified by PCR for di- and tetranucleotide repeat markers (Table I). PCR was carried out in 20 µl volume reactions, containing 1 × Buffer II (Applied Biosystems, Stockholm, Sweden), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM of each primer (one primer of each pair was Cy5-labelled), 2 U AmpliTaq Gold (Applied Biosystems, Stockholm, Sweden), and 1-5 µl DNA template. The PCR conditions were 95°C × 5 min followed by 40 cycles of 95°C × 1 min, 55°C × 30 s, and 72°C × 45 s. A final extension step was carried out at 72°C for 5 min.

Detection of LOH and MSI

PCR products were run on a denaturing 6% polyacrylamide gel using ReproGel high resolution kit and an ALFexpress™ II DNA analysis system (Amersham Biosciences, Uppsala, Sweden). The results were analysed using ALFwin™ Fragment Analyzer software (Amersham Biosciences, Uppsala, Sweden). A reduction by > 50% for an allele in tumour DNA relative to the corresponding normal DNA was interpreted as LOH (Figure 1). Microsatellite instability (MSI) was scored when tumour DNA was observed to have additional bands that were not present in corresponding normal tissue. Cases showing LOH or MSI were repeated from a new PCR reaction at least once to confirm the change. All the data generated from the ALFwin™ Fragment Analyzer were read blindly, without knowledge of histological grade or outcome for the patients (JS). In addition, some samples were initially and blindly read by four observers. In those instances where a dissimilar result was

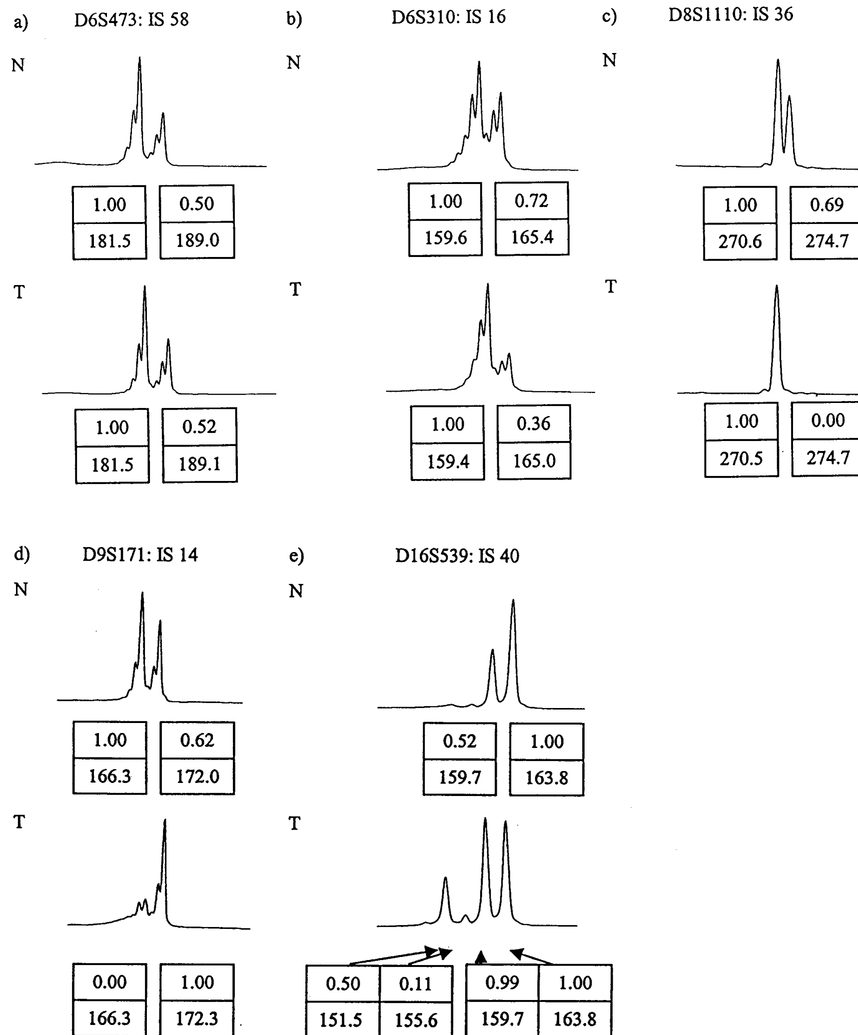

Figure 1. Examples of microsatellite analyses. (a) Retention of heterozygosity, (b–d) LOH, and (e) MSI in the DCIS samples. N and T denote DNA from normal and corresponding tumour tissue, respectively. The microsatellite markers and DCIS numbers are given above each graph. The numbers beneath each peak give the allele size and relative peak area.

obtained, a final consensus was closed between two observers (JS, TN).

Statistical analysis

The statistical significance of differences in comparisons of LOH at different chromosomal loci in DCIS grade 1 versus grade 3 lesions was determined by Fisher's exact test and the significance level was set at 5%. Fractional allelic loss (FAL) was calculated for each DCIS sample as the ratio of chromosomal arms having allelic loss divided by the total number of informative chromosomal arms. A weighted F-test was used for comparison of FAL values between the groups. The weights equal the estimated variances of the FAL observations.

Results

Detection of LOH and MSI

Of the 30 DCIS lesions, 23 were analysed for almost all markers while 7 could only be analysed for a few markers (Figure 2). Problems with the PCR amplification were due to the poor quality of DNA, which was extracted from paraffin-embedded tissues.

Comparing tumours of different grades, the grade 3 tumours had higher frequencies of LOH than grade 1 DCIS for chromosomes 6q, 11p, 17p, and 17q (Table II). The frequency of loss was higher in grade 1 samples for the other chromosomes (3p, 8p, 8q, 9p, 11q, and 16q). The LOH frequency on chromosomes 11q and 16q in DCIS grade 1 lesions was significantly higher compared with grade 3 lesions ($p = 0.009$ for chromosome 11q and $p = 0.005$ for chromosome 16q). However, the number of samples in each of the groups was small. Two DCIS tumours showed MSI for two different markers and two tumours for one marker each.

A high FAL value might indicate a high overall genetic instability in the tumour. The mean FAL value for the 19 loci was 0.35 in grade 1 DCIS and 0.20 in grade 3. The difference was statistically significant ($p = 0.0225$, $F > 5.84$; the weighted least square estimates were 0.37 for grade 1 and 0.15 for grade 3).

Correlation between LOH and MSI alterations in DCIS and their subsequent breast cancer events

New events from 6 women with a primary DCIS diagnosis were analysed, without knowledge about the matching primary DCIS. Ipsilateral events occurred in individuals with primary DCIS labelled IS14, IS28, and IS36, whereas individuals with primary DCIS IS44, IS52, and IS58 had contralateral new events (Figure 3). Difficulties with amplification for markers in some of the samples, however, reduced the number of possible comparisons in the LOH pattern. DCIS sample IS14 showed LOH for marker D17S921 while its corresponding ipsilateral new event R14 showed heterozygosity. This would indicate a different clonal origin of the new event. Similarly, DCIS sample IS36 showed LOH for marker D8S1110 while the ipsilateral new event R16 was heterozygous for the marker. As several markers could not be analysed in ipsilateral R6 and R8 that corresponded to DCIS sample IS28, it was not possible to determine whether the LOH pattern was similar or not in the primary and the new events. Also, in DCIS samples IS44 and IS52 most markers could not be evaluated, and hence no major comparison could be carried out with the markers in the corresponding contralateral events R4 and R2, respectively. The contralateral *in situ* and invasive tumour components R10 and R12 showed LOH patterns different from corresponding DCIS sample IS58. MSI appeared more frequently in the new events than in the primary DCIS lesions. Interestingly, the *in situ* and invasive tumour components R10 and R12 corresponding to DCIS sample IS58 differed in at least three of the markers.

Comparison of the rates of LOH for the different chromosomes in DCIS patients with no further recorded event versus those with a new event demonstrated a tendency for some of the markers to have a higher LOH frequency in the latter group, although the number of patients were too few for a meaningful statistical analysis (Table III).

Discussion

The main finding in this study was a significantly higher FAL in DCIS grade 1 than in DCIS grade 3 for 19 analysed loci. Statistically significant differences for LOH between DCIS grade 1 and 3 were observed for chromosomes 11q and 16q, where LOH was more frequent in DCIS grade 1 than in grade 3 lesions ($p = 0.009$ for chromosome 11q and $p = 0.005$ for chromosome 16q). We detected MSI in only four of the 30 DCIS samples, at five markers on chromosomes 3, 8, 11, and 16. The low frequency of MSI in the present material is consistent with the proportion of MSI found in DCIS and invasive breast cancer in other studies [8,29,30].

The DCIS tumours in the current study were selected from a population-based cohort and represented well-defined subgroups of this entity. The complete clinical follow-up and the available corresponding new events made the material even more valuable. *In situ* material offers interesting possibilities to evaluate alterations in early breast cancer and to make comparisons with later stages of breast

	Sample	D3S1766	D3S2432	D3S2387	D6S417	D6S310	D6S473	D8S264	D8S1110	D9S171	D11S904	D11S1999	D11S4175	D11S1986	D16S753	D16S2622	D16S2624	D16S539	D17S921	D17S787
Grade 3	IS2	NI	HET	HET	NI	HET	HET	HET	HET	NI	HET	HET	LOH	NI	NI	HET	HET	HET	HET	NI
	IS4	HET	HET	HET	HET	HET	HET	HET	HET	HET	NI	NI	HET	HET	HET	NI	NI	NI	NI	NI
	IS6	HET	HET	NI	NI	NI	HET	MSI	MSI	HET	NI	HET	HET	HET	NI	HET	HET	HET	NI	NI
	IS8	HET	HET	HET	NI	HET	NI	NI	HET	HET	HET	HET	NI	HET	HET	NI	NI	HET	HET	LOH
	IS10	HET	NI	HET	HET	NI	HET	NI	HET	HET	NI	HET	NI	HET	HET	NI	HET	HET	HET	NI
	IS12	HET	MSI	LOH	NI	NI	LOH	NI	HET	HET	HET	MSI	HET	NI	NI	HET	HET	HET	NI	NI
	IS14	LOH	NI	HET	HET	NI	HET	HET	HET	LOH	HET	HET	HET	LOH	HET	NI	HET	HET	LOH	HET
	IS16	HET	HET	HET	HET	LOH	NI	HET	HET	HET	HET	HET	HET	HET	NI	HET	NI	NI	NI	HET
	IS18																			LOH
	IS20	NI	NI	HET	LOH	NI	HET	HET	NI	LOH	NI	HET	HET	HET	HET	HET	HET	HET	HET	HET
	IS22	HET	HET	HET	NI	HET	HET	NI	HET	HET	NI	HET	HET	NI	HET	NI	HET	HET	HET	HET
	IS24										NI									HET
	IS26	HET	LOH	LOH										LOH	NI	HET	HET			
	IS28	NI	HET	HET	LOH	HET	HET	HET	HET	HET	HET	HET	HET	NI	HET	HET	NI	HET	HET	HET
	IS30	HET	HET	NI	HET	HET	LOH	HET	HET	HET	HET	HET	HET	NI	HET		HET	HET	NI	HET
	IS32	HET	NI	HET	NI	HET	HET	HET	HET	HET	HET	HET	HET	HET	NI	HET	HET	HET	HET	NI
	IS34	HET	HET	HET	NI	HET	HET	HET	HET	NI	NI	HET	HET	HET	HET	HET	NI	HET	NI	NI
	IS36	HET	NI	HET	NI	HET	LOH	HET	LOH	NI	NI	HET	HET	HET	HET	HET	HET	HET	HET	NI
	IS38	HET	HET	LOH	HET	HET	HET	NI	LOH	HET	LOH	HET	HET	HET	HET	HET	HET	NI	NI	LOH
Grade 1	IS40	LOH	HET	HET	NI	HET	HET	HET	NI	NI	NI	HET	LOH	HET	NI	HET	HET	MSI	HET	HET
	IS42	HET	HET	HET	NI	HET	NI					HET		NI	HET	NI	HET	HET		
	IS44							NI								HET				
	IS46	NI		LOH		NI		LOH		NI	NI		NI			HET			NI	
	IS48	LOH	HET	NI	NI	NI	HET	NI	NI	LOH	NI	HET	LOH	HET	NI	HET	NI	LOH	NI	NI
	IS50	NI		HET				MSI	HET	HET		NI				LOH				NI
	IS52	LOH						NI		NI	NI							HET		
	IS54	HET	HET	HET	LOH	HET	NI	NI	HET	LOH	NI	NI	HET	LOH	LOH	HET	HET	HET	HET	NI
	IS56	HET	HET	LOH	LOH	NI	LOH	HET	HET	LOH	HET	HET	LOH	HET	HET	HET	HET	LOH	HET	HET
	IS58	HET	HET	HET	NI	HET	HET	HET	HET	HET	HET	NI	HET	HET	HET	HET	HET	LOH	NI	HET
	IS60	LOH	HET	NI	NI	HET	HET	NI	LOH	HET	HET	HET	HET	LOH	HET	NI	HET	NI	NI	NI

Figure 2. Schematic representation of results from analysis of 19 microsatellite markers. The bold line divides the samples into DCIS tumours of grade 3 (IS2 to IS38) and grade 1 (IS40 to IS60). Tumour samples were given even numbers, while corresponding normal samples with odd numbers are not shown in the figure. LOH = loss of heterozygosity, MSI = microsatellite instability, HET = heterozygous (informative), NI = non-informative. Grey coloured empty boxes indicate samples that could not be analysed.

Table II. The proportion of LOH on different chromosome arms and percentage of positive staining for previously studied markers in DCIS tumours of grade 1 and 3.

Marker	Grade 1 (n = 11)	Grade 3 (n = 19)
LOH 3p	6/10 (60%)	4/17 (24%)
LOH 6q	2/7 (29%)	6/16 (38%)
LOH 8p	1/4 (25%)	0/11 (0%)
LOH 8q	1/5 (20%)	2/14 (14%)
LOH 9p	3/6 (50%)	2/13 (15%)
LOH 11p	0/6 (0%)	1/15 (7%)
LOH 11q[1]	5/6 (83%)	3/17 (18%)
LOH 16q[2]	5/11 (45%)	0/17 (0%)
LOH 17p	0/3 (0%)	1/9 (11%)
LOH 17q	0/3 (0%)	3/10 (30%)
p53	0/11 (0%)	13/19 (68%)
c-erbB-2	4/11 (36%)	14/19 (74%)
ER	10/11 (91%)	8/18 (44%)
PR	9/11 (82%)	4/18 (22%)
Bcl2	9/11 (82%)	4/18 (22%)
Ki67	0/11 (0%)	2/19 (11%)

[1]The frequency of LOH was statistically significantly higher in grade 1 DCIS; p = 0.009 (Fisher's exact test).
[2]The frequency of LOH was statistically significantly higher in grade 1 DCIS; p = 0.005 (Fisher's exact test).

cancer. However, technical difficulties consisting in problems with PCR amplification of the formalin-fixed and paraffin-embedded samples occurred in several cases due to the poor quality of DNA as previously reported by us [31]. For some of the samples very few markers could be amplified although repeated experiments were carried out. Additional microdissections and amplifications were performed for problematic samples but the material was used up without obtaining successful results in some of the cases. Many of the selected markers were tetranucleotide markers, which are less prone to produce stutter bands and PCR artefacts than dinucleotide markers [32].

Our study was not designed to study the distribution of LOH and MSI in DCIS but rather to contrast two different subsets of DCIS with known different natural history. Others, like us, have previously reported different local recurrence rates after 5 to 8 years for low grade DCIS (0–4%) and high grade DCIS (20–30%) [2,5,33]. However, different histopathological classification systems for DCIS were used in different studies. The 19 microsatellite markers that were analysed in the two DCIS groups were selected from chromosomal regions with implication in invasive breast cancer [34].

It has been suggested that ipsilateral recurrent DCIS shares a common genetic pathway with the initial DCIS, while genetic alterations of contralateral tumours have been thought to be unrelated to those in the initial lesion [35]. We cannot tell whether the ipsilateral subsequent events in this

Sample	Type of relapse	D3S1766	D3S2432	D3S2387	D6S417	D6S310	D6S473	D8S264	D8S1110	D9S171	D11S904	D11S1999	D11S4175	D11S1986	D16S753	D16S2622	D16S2624	D16S539	D17S921	D17S787
IS14		LOH	NI	HET	HET	NI	HET	HET	HET	LOH	HET	HET	HET	LOH	HET	NI	HET	HET	LOH	HET
R14	Ipsilat invasive after 10 mon	MSI	HET	LOH				HET	NI		MSI	LOH	HET	NI	NI	NI	HET	HET	HET	NI
IS28		NI	HET	HET	LOH	HET	HET	HET	HET	HET	HET	HET	HET	NI	HET	HET	NI	HET	HET	HET
R6	Ipsilat in situ after 51 mon	HET						NI	NI			HET				HET	NI			HET
R8	Ipsilat invasive after 51 mon	LOH	HET					HET	NI	NI	HET	NI		NI	HET	HET	NI	HET	NI	NI
IS36		HET	NI	HET	NI	HET	LOH	HET	LOH	NI	NI	HET	HET	HET	HET	HET	HET	HET	HET	NI
R16	Ipsilat invasive after 19 mon	NI						NI	HET			NI	HET			LOH		NI	HET	NI
IS44								NI								HET				
R4	Contralat invasive after 41 mon	HET	NI	HET	NI	NI		NI		NI	NI	MSI	NI	NI	HET	HET	NI	NI	NI	MSI
IS52		LOH						NI		NI	NI							HET		
R2	Contralat in situ after 46 mon	MSI	NI	NI	NI	NI	LOH	NI			HET	HET	NI	NI		HET	HET	HET	HET	HET
IS58		HET	HET	HET	NI	HET	HET	HET	HET	HET	HET	NI	HET	HET	HET	HET	HET	LOH	NI	HET
R10	Contralat in situ after 27 mon	MSI	HET	HET	LOH	MSI	MSI	HET	NI	NI	HET	NI	HET	HET	NI	HET	HET	HET	NI	HET
R12	Contralat invasive after 27 mon	LOH	HET	HET		MSI		LOH			NI	NI	NI	NI	LOH	HET	HET	MSI	NI	HET

Figure 3. Comparison of LOH pattern in DCIS tumours with corresponding ipsi- and contralateral new tumours (R2 to R16). In two of the patients the new events contained two components, *in situ* and invasive tumour components (R6 and R8 in the same patient as DCIS tumour IS28, and R10 and R12 in the patient with DCIS tumour IS58). Abbreviations as for Figure 1.

Table III. Comparison of LOH frequencies in DCIS tumours with new events with those without new events.

Sample type	3p	6q	8p	8q	9p	11p	11q	16q	17p	17q
DCIS without recurrence (n = 23)	7/21[1] (33%)	5/18[1] (28%)	1/10 (10%)	2/14 (14%)	3/15 (20%)	1/16[1] (6%)	6/18[1] (33%)	3/21[1] (14%)	0/8 (0%)	3/9 (33%)
DCIS with recurrence (n = 7)[2]	3/6 (50%)	3/5 (60%)	0/5 (0%)	1/5 (20%)	2/4 (50%)	0/5 (0%)	2/5 (40%)	2/7 (29%)	1/4 (25%)	0/4 (0%)

[1]Number of tumours showing LOH for at least one marker on the chromosome.
[2]New events occurred in 7 patients, out of which material for LOH analysis was available for 6. DCIS patient IS56 had an ipsilateral event, which was not analysed.

study were true recurrences or new cancers. Clinical data from the medical records did not specify whether the new events developed in the same quadrant as the primary DCIS. One of the new events that were analysed in the present study was ipsilateral *in situ* (R6) but the lesion did not show LOH for any marker. The matching primary DCIS (IS28) showed LOH only for marker D6S417, which could not be determined in R6. No similarities in the LOH pattern were detected between DCIS and their corresponding new events. These observations suggest that both the ipsilateral and contralateral new tumours in this study developed from different cell clones.

It was recently demonstrated that a higher proportion of LOH detected in benign breast epithelial cells correlated with an increased breast cancer risk [36]. The present LOH study, however, did not show that an overall increased frequency of LOH would indicate a higher risk of recurrences or new subsequent breast cancers, as allelic loss was more common in DCIS grade 1 than in DCIS grade 3. However, higher frequencies of LOH in DCIS grade 3 were found for chromosomes 6q, 11p, 17p, and 17q. Potential involvement in the progression to invasive cancer has been assigned loci on chromosomes 2p, 11p, and 17q [12]. Considering the limited number of chromosome arms analysed in the present study, we cannot rule out that additional loci might be of importance for the DCIS grade 3 tumours. Vos et al. tested 76 markers dispersed on all chromosome arms and detected more frequent LOH on chromosome 17 in 35 high-grade DCIS tumours (70%) than in 26 low-grade DCIS tumours (17%). The reverse was seen for chromosome 16q, where 66% LOH was found in low-grade DCIS and 39% in high-grade DCIS [14]. We analysed one marker on locus 17p12-11.2 and another on 17q22, showing LOH in 11% and 30% of the grade 3 DCIS tumours, respectively. None of the markers showed LOH for any of the DCIS grade 1 tumours. In agreement with our results, others have also reported more frequent LOH on chromosome 16q in low- and intermediate-grade DCIS and on 11p and 17p in high-grade DCIS [26,27]. Buerger et al. identified a higher frequency of LOH on 16q in low- and intermediate-grade DCIS but found a higher average number of genetic imbalances in high-grade DCIS [25]. More extensive LOH has been found for several chromosomes in pure DCIS compared with DCIS containing adjacent IDC [10,37]. A study of 9p LOH in breast cancer showed a significantly higher LOH level in DCIS tumours than in invasive breast carcinomas [9]. In the present study, 3 of 6 DCIS grade 1 lesions demonstrated LOH on chromosome 9p and only 2 of 13 grade 3 lesions.

All DCIS tumours in the present study were previously analysed as part of a study assessing the status of p53, c-erbB-2, Ki-67, ER, PR, Bcl-2, and angiogenesis in DCIS and invasive carcinomas (see Table II) [28]. These markers correlated with grade as the DCIS grade 3 tumours had a more malignant pattern for all of them. In contrast with this the quantity of LOH did not correlate with the clinically validated grading of aggressiveness, nor did we identify a specifically crucial locus for LOH. However, our study design did not allow analysis of markers on all chromosome arms owing to scarcity of tumour material, and the results would need to be confirmed in a larger dataset to further investigate the involvement of different loci in DCIS tumours of different grades.

In conclusion, DCIS grade 1 lesions contain a higher frequency of LOH for some chromosomes compared with DCIS grade 3 lesions. On the other hand, LOH on chromosomes 6q, 11p, 17p, and 17q, respectively, tended to be more frequently involved in DCIS grade 3 lesions. These chromosomes have also shown frequent LOH in invasive breast cancer, indicating these regions to be potentially involved in the progression from *in situ* to invasive breast cancer lesions. An emerging hypothesis is that a DCIS must have some crucial malignant genetic alteration—but at the same time not be too genetically unstable—to ‘survive’ to the state of invasiveness.

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