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Refined deletion mapping in sporadic breast cancer at chromosomal region 8p12-p21 and association with clinicopathological parameters

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Abstract

We have further refined the loss of heterozygosity (LOH) pattern on the human chromosomal region 8p12-p21 using 15 well characterised microsatellite markers in a panel of 50 breast carcinomas. The allelic loss pattern of these tumours suggests the presence of five commonly deleted regions on 8p12-p21. The most commonly deleted region was located between markers *D8S1734* and *D81989*, spanning a distance of approximately 3 cM and reaching 56% LOH at locus *NEFL*. LOH at 8p12-p21 was significantly correlated with large tumour size (T > 5 cm). Patients with the age at diagnosis of breast cancer between 45 and 55 years showed significantly more LOH than patients older than 55 years or younger than 45 years. No correlation was observed between 8p12-p21 alterations and histological tumour type, grade and the presence of lymph node metastases.

Keywords: Breast cancer; Chromosomal region 8p12-p21; Loss of heterozygosity; Clinicopathological parameters

1. Introduction

The short arm of chromosome 8 shows frequent loss of heterozygosity (LOH) in a variety of human malignancies including breast cancer [1–3].

We recently reported 8p LOH occurred at a high frequency within the 8p12-p21 region in a panel of familial (86% LOH) and sporadic (74% LOH) breast tumours [1]. The two most deleted regions were defined around marker *D8S133* and in a broader centromeric region bounded by markers *D8S137* and *D8S339*. Similar regions of loss have been described in recent studies [4–6], but also more complex patterns of LOH, including additional subregions, have been reported [2]. In addition, there are strong indications that the 8p12-p21 region may be involved in inherited breast cancer [4,5,7].

To date, no credible tumour suppressor candidates have been identified on the basis of 8p12-p21 allelic loss. One major reason may be the use of different, poorly characterised polymorphic markers in the respective studies.

Allelic loss on 8p12-p21 has been shown to be associated with invasive behaviour in breast cancer [6,8]. However, the results of such studies have been few and are rather contradictory.

The aim of this study was to define more precisely the location of a putative tumour suppressor gene(s) by LOH analysis of the 8p12-p21 region using a well characterised panel of polymorphic markers. In addition, we investigated whether particular 8p12-p21 regions of loss were associated with specific clinicopathological characteristics.

2. Patients and methods

2.1. Patient material

For LOH analysis, 50 tumour specimens and corresponding non-tumorous epithelial tissues were utilised

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from breast cancer patients who had undergone tumour-specific surgery between October 1996 and January 1998 at the Robert Roessle Hospital in Berlin (see Table 1).

DNA was extracted using standard phenol/chloroform methods.

The study was performed with the approval of the local ethics committee.

2.2. LOH analysis

The primers for the 15 polymorphic markers used in this study were synthesised according to published primer sequences (Genome Data Base, 1998). The order of the markers was derived from existing consensus maps (Cedar Genetics, 1999 *http://cedar.genetics.soton.ac.uk/pub/chrom8* and [2]. Polymerase chain reactions (PCRs) were carried out with 40 ng DNA in PCR buffer (Perkin Elmer, Foster City, CA, USA), 0.25 μM of each primer (one primer of each pair was fluorescence-labelled), 375 μM of dNTPs and 0.25 units Taq-polymerase (Perkin-Elmer, Foster City, CA, USA). After 5 min denaturation, 30–35 cycles of amplification were carried out using the following cycling parameters: 30 s at 94°C, 30 s at the annealing temperature (between 52°C and 65°C) and 30 s or 1 min at 72°C, with a final extension step of 10 min at 72°C. The fluorescent PCR products were mixed with an internal standard size marker and fractionated on a denaturating 6% polyacrylamide gel using an ABI 373A or ABI 377 DNA sequencer (Fig. 1). LOH data were analysed automatically by comparing non-tumorous and tumour tissue allele peak sizes, heights and area ratios. Intensity or signal ratio differences of 35% or more were considered sufficient for LOH assignment.

2.3. Statistical analysis

All statistical analyses were done using the statistical program SPSS. For the evaluation of associations between LOH and clinicopathological variables in the case of categorical variables the Fisher's exact test (Crosstabs statistics) and in the case of non-categorical variables the Mann–Whitney test or the H-test of Kruskal and Wallis were used. Survival curves were calculated by Kaplan–Meier estimations and compared according to the log rank test [9].

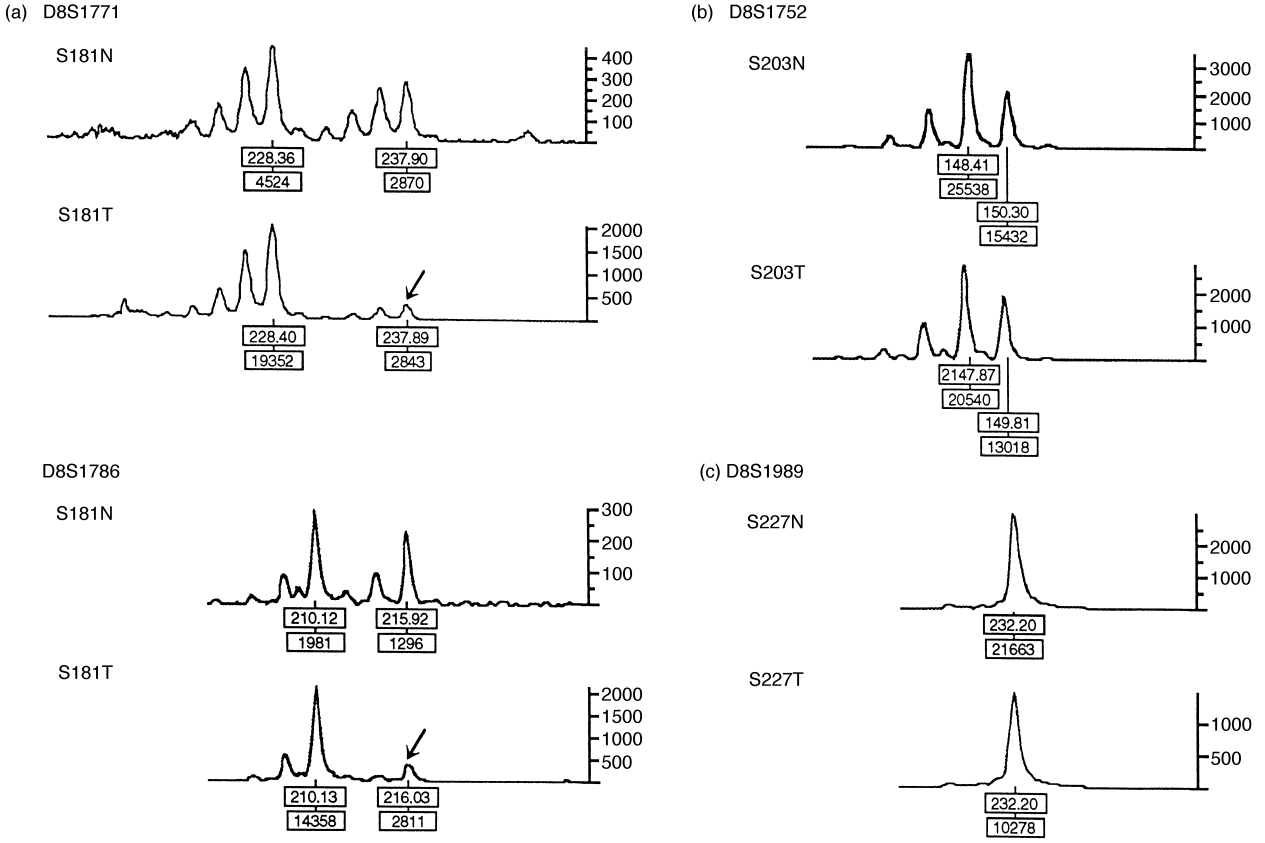

Fig. 1. Representative examples of: (a) loss of heterozygosity (LOH); (b) retention of heterozygosity; and (c) homozygosity at different loci at chromosomal region 8p12-p21 in sporadic breast cancer are shown for paired non-tumorous (N) and tumour (T) specimens. Arrows indicate the alleles showing loss. On top of the peaks the marker names and tumour numbers are indicated. The numbers beneath each peak give the allele fragment length and peak area.

3. Results

3.1. Loss of heterozygosity in the 8p12-p21 region

DNA isolated from 50 paired breast cancer and adjacent non-tumorous breast tissues were analysed for LOH using 15 microsatellite markers which had been oriented along the chromosomal region 8p12-p21 and with an average marker distance of 0.8 cM. 29 of 50 tumours (58%) had lost at least one locus of 8p. Frequencies of LOH in this series of tumours varied from 17% (*D8S298*) to 56% (NEFL) (Figs. 2 and 3). We have found deletions of all sizes, ranging from single loci to extensive deletions, including most of the chromosomal region (Fig. 2). Generally, tumours could be classified into three groups. The first group of tumours (Group A) showed deletions at only one or two (at the adjacent marker) loci and retention of heterozygosity at others. The second group of tumours (Group B) showed more complex patterns of loss and multiple alternating regions of deletions and retention were observed in single tumours. Remarkably, many of the deletions at several markers fell within the subregions of loss defined by group A tumours.

A third group of tumours (five tumours) showed loss of large parts or the whole 8p region investigated. Their pattern could not be used to define subregions of loss. These tumours were not shown in Fig. 2.

From the deletion patterns, five subregions of minimal loss were identified. The most conspicuous region (subregion II) is located between markers *D8S1734* and *D8S1989*, reaching 56% LOH at locus *NEFL*. This region was defined by ten tumours (Group A: S196, S205, S207, S202, S200, S184, S183A and Group B: S182, S194, S217), spanning a distance of approximately 3 cM. Most of the Group B tumours showed extensive deletions within this subregion. In the majority of tumours, centromeric to subregion II, a broad region of allelic loss was identified (locus *D8S1739–D8S339*), reaching 43% LOH at marker *D8S1739*. However, specific LOH patterns allowed three subregions to be defined within this chromosomal area: subregion III

Table 1
Clinical and histopathological features

Characteristics	*n* of cases (%)
Tumour size (cm)	
< 2 (pT1)	15 (30)
2–5 (pT2)	20 (40)
> 5 (pT3/pT4)	14 (8/6) (28)
Unknown	1 (2)
Histological type	
Intraductal	2 (4)
Invasive ductal with a predominant intraductal component	2 (4)
Invasive ductal	26 (52)
Invasive lobular	15 (30)
Medullary	1 (2)
Papillary	1 (2)
Unknown	3 (6)
Grade	
Well differentiated (G1)	3 (6)
Moderately differentiated (G2)	22 (44)
Poorly/not differentiated (G3/G4)	23 (22/1) (46)
Unknown	2 (4)
Lymph node metastases	
Node-positive	21 (42)
Node-negative	16 (32)
Unknown	13 (26)
Age at diagnosis (years)	
< 45	8 (16)
45–55	13 (26)
> 55	28 (56)
Unknown	1 (2)

Table 2
Statistically significant associations between clinicopathological parameters and loss of heterozygosity (LOH) at a particular chromosome 8p12-p21 locus

Clinicopathological parameters	Tumours with loss/no loss	% LOH	*P* value
Age at diagnosis (years)			
D8S1786			0.018
< 45	1/5	17	
45–55	6/5	55	
> 55	6/17	26	
D8S1771			0.027
< 45	1/5	17	
45–55	4/3	57	
> 55	5/16	24	
D8S1839			0.012
< 45	2/5	29	
45–55	4/3	57	
> 55	3/18	14	
Tumour size (cm)			0.024
D8S136			
< 2	1/12	8	
2–5	3/11	21	
> 5	4/6	40	
D8S298			0.015
< 2	0/12	0	
2–5	2/11	15	
> 5	3/7	30	
D8S1786			0.017
< 2	1/12	8	
2–5	5/10	33	
> 5	7/5	58	
D8S137			0.012
< 2	1/7	13	
2–5	1/11	8	
> 5	5/5	50	
D8S339			0.037
< 2	2/9	18	
2–5	4/6	40	
> 5	3/2	60	

around marker *D8S1839* and defined by tumours S213 (Group A), S182 and S221 (Group B); subregion IV at marker *D8S137* and defined by the tumour S191 (Group A); subregion V around marker *D8S339* and defined by two Group B tumours (S221, S198). The most telomeric region (subregion I) around marker *D8S1786* was defined by tumours S227 and S193 (Group A) and S182 and S221 (Group B).

3.2. Correlation of allelic loss at 8p12-p21 with clinical and pathological features

We looked for associations between LOH at specific loci and particular clinicopathological features, including tumour size, histological type, grade, node status and age at diagnosis. Statistically significant associations were observed and are summarised in Table 2. Tumours with larger size (T > 5 cm) revealed significantly more LOH with five markers: *D8S136* ($P = 0.024$), *D8S298* ($P = 0.015$), *D8S1786* ($P = 0.017$), *D8S137* ($P = 0.012$) and *D8S339* ($P = 0.037$). Significantly more LOH was identified in tumours from patients at the age between 45 and 55 years with the markers *D8S1786* ($P = 0.018$), *D8S1771* ($P = 0.027$) and *D8S1839* ($P = 0.012$) (Table 2, Fig. 4). Histological tumour type, grade and the presence of lymph node metastases were not correlated to LOH with any marker used in this study.

4. Discussion

Previous allelotyping studies have shown that allelic losses on the short arm of chromosome 8 are frequently associated with a variety of tumours, including breast cancer, supporting the hypothesis that one or several putative tumour suppressor genes may play a role in breast carcinogenesis. However, their precise location has proved to be difficult. One major reason is that different and often poorly characterised polymorphic markers have been used in most of these studies.

In this study, we have attempted to define more precisely the LOH pattern of the 8p12-p21 region using 15 well characterised microsatellite markers in a panel of 50 breast tumours.

In total, 58% of informative cases showed LOH with at least one marker. The results of our study suggest that there are five commonly deleted regions on 8p12-p21. The chromosomal segment, defined in our LOH analysis as subregion II proved to be the most commonly deleted region. Subregions II–IV may correspond to the region(s), defined by us and others in sporadic

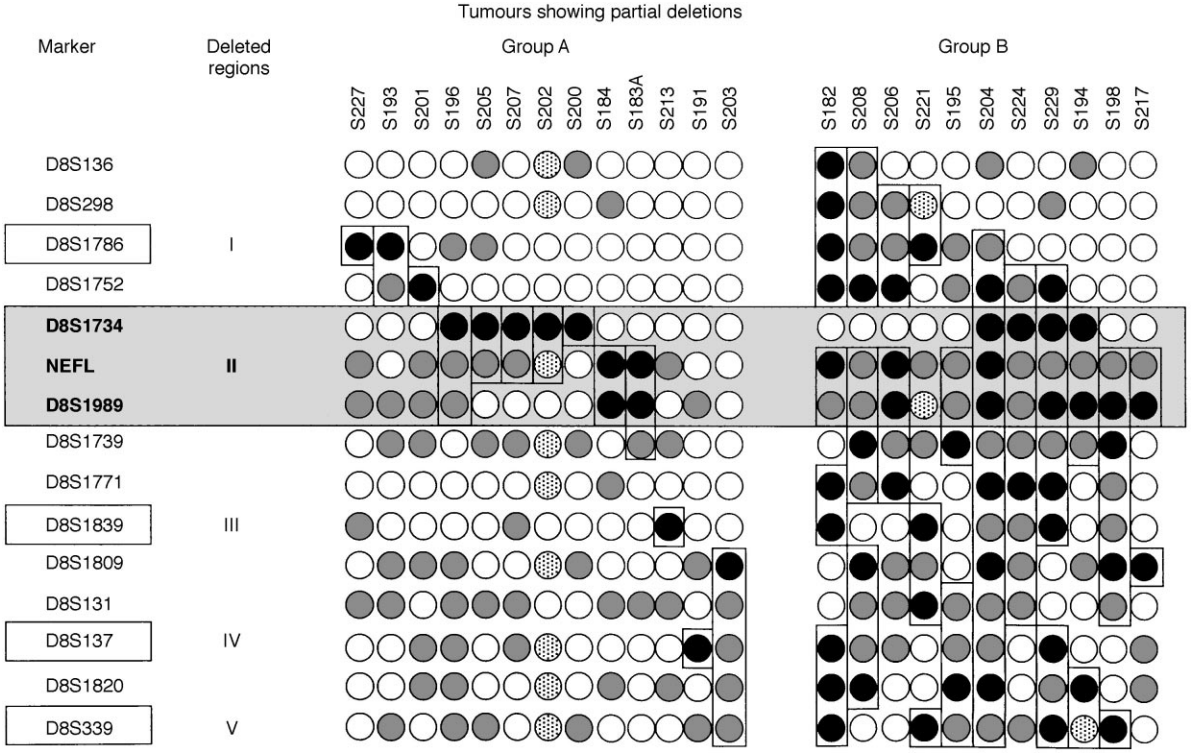

Fig. 2. Schematic representation of independently deleted regions (regions I–V) in 24 breast tumours showing partial deletions at 8p12-p21. On the left microsatellite markers are listed from telomere to centromere. Numbers at the top identify the tumours. The most prominent loss of heterozygosity (LOH) region (region II) is outlined by a grey box. Closed circles denote LOH; open circles, retention of both alleles in the tumour; grey circles, homozygous loci; and open circles with black points represent untested tumours. Group A tumours are characterised by single loci deletions, group B tumours by extensive and/or alternating deletions.

and familial breast cancer as harbouring a putative third breast cancer susceptibility gene [1,4,5,7].

However, our data presented here strongly suggest that the critical region for such a gene is more likely to be located between markers *D8S1734* and *D8S1989* (subregion II).

In recent studies, amplification of genes from the 8p11.1-p12 region was found in approximately 10–15% of human breast carcinomas [2,10]. The amplicon was localised between markers *D8S105* and *POLB*, approximately 3 cM distant from the most centromeric LOH subregion V, defined in our study. Although we cannot completely exclude that allelic imbalance may in some cases be due to gene amplification, recent mapping results and molecular alterations in breast cancer suggest that this might be a rather rare event in the region of investigation.

Allelic loss on 8p has been associated with advanced clinical state and poor patient prognosis in a variety of tumours such as prostate [11,12], lung [13], colon [14] and hepatocellular [15] carcinoma. To evaluate the contribution of distinct 8p12-p21 deleted regions to the development and/or progression of breast cancer, we compared clinicopathological characteristics of patients with LOH at a particular locus with those patients which were informative but showed no LOH. Recent studies have indicated that allelic loss on 8p12-p21 might be associated with invasive behaviour in breast cancer. Yaremko and colleagues [16] refine this impression and suggest that 8p LOH could play a role in breast cancer development at the stage where tumours progress from clinical stage TIS to T1 and higher. We found 8p deletions at a particular 8p12-p21 locus were associated with a more advanced tumour phenotype. Specifically these deletions were significantly associated with invasive T3–T4 tumours at *D8S136* ($P=0.024$), *D8S298* ($P=0.015$), *D8S1786* ($P=0.017$), *D8S137* ($P=0.012$) and *D8S339* ($P=0.037$) (Table 2). This was in agreement with a recent report by Anbazhagan and colleagues [8] and we interpret these data as suggesting that 8p LOH is not the first event in breast cancer development and that several 8p genes might be involved in these processes. In this respect, putative candidate genes such as the *FEZ1* gene, encoding a leucine–zipper protein, the Frizzle-related *FRP1/FRZB* gene and the death receptor gene *DR5*, a potential mediator in p53-dependent apoptosis which were mapped within the 8p12-p22 region are of interest [17–19].

To date, studies with comparable findings to those outlined in this study have been few and rather contradictory. In consequence, these findings clearly warrant further study, particularly the significant association

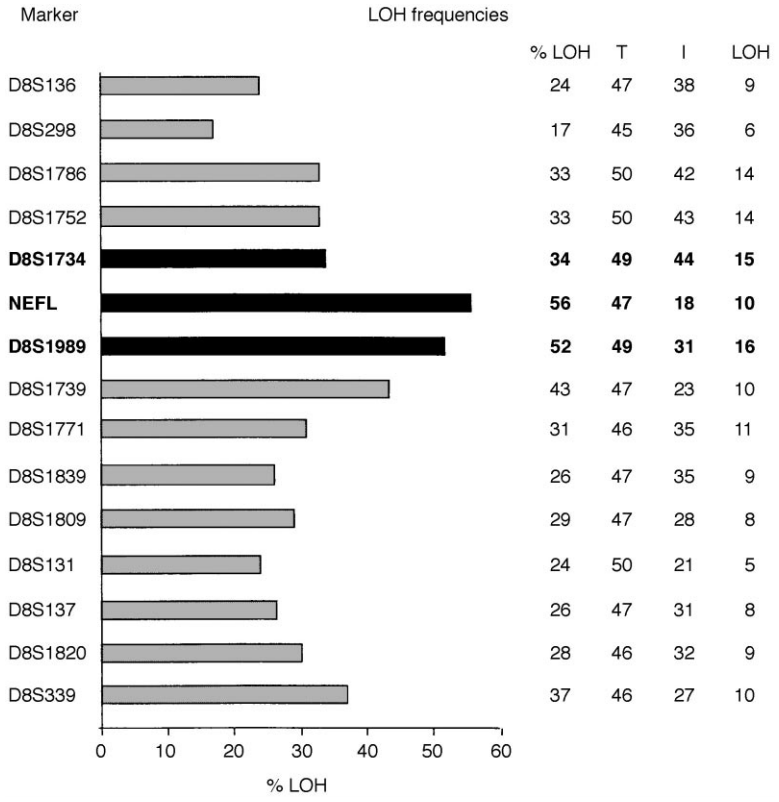

Fig. 3. Schematic representation of loss of heterozygosity (LOH) frequencies in sporadic breast tumours at chromosomal region 8p12-p21. On the left, microsatellite markers used and their relative order are listed. The bars indicate the LOH frequencies (% LOH). From right to left are the LOH frequencies (% LOH), the number of tumours tested (T), the number of informative tumours (I) and the number of tumours showing LOH (LOH) are documented. The most prominent region around *NEFL* is bolded.

between age 45–55 years at diagnosis and loss of 8p. Interestingly, 56% of the tumours lost at least one of the three markers that showed significant LOH associated with patients aged 45–55 years but only 29% of the tumours from patients aged older than 55 years were tumours with a larger tumour size (>5 cm). These data could indicate an overlap between tumour size and age at diagnosis and/or that tumorigenesis in a subset of patients may arise via another pathway. No significant correlation was observed between 8p12-p21 alterations and the other clinicopathological characteristics.

In conclusion, we have narrowed down five relevant regions on chromosome 8p12-p21 where LOH is observed in breast cancer tumour tissues. Subregion II between markers *D8S1734* and *D8S1989* may be the main region harbouring a putative tumour suppressor gene.

Our results further indicate that allelic loss is associated with different subsets of tumours, with age at diagnosis and tumour size being significant factors.

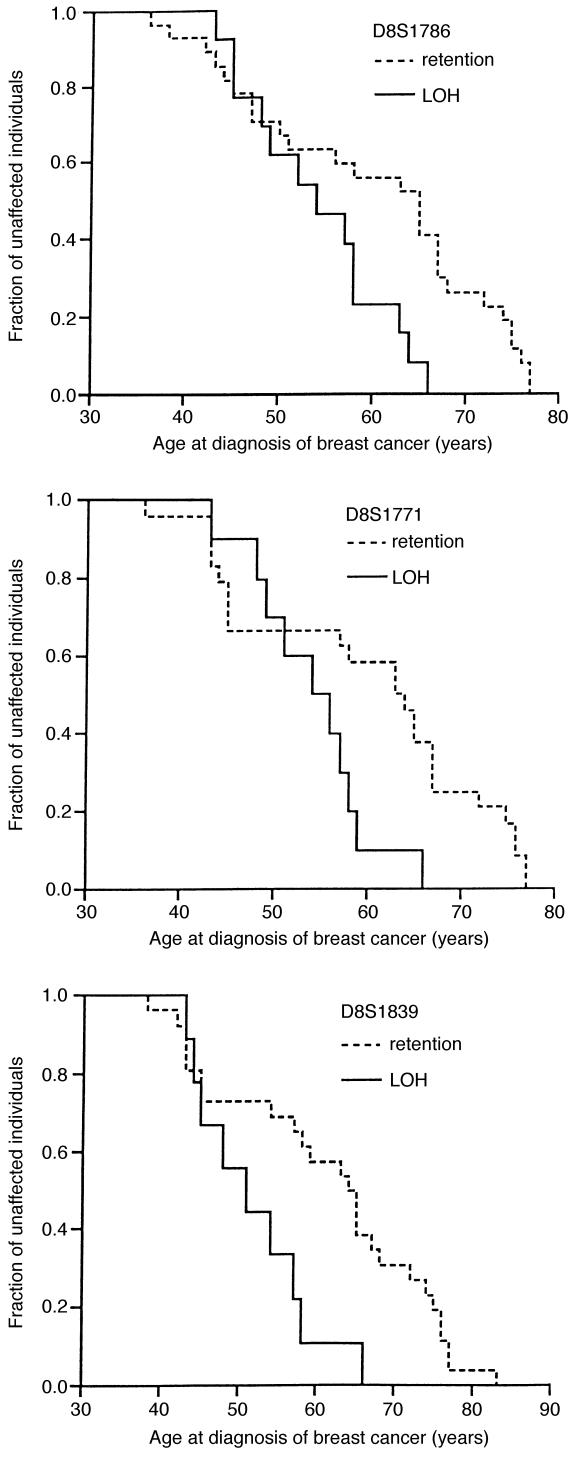

Fig. 4. Age at diagnosis of breast cancer (Kaplan–Meier plot) in patients without LOH (----) and with LOH (—) at microsatellite loci *D8S1786, D8S1771* and *D8S1839*.

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