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Chromosome 11q in sporadic colorectal carcinoma: patterns of allele loss and their significance for tumorigenesis

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Abstract

***Aims*—To analyse the frequency of loss of heterozygosity (allele loss, LOH) in a large sample of colorectal carcinomas using highly informative markers along chromosome 11q.**

***Methods*—One hundred paired samples of colorectal cancer and normal tissue were genotyped at six microsatellite markers on chromosome 11q (cen-D11S1313-D11S901-DRD2/NCAM-D11S29-D11S968-tel). The high levels of heterozygosity at these markers allow allele loss to be determined in about 80% of cases at any one locus. The frequency of replication errors (RERs, microsatellite instability) has also been determined.**

***Results*—LOH was found at frequencies of 25% and 29% at the distal D11S968 (11qter) and D11S29 (11q23.3) loci, slightly above the accepted baseline of 0–20%. Allele loss at NCAM, DRD2, D11S901, and D11S1313 was not raised above baseline levels. The probable genetic mechanism of allele loss—chromosomal non-disjunction, mitotic recombination, deletion, or gene conversion—seemed to vary between tumours and no consistent mechanism of mutation was found. Microsatellite instability was found in 23 (23%) tumours. No associations were found between LOH and clinical data (patient sex, age at presentation, tumour site, and Dukes' stage).**

***Conclusions*—Although gene(s) on 11q may have a role in the development of a minority of colorectal carcinomas, this study provides evidence against the general importance of allele loss on chromosome 11q in the pathogenesis of colorectal cancer. The results also have implications for the importance of 11q in other cancers: it seems less likely that a single tumour supressor gene at this location promotes the growth of all types of tumour when lost. Rather, one or more genes with tissue specific effects may be involved.**

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There are well defined chromosomal regions and genetic loci that undergo frequent loss of heterozygosity (LOH, allele loss) in colorectal cancer. The DCC locus on chromosome 18q,[1] the APC locus (5q),[2] the RB1 locus (13q),[3] the TP53 locus (17p),[4] and chromosome 8p[5] are the best known examples. Frequent LOH has, however, been reported at other loci in sporadic colorectal cancer, suggesting that colorectal tumorigenesis is more complex than the basic model of Fearon and Vogelstein.[6] One region where frequent LOH has been suggested using cytogenetic methods is chromosome 11q.[7–9]

Chromosome 11q, particularly distal to band q22, is an interesting genetic region in many cancers. Many haematological malignancies show chromosomal rearrangements at this site.[10] In addition, allele loss occurs at moderate-to-high frequencies distal to 11q22 in breast cancers,[11–14] cervical cancers,[15 16] ovarian tumours,[17] and melanomas.[18] It is not known whether the same loci are involved in all these tumours, but the possibility exists that there is a locus between 11q22-qter that provides a selective advantage when "lost" in many types of tumour.

Therefore, there are two reasons for studying LOH on the long arm of chromosome 11 in colorectal cancer: first, to determine whether this region really is important in colorectal cancer using a large sample of tumours and several marker loci; and second, to provide data to determine whether allele loss on 11q is a general phenomenon in cancers, or specific to certain tumour types.

LOH has been searched for in 100 cases of sporadic colorectal carcinoma using six microsatellite markers on chromosome 11q (table 1, fig 1). These were chosen to extend from the centromere to the telomere (cen-D11S1313-D11S901-DRD2/NCAM-D11S29-D11S968-tel). The DRD2 (dopamine receptor D2) and NCAM (neural cell adhesion molecule) loci were both chosen despite their close map positions, in order to provide comparisons with previous studies, in order to increase the number of informative (heterozygous) cases at 11q22 and to test NCAM directly, as it is a candidate locus in colorectal tumorigenesis.[19] The allele loss data can be used to suggest how often LOH occurs by chromosomal non-disjunction and by other mechanisms (mitotic recombination, deletion and gene conversion) in the colorectal tumours studied. Using these six dinucleotide repeat loci, it was also possible to determine the frequency of replication errors (RERs, microsatellite instability) in the tumours studied. The following clinical data

Table 1 The loci studied

Name	*Map position*	*Repeat unit*	*Size of alleles (bp)*	*Heterozygosity*
D11S1313	11cen	CA	184–204	0·85
D11S901	11q	CA	160–176	0·82
DRD2	11q22–q23	CA	80–86	0·62
NCAM	11q22–q23	CA	101–119	0·79
D11S29	11q23·3	CA	143–163	0·77
D11S968	11qter	CA	137–155	0·81

Table 2 Frequency of allele loss at each of the loci studied

Locus	*No. of informative cases*	*No. of cases with LOH (%)*
D11S1313	70	11 (16%)
D11S901	82	11 (13%)
DRD2	61	11 (18%)
NCAM	70	10 (14%)
D11S29	85	25 (29%)
D11S968	59	15 (25%)

were also known: patient sex, age at presentation, tumour site, and Dukes' stage. Using standard statistical techniques, associations were searched for between the clinical data and allele loss.

Methods

Paired samples of genomic DNA from peripheral blood or normal tissue and from carcinomas were extracted using standard methods. All carcinomas consisted entirely of tumour tissue at the macroscopic level, although tumours were not microdisssected. PCR reactions were performed at each microsatellite ((CA)n repeat) locus on each pair of tumour/normal DNAs, using the following conditions: 50–250 ng DNA was used as template in a final volume of 50 µl with reaction concentrations of 1 × standard PCR buffer (Promega, Madison, Wisconsin, USA), 1·5 mM Mg^{2+}, 0·5 mM dNTPs, and 0·4 mM of each specific oligonucleotide primer. One unit Taq polymerase was added per reaction. For D11S968, D11S901 and D11S1313, amplification was carried out using a protocol of one cycle of 94°C (one minute), 35 cycles of 94°C (one minute)/55°C (one minute), and one cycle of 72°C (five minutes).[20] For D11S29, amplification was carried out using a protocol of one cycle of 94°C (one minute), 30 cycles of 94°C (one minute)/55°C (one minute)/72°C (one minute), and one cycle of 72°C (10 minutes).[21] For NCAM, amplification was carried out using a protocol of one cycle of 94°C (one minute), 30 cycles of 94°C (one minute)/50°C (one minute)/72°C (one minute), and one cycle of 72°C (five minutes).[22] For DRD2, amplification was carried out using a protocol of one cycle of 94°C (one minute), 30 cycles 94°C (30 seconds)/58°C (30 seconds)/74°C (30 seconds), and one cycle of 74°C (five minutes).[23] PCR products were heated to 90°C for five minutes and electrophoresed on a 6% acrylamide sequencing gel (Sequagel) under denaturing conditions for two to four hours. DNA was transferred by blotting onto Hybond N+ membranes (Amersham, Little Chalfont, UK) for four to 16 hours. PCR products were detected by the enhanced chemiluminescence technique (Amersham), using a randomly elongated oligonucleotide primer as a specific probe for each locus. Products were visualised by exposing membranes to Hyperfilm (Amersham) for one minute to one hour. In informative cases, allele loss was scored by eye. In this way, only unequivocal cases were scored as loss. Extra bands in tumour samples that differed by multiples of 2 base pairs (bp) from their normal counterpart were scored as RERs (replication errors). Loci at which RERs were found were removed from the LOH analysis and classed as not informative. Other loci from the same tumour were included in the LOH analysis. Clinical data were determined from patients' notes, histopathology reports and laboratory records. χ^2 and *t* tests were used to determine the statistical significance of any associations between the molecular data and clinical variables.

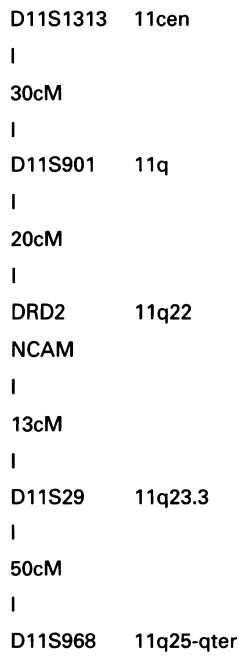

Figure 1 Approximate genetic map of the loci studied. The order of DRD2 and NCAM is still uncertain.

Results

LOH was found at frequencies of 25% and 29% at the distal D11S968 (11qter) and D11S29 (11q23·3) loci, respectively (table 2). This is only a slightly elevated frequency of LOH over the generally accepted baseline (0–20%). Lower frequencies of allele loss were observed at NCAM, DRD2, D11S901, and D11S1313 (table 2). Representative cases of LOH are shown in fig 2.

The patterns of LOH at each of the six loci are shown in fig 3 for all cases which had lost an allele at one of the loci studied. Of the 100 cases studied, 36 showed allele loss at one or more loci. The genetic mechanism of allele loss—chromosomal non-disjunction, mitotic recombination, deletion, or gene conversion—seems to vary between tumours. Figure 3 gives what we suggest to be the most probable mechanism underlying the observed mutations. Six cases showed probable chromosomal non-disjunction, because the pattern of LOH observed was consistent with loss of the entire chromosome 11. Seventeen tumours showed terminal deletion or mitotic recombination (extending a variable distance from the telomere along the long arm of the chromosome) and two cases were constitutional homozygotes at D11S1313 and hence consistent with both non-disjunction and terminal deletion or mitotic recombination. The remaining 11 cases showed LOH at proximal loci with retention of distal markers (interstitial allele loss). In two of these 11 cases two separate regions of allele loss could be identified on the chromosome arm.

Microsatellite instability was found in 23 (23%) tumours. Examples are shown in fig 2. Of these 23 cases, 16 showed novel alleles at only one of the six loci, three showed instability at two loci and four were unstable at three loci. These figures are consistent with other studies in which 7–30% of sporadic colorectal cancers show microsatellite instability.[24–28] Cases with microsatellite instability tended to have a low frequency of allele loss (details not shown).

Patients presented at a mean (SD) age of 65·8 (11·3) years. The female:male ratio was 1·2:1. Of the cancers, 80% were left-sided, 10% were right-sided and 10% were of unknown site in the colon. Twelve per cent of patients presented as Dukes' stage A, 45% were stage B and 43% were stage C. None of the clinical data showed a significant association (χ^2 and *t* tests, one-tailed, $p>0·05$) with LOH at any of the loci studied, or with the imputed mechanism of LOH (non-disjunction or mutations affecting only part of the chromosome arm).

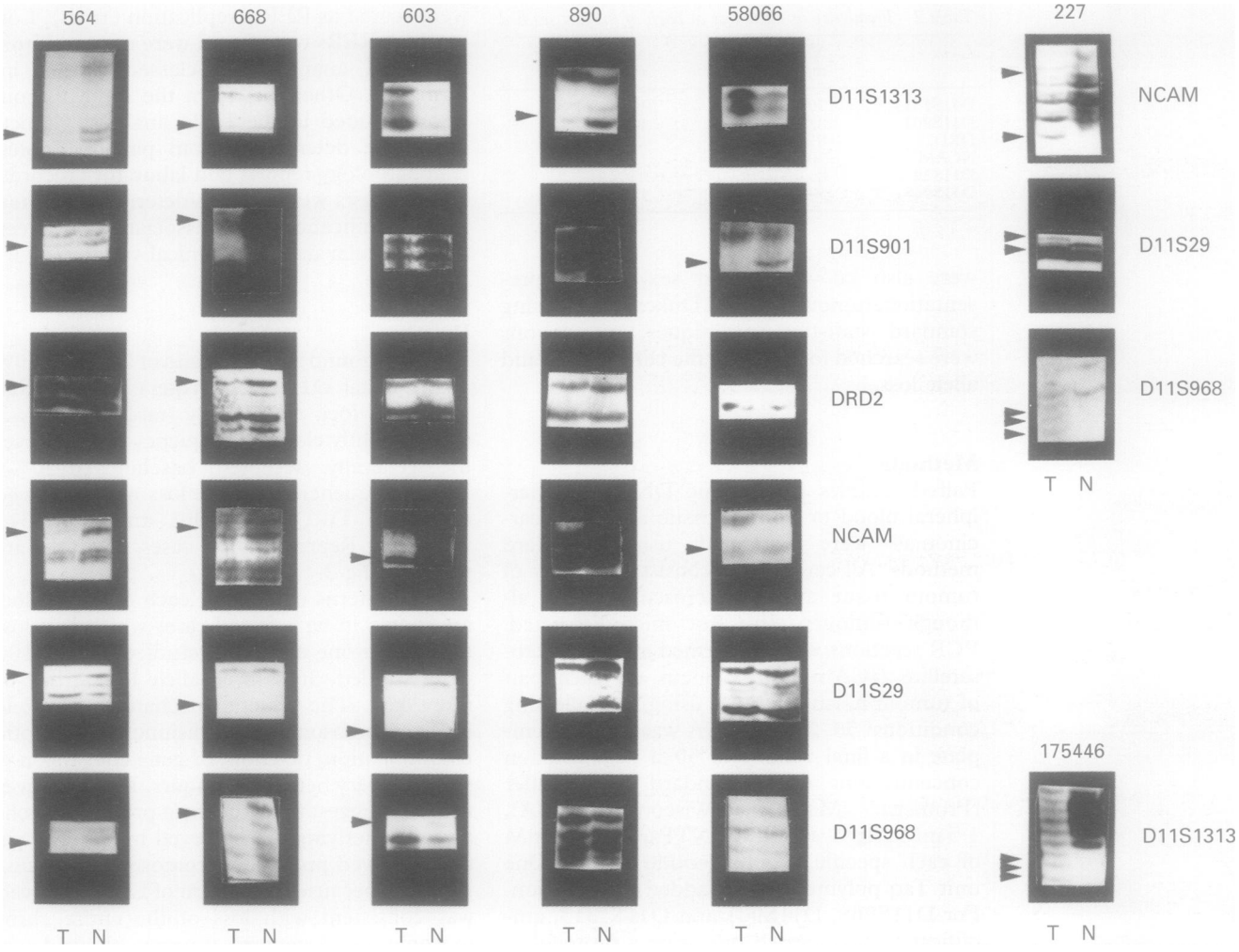

Figure 2 Examples of allele loss and microsatellite instability. Tumour(T)/normal(N) pairs are shown. Arrows denote allele loss or, in cases 227 and 175446, extra allelic bands indicative of microsatellite instability. Cases 564 and 668 show probable non-disjunction in the tumour and case 603 is a case of probable terminal deletion or mitotic recombination distal to DRD2. Case 890 shows two regions of interstitial LOH and case 58 066 shows a probable single region of interstitial LOH (although the patient is non-informative at DRD2). Case 227 shows extra bands indicative of microsatellite instability at three of the six loci, with classic allelic ladders differing by 2 bp at NCAM and D11S968 and a somewhat atypical pattern at D11S29. Case 175446 shows the classic allelic ladder of microsatellite instability at just one of the six loci (D11S1313).

For the sake of brevity, details of the statistical analyses are not shown. Given a study of LOH at six loci and two possible mechanisms underlying that LOH, together with four clinical variables (patient sex, age at presentation, tumour site (left- or right-sided) and Dukes' stage), it would actually be expected that one or two associations would be significant at the 5% level of probability by chance alone.

Discussion

This study provides evidence against the hypothesised importance of allele loss on chromosome 11q in the pathogenesis of colorectal cancers in general. LOH at the NCAM, DRD2, D11S901, and D11S1313 loci occurred at frequencies within the generally accepted background range of 0–20%. LOH at the D11S29 and D11S968 loci was detected at frequencies slightly above background and it remains possible that a gene near these loci is important in a minority of colorectal tumours. These data do not, however, support the findings of Keldysh *et al*[9] that allele loss on chromosome 11 occurs in over half of all colorectal carcinomas. In comparison with the data of Gustafson *et al*[29] published in the interim and which analysed only the DRD2 locus, this study found consistent results, but a lower frequency of LOH (table 3).

It is possible that differences in experimental method can account for the differences between the results of Keldysh *et al*[9] and the results presented here. Keldysh *et al* initially studied tumours by cytogenetic means, which may be prone to different types and frequencies of error than studies at the DNA level. Keldysh *et al* then used restriction fragment length polymorphism (RFLP) analysis to detect LOH, rather than the simple sequence length polymorphism (SSLP) analysis with microsatellite markers used here. It is possible that erroneously low frequencies of LOH may have been detected in this study owing to con-

Table 3 Comparison of LOH at the DRD2 locus in the studies of Gustafson et al[29] *and the present study*

Study	*LOH*	*No LOH*	*Total*
Gustafson *et al*	23	45	68
The present study	13	48	61
Total	36	93	129

Association $\chi^2_1 = 2{\cdot}5$ (NS).

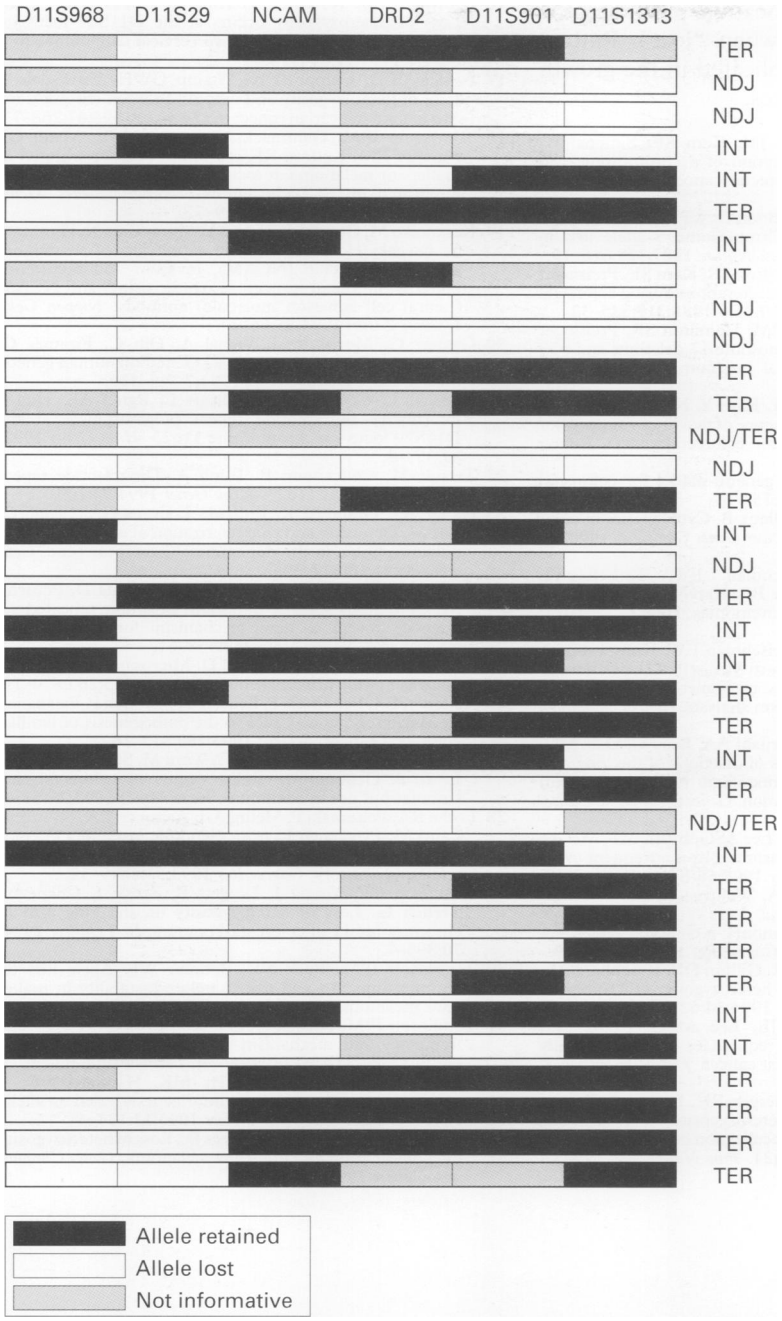

Figure 3 The patterns of allele loss on chromosome 11q. The annotation denotes the suggested cause of the observed data. NDJ = chromosomal non-disjunction; TER = terminal deletion or mitotic recombination; and INT = interstitial allele loss caused by gene conversion, deletion or mitotic recombination. Clearly, the hypothesised mechanisms of LOH are not the only ones that can explain the data. Those presented are generally the simplest mutations that can produce the observed patterns of allele loss in the smallest number of steps.

tamination of tumours with normal tissue and the scoring of mutations by eye. However, the consistency of the results reported here with those of Gustafson *et al*[29] (who also used SSLP analysis) suggests that such errors were rare. We suggest that the most important difference between this study and that of Keldysh *et al*[9] may have been between the use of SSLP analysis and RFLP analysis, respectively. The latter method uses markers that sometimes have levels of informativeness approaching the 0·80 typical of microsatellites, but frequently have much lower heterozygosity. At D11S29—for example, the RFLP based marker has heterozygosity of about 0·30, but the SSLP marker's heterozygosity is about 0·75. Recently, dense genetic maps of microsatellite markers have been made available by organisations such as Genethon.[20] This has allowed allele loss mapping to become much more efficient, both in utilising information from most cancers sampled and in delimiting the extent of LOH. In general, SSLP analysis produces a lower frequency of LOH than comparable RFLP studies (for example, Tomlinson *et al*[14] compared with Hampton *et al*[12]). This difference probably results from the larger effective sample size caused by the use of highly polymorphic SSLP markers and publication bias towards positive results. We believe this to be the likely cause of differences between our results and those of Keldysh *et al*[9]: suggestions—for example, that PCR based techniques may obscure allele loss are unlikely to be true, as amplification techniques such as PCR should actually exaggerate differences in allele dosage.

No consistent mechanism of allele loss can account for the observed data (fig 3), hence the finding that 36% of all tumours had lost an allele at one of the six loci, whereas the maximum frequency of allele loss at an individual locus was 29%. This is similar to LOH on 11q in melanoma (which occurs at a somewhat higher frequency),[30] but contrasts with allele loss on 11q in breast cancer,[31] which can mostly be accounted for either by chromosomal non-disjunction or by mutations involving the whole of the chromosome arm distal to band q22. The frequency of any mutation in a set of tumours reflects the mutation rate and its selective advantage. As there is no reason to suppose that one type of LOH causing mutation occurs more frequently in breast cancers than in colorectal cancer, the results from breast cancer suggest a particular selective advantage to the specific types of 11q mutation that occur in those tumours. In the colorectal cancers, by contrast, the existence of varied types of allele loss suggests no such selective advantage and provides evidence against an important role for 11q in colorectal tumorigenesis. In breast cancers[14] and melanoma,[32 33] moreover, there are data that suggest that 11q alleles are lost relatively late in tumour development. The absence of associations in this study between allele loss at any of the loci studied and the clinical data, in particular Dukes' stage, also provides evidence against an influential tumour suppressor gene on 11q in colorectal cancers.

In conclusion, there is little evidence that there are loci on chromosome 11q that are important in the pathogenesis of colorectal cancer. It remains possible, however, that allele loss towards the telomere of the long arm inactivates a tumour suppressor gene in a minority of tumours. If this is actually the case, adhesion loci such as NCAM and genes involved in maintaining genomic stability (such as *ataxia telangiectasia*) are candidate targets for 11q LOH. As well as some significance for colorectal neoplasms, however, these results have implications for the importance of chromosome 11q in other cancers. If colorectal cancers do not frequently lose alleles on 11q, it becomes less probable that a single tumour

supressor gene at this location promotes the growth of all tumours when "lost". Rather, different loci may be implicated in the growth of cancers of different sites.

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