Chromosome 11 allele imbalance and clinicopathological correlates in ovarian tumours

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Summary Allele imbalance on chromosome 11 loci in ovarian cancer is a frequent event, suggesting the presence of tumour-suppressor genes for ovarian carcinogenesis on this chromosome. Ten highly polymorphic (CA) repeat microsatellites were used to determine allele imbalance in 60 primary ovarian tumours, including 47 epithelial ovarian cancers (EOCs). Forty EOCs (85%) showed allele imbalance at one or more loci, and in 39 of these (83%) the data suggested subchromosomal deletions: eight of 11p only; six of 11q only; and 25 of both 11p and 11q. Three consensus regions of deletion were indicated at 11p15.5–p15.3, 11q12–q22 and 11q23.3–q24.1. Allele imbalance at the 11q subtelomeric region (D11S912) correlated significantly with adverse survival, while imbalance at 11q14.3 and retention of heterozygosity at 11q22 (close to the site of the progesterone receptor gene) were associated with favourable clinicopathological features. The findings allow development of a preliminary model for the molecular evolution of epithelial ovarian cancer.

Keywords: chromosome 11; tumour-suppressor genes; ovarian cancer; loss of heterozygosity

Epithelial ovarian cancer is the main cause of death from gynaecological cancer in British women, primarily because of its late presentation, which carries a poor prognosis despite available treatment modalities. Interest is focusing on the molecular basis of ovarian cancer in order to uncover new and hopefully effective management strategies.

Strong circumstantial evidence for the location of tumour-suppressor genes (TSGs) can be obtained by observed allele imbalance (loss of heterozygosity, LOH) in tumour DNA compared with a matched constitutional DNA specimen at defined chromosomal loci (Ponder, 1988). Minimum consensus regions of allele imbalance may lead to isolation and cloning of these 'deleted' genes, and LOH studies in many tumour types have suggested putative TSGs located on chromosome 11.

In ovarian cancer, cytogenetic analysis has demonstrated partial deletions of chromosome 11 affecting both the long and short arms (Bello and Rey 1990; Pejovic et al., 1992; Jenkins et al., 1993). Frequent LOH has been demonstrated at 11p15 (Lee et al., 1989; Ehlen and Dubeau, 1990; Eccles et al., 1992a; Gallion et al., 1992; Vandamme et al., 1992; Viel et al., 1992; Kiechleschwarz et al., 1993), although not all studies have confirmed this high level of loss (Sato et al., 1991; Zheng et al., 1991; Yangfeng et al., 1992). A proximal locus at 11p13 (site of WT1) exhibits lower rates of LOH in ovarian cancer (Call et al., 1990; Vandamme et al., 1992; Viel et al., 1992; Bruening et al., 1993). A minority of these studies have proposed a correlation of 11p LOH with poorly differentiated (Zheng et al., 1991; Kiechleschwarz et al., 1993) and more advanced (Viel et al., 1992) tumours. Molecular studies of the proximal 11q region have shown low rates of both LOH and amplification of the 11q13 amplicon in ovarian cancer (Lee et al., 1989; Li et al., 1991; Sato et al., 1991; Viel et al., 1992; Foulkes et al., 1993). In contrast, the only study that has looked specifically at the subtelomeric region of 11q (Foulkes et al., 1993) recorded a high rate of allele imbalance at 11q23.3–qter in a small sample of tumours. The advent of highly polymorphic, well-mapped, microsatellites distributed evenly throughout the genome

(Weissenbach et al., 1992; Gyapay et al., 1994) and amenable to polymerase chain reaction (PCR) amplification has allowed rapid LOH analysis using small amounts of DNA (Futreal et al., 1992), which can be derived, if necessary, from archival material.

We have used 10 (CA)$_n$ polymorphic microsatellites to determine allele imbalance on chromosome 11 in ovarian tumours removed from 60 women [47 epithelial ovarian cancers (EOCs), five borderline malignancies, three adenofibromas, two mixed mesodermal tumours, two granulosa cell tumours and one teratoma]. The data have been analysed in relation to clinicopathological findings.

Materials and methods

Clinical specimens

Fresh primary ovarian tumour tissue from 60 patients was transferred directly to dry ice or liquid nitrogen and stored at $-70°C$. The normal tissue for comparison was blood in 39 patients and normal regions from formalin-fixed blocks in 21 patients. FIGO staging, histopathology and differentiation state were determined and reviewed in a standardised fashion at a multidisciplinary combined gynaecological oncology clinic. Treatment was in accordance with standard protocols, which consisted of the best possible surgical debulking followed by adjuvant/palliative chemotherapy. Minimum follow-up from diagnosis is 24 months, with median follow-up (by reverse Kaplan–Meier method) of 47 months. All deaths that have occurred have been due to ovarian cancer. Patient characteristics are outlined in Table I.

DNA extraction

DNA from fresh-frozen tissue and blood was extracted by a standard technique as previously described (Eccles et al., 1990). DNA extraction from fixed specimens was performed by cutting $3 \times 10\,\mu m$ sections, dewaxing in xylene, washing in 100% ethanol and desiccating the specimen. Proteinase K ($200\,\mu g\,ml^{-1}$) digestion was performed overnight at 37°C followed by heat inactivation. Debris was removed by centrifugation, providing a preparation containing adequate DNA template for PCR.

Table I Clinicopathological characteristics of the 60 patients with ovarian tumours

Number of patients	60
Ovarian adenocarcinoma	47
Histology	
Serous	25
Endometrioid	14
Mucinous	5
Clear cell	3
Differentiation	
Well	3
Moderate	14
Poor	25
Not known	5
Stage	
I/II	16
III/IV	29
Not known	2
Surgical treatment	
Completely debulked	32
Incompletely debulked	15
Not known	2
Chemotherapy	
Chlorambucil	
Adjuvant	3
Palliative	6
Cis-platinum	
Adjuvant	11
Palliative	8
Carboplatinum	
Palliative	2
None	13
Borderline malignant potential	5
Mixed mesodermal tumour	2
Granulosa tumour	2
Teratoma	1
Benign adenofibroma	3

Table II Polymorphic chromosome 11 microsatellite markers used in this study: identity and location

Locus	Location	Name	References[a]
D11S922	11p15.5	AFM217yb10	1,2
D11S569	11p15.3	cCIII-434	2,3
D11S929	11p14.1	AFM234xc3	1,2
D11S935	11p13	AFM254zb9	1,2
D11S905	11p13-12	AFM105xb10	1,2
D11S873	11q14.3	Mfd127	GDB ID no. 32638
D11S35	11q22	Phage2-22	2,4
D11S897	11q23.1	Mfd231	GDB ID no. 34742
D11S925	11q23.3	AFM220yb6	1,2
D11S912	11q24.1	AFM157xh6	1,2

[a]1, Weissenbach *et al.* (1992), Gyapay *et al.* (1994), Couillin *et al.* (1994). 2, Litt *et al.* (1993). 3, Phromchotikul *et al.* (1992). 4, Litt *et al.* (1990). GDB, genome database.

Oligonucleotide primers

Primers were selected on the basis of recently generated microsatellite index maps for locus, informativeness and spacing. Table II shows these primers and associated inform-ation. A high-resolution radiation hybrid map allowed reas-onable estimates of physical distance separating these markers (Figure 1) (James *et al.*, 1994).

Polymerase chain reaction and polymorphic microsatellite detection

PCR was performed under conditions specified in the original papers. A 10 µl volume of the PCR reaction product was loaded onto 8% denaturing polyacrylamide gel, separated by electrophoresis, passively transferred to Hybond nylon and probed with a ^{32}P end-labelled poly(CA) probe as previously described (Cohen *et al.*, 1992). Two observers visually analysed the autoradiographs and recorded allele imbalance when there was a clear reduction in the intensity of one allele in tumour DNA.

Statistical analysis

The two-tailed Fisher exact test was used. Since numerous analyses were performed, significance was set at $P = 0.01$, but we have included trends towards significance in the region of $0.07 > P > 0.01$ where they have supported or suggested biological hypotheses. Kaplan–Meier curves and log-rank analysis were performed (ICRF ICNET PDPLOT actuarial survival program, W Gregory) to determine LOH–survival relationships. Multivariate analysis was not performed because of the small sample number.

Results

Molecular analysis

Clinicopathological characteristics of the patient cohort are outlined in Table I. Table III shows the allele imbalance results for all markers and subgroups in this study.

Eighty-seven per cent of all ovarian tumours (52/60) and 85% of EOCs (adenocarcinomas excluding borderline malig-nancies) (40/47) had evidence of LOH involving at least one locus on chromosome 11. Only one EOC had LOH at all informative loci, and seven EOCs (15%) had no detectable LOH. Examples of allele loss for each of the markers are shown in Figure 1.

Analysis of consensus regions of allele imbalance in ovararian EOCs Figure 2 is a graphic representation of the data from Table III showing that serous, poorly differentiated and advanced stage EOCs have particularly high levels of LOH at both the 11p and 11q subtelomeric regions. Conversely, EOCs which are early stage or moderately/well differentiated appear to have high levels of LOH at the 11q14.3–q22 region.

Figure 3 shows those tumours that have partial losses on chromosome 11. Deletions are shown in shaded bars and are limited by the next heterozygous locus. In cases where a locus with allele loss is separated by an uninformative locus from a locus that remains heterozygous, that uninformative locus is included within the shaded bars as part of the deletion (since this region could be deleted). Eight tumours had only 11p loss, six tumours had only 11q loss and 25 tumours had partial loss of both arms. This type of analysis suggests three shortest regions of overlap (SROs) correspond-ing to three consensus regions of deletion/allele imbalance at 11p15.5–15.3, 11q23.3–qter and 11p12–q22.

11p loss of heterozygosity LOH was observed for at least one short arm locus in 77% (46/60) of all informative tumours, including 72% of EOCs (34/47).

For all ovarian tumours, high levels of LOH (>40%) were found for three loci (see Table III): D11S922 at 11p15.5 in 24/47 informative tumours (51%) and 16/36 EOCs (44%); D11S569 at 11p15.3 in 23/43 informative tumours (54%) and 14/30 EOCs (47%); and at D11S905 at 11p13–12 in 21/45 informative tumours (47%) and 15/33 EOCs (45%). When considering only those tumours that were informative at both loci telomeric to 11p15.3 (D11S569 and D11S922), the rate of 11p subtelomeric LOH was 16/24 (67%).

The lowest frequencies of allele loss on 11p were detected at D11S929 (11p14.1), with only 28% LOH in ovarian tumours and 24% LOH in EOCs.

11q loss of heterozygosity 11q LOH was observed for at least one locus in 65% (39/60) of all informative tumours, including 66% of EOCs (31/47).
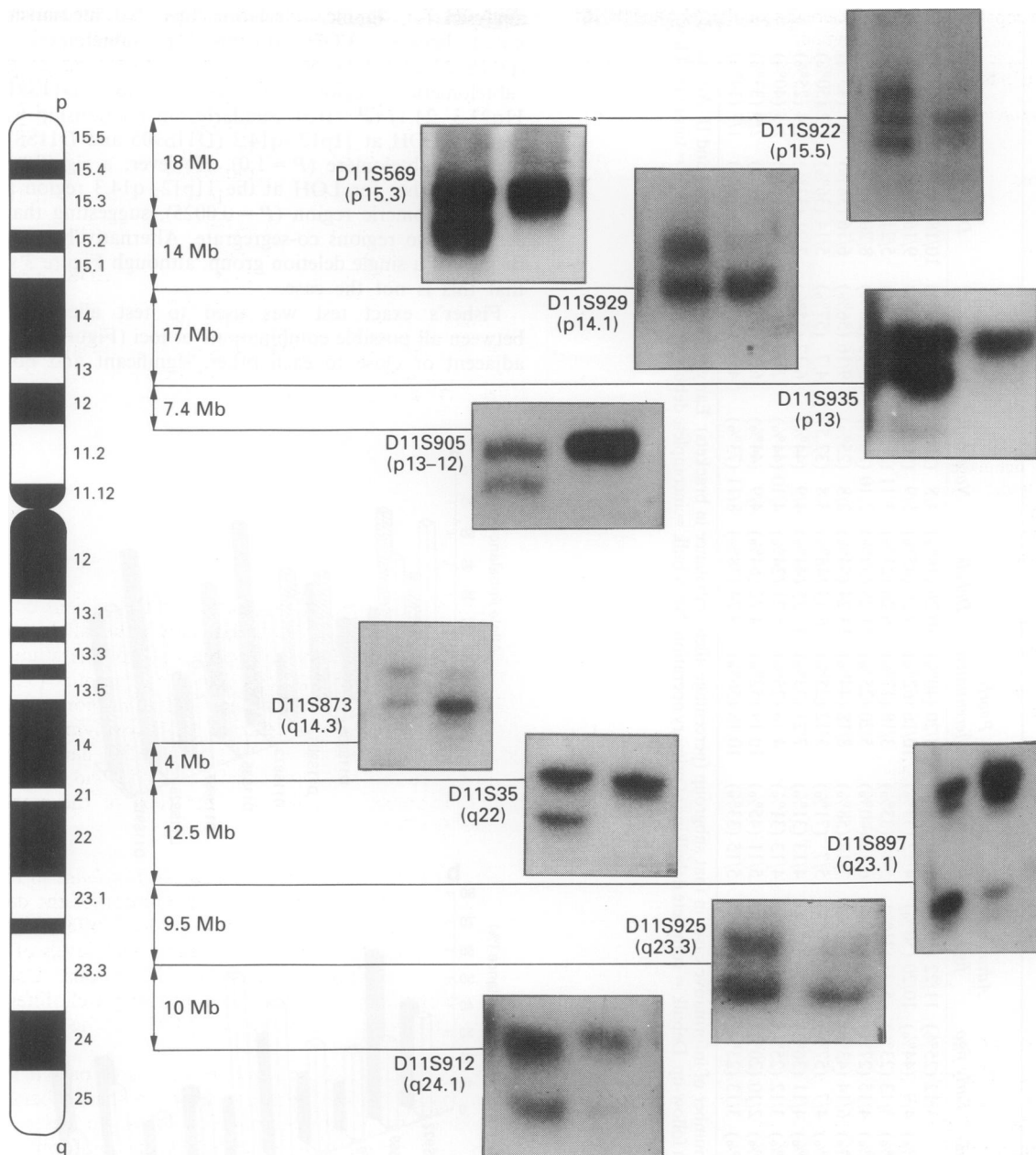
Figure 1 Idiogram of chromosome 11 with examples of allele imbalance. Constitutive DNA (left lane) and tumour DNA (right lane) for each microsatellite locus are shown with approximate distances between loci.

For all ovarian tumours, high levels of LOH were seen in three loci (see Table III): D11S873 at 11q14.3 in 11/25 informative tumours (44%) and 9/22 EOCs (41%); D11S925 at 11q23.3 in 24/45 informative tumours (53%) and 18/33 EOCs (54%); and D11S912 at 11q24.1 in 23/49 informative tumours (47%) and 18/37 EOCs (49%).

When considering only those EOCs that were informative at both loci telomeric to 11q23.3 (D11S925 and D11S912), the LOH rate was 18/27 (67%).

The lowest frequencies of allele loss were detected at D11S897 (11q23.1), with only 32% LOH in ovarian tumours and 28% LOH in EOCs.

Allele imbalance in other ovarian tumour types The non-EOC tumour numbers were too small for statistically valid conclusions. We considered benign and borderline (low maligant potential, LMP) tumours together. Only 1/8 benign or borderline tumours had LOH at D11S569 (11p15.3) and 1/7 had loss at D11S912 (11q24.1). However, 3/5 benign or borderline tumours had LOH at D11S922 (11p15.5). Both the mixed mesodermal tumours had LOH at both 11p15.5–p15.3 and 11q23–qter. One granulosa cell tumour had LOH

at all loci on 11p, suggesting whole arm loss. The ovarian teratoma in our series was hemizygous at all nine informative loci, and this is compatible with the usual description of these tumours as being parthenogenetic.

Microsatellite instability Microsatellite instability (MSI) (Aaltonen *et al.*, 1993; Thibodeau *et al.*, 1993) was noted in only 6.4% of EOCs (3/47). Both granulosa cell tumours had evidence of MSI, and one of these tumours had evidence of instability at three loci. There were no cases of MSI in five borderline, three benign and two mixed mesodermal tumours.

Statistical analysis

Fisher's exact test was used to analyse the relationship for allele imbalance between specific loci and also the relationship between imbalance for specific loci and clinicopathological parameters for EOCs.

Relationship of allele imbalance between different loci The three regions of deletion determined from Figure 3 were

Table III Allele imbalance rates for all subgroups in this study

Locus	All tumours	Adenocarcinoma of ovary	Serous	Endometrioid	Mucinous	Early figo	Advanced figo	Moderately/well differentiated	Poorly differentiated	Debulk	No debulk	Alive	Dead	Benign/LMP
D11S922	24/47 (51%)	16/36 (44%)	11/20 (55%)	4/11 (36%)	0/2	3/12 (25%)	11/22 (50%)	5/13 (38%)	8/20 (40%)	10/26 (38%)	4/8 (50%)	5/17 (29%)	10/18 (56%)	3/5 (60%)
D11S569	23/43 (54%)	14/30 (47%)	9/19 (47%)	3/5 (60%)	1/4 (25%)	4/9 (44%)	10/20 (50%)	4/12 (33%)	10/16 (62%)	9/20 (45%)	5/9 (56%)	5/13 (38%)	9/16 (56%)	1/8 (12.5%)
D11S929	13/47 (28%)	9/38 (24%)	3/20 (15%)	3/11 (27%)	1/4 (25%)	3/13 (23%)	5/23 (21%)	5/15 (33%)	3/18 (17%)	6/26 (23%)	3/11 (27%)	4/16 (25%)	5/21 (24%)	2/5 (40%)
D11S935	18/50 (36%)	13/39 (33%)	4/18 (22%)	6/13 (46%)	2/5 (40%)	4/15 (27%)	8/23 (34%)	6/15 (40%)	5/20 (25%)	11/28 (39%)	2/10 (20%)	5/19 (26%)	8/20 (40%)	1/6 (17%)
D11S905	21/45 (47%)	15/33 (45%)	7/16 (44%)	5/10 (50%)	1/5 (20%)	6/14 (43%)	9/18 (50%)	7/12 (58%)	8/18 (44%)	13/24 (54%)	2/8 (25%)	9/16 (56%)	6/16 (38%)	3/7 (43%)
D11S873	11/25 (44%)	9/22 (41%)	5/12 (42%)	0/5	2/3 (66%)	4/7 (57%)	5/14 (36%)	5/7 (71%)	3/12 (25%)	6/13 (46%)	3/8 (37.5%)	4/7 (57%)	5/14 (36%)	1/2 (50%)
D11S35	16/50 (32%)	14/39 (36%)	9/23 (39%)	0/9	2/4 (50%)	4/11 (36%)	9/26 (35%)	4/13 (31%)	7/21 (33%)	11/25 (44%)	4/9 (44%)	6/17 (35%)	7/21 (33%)	1/4 (25%)
D11S897	13/40 (32%)	9/32 (28%)	6/18 (33%)	1/6 (17%)	1/4 (25%)	3/12 (25%)	6/19 (32%)	4/13 (31%)	4/16 (25%)	5/21 (24%)	4/10 (44%)	2/15 (13%)	7/16 (43%)	2/5 (40%)
D11S925	24/45 (53%)	18/33 (54%)	11/19 (58%)	3/8 (38%)	2/3 (66%)	2/10 (20%)	13/21 (62%)	5/11 (45%)	10/19 (52%)	14/22 (64%)	4/9 (44%)	6/15 (40%)	9/17 (53%)	2/9 (33%)
D11S912	23/49 (47%)	18/37 (49%)	12/21 (57%)	3/7 (43%)	2/4 (50%)	3/13 (23%)	14/22 (64%)	5/15 (33%)	10/18 (56%)	9/24 (38%)	8/11 (73%)	4/17 (27%)	14/19 (74%)	1/7 (14%)

Values given are number of cases with allele imbalance in that subgroup/total number of informative cases in that subgroup (percentage allele imbalance in brackets). Early FIGO = FIGO stage I and II. Advanced FIGO = FIGO stage III and IV. Alive/dead refers to this status at 2 years' minimum follow-up. Debulk = complete debulking at primary operation. No debulk = incomplete debulking at operation. LMP = tumours of low malignant potential (borderline tumours).

analysed for significant relationships. No relationship was noted between LOH at the 11p subtelomeric region (D11S922 and D11S569 at 11p15.5–15.3) and at the 11q subtelomeric region (D11S925 and D11S912 at 11q23.3–24.1) ($P = 0.5$). Similarly, no relationship was seen between LOH at 11p12–q14.3 (D11S905 and D11S873) and the 11p subtelomere ($P = 1.0$). However, a significant relationship exists for LOH at the 11p12–q14.3 region and the 11q subtelomeric region ($P = 0.0025$), suggesting that losses at these two regions co-segregrate. Alternatively, they could be part of a single deletion group, although Figure 3 suggests that this is not the case.

Fisher's exact test was used to test allele imbalance between all possible combinations of loci (Figure 4). For loci adjacent or close to each other, significant and borderline
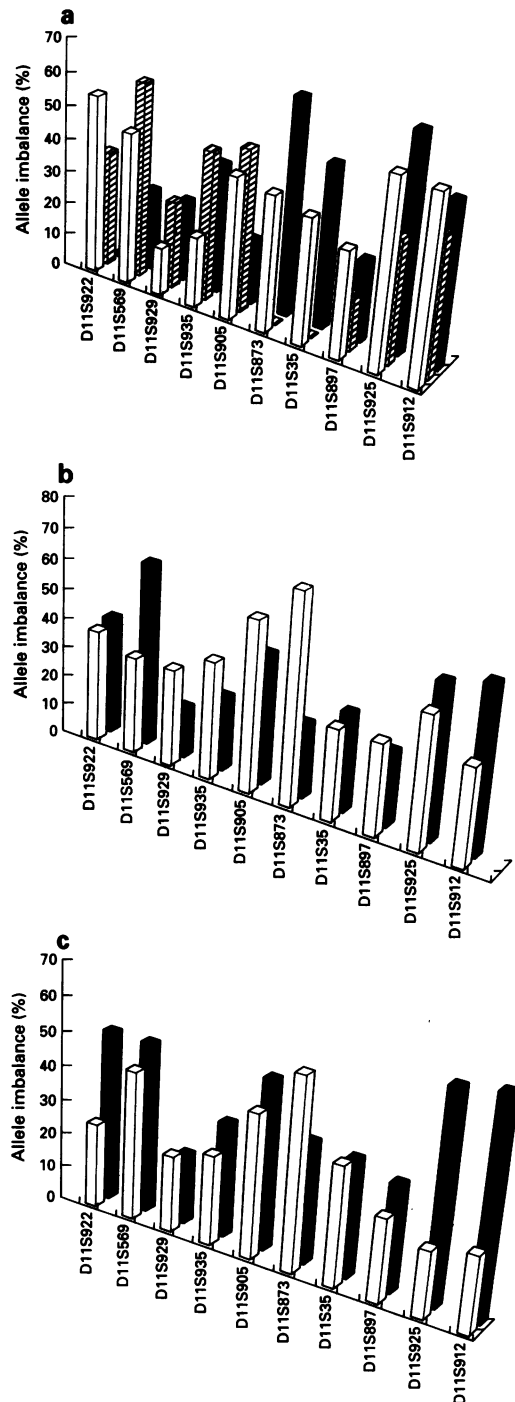

Figure 2 Graphic representation of allele imbalance rates from Table III for various clinicopathological parameters. (a) □, Serous; ▨, endometrioid; ■, mucinous. (b) □, Moderately/well differentiated; ■, poorly differentiated. (c) □, Early FIGO; ■, advanced FIGO.

findings are likely to reflect association simply as part of substantial subchromosome deletions which may include a tumour-suppressor gene. For loci distant from each other, D11S912/D11S935 LOH showed a significant statistical relationship ($P = 0.0073$) and the relationship for D11S935/D11S922 was of borderline signifiance ($P = 0.046$), suggesting the possibility that these loci harbour genes which may be inactivated cooperatively.

Relationship between allele imbalance and clinicopathological parameters Table IV shows Fisher's test *P*-values with significance trends for clinicopathological parameters at the loci tested.

Allele imbalance and histology No significant difference was seen at any locus, comparing serous EOCs with other histologies. However, of nine informative endometrioid tumours,
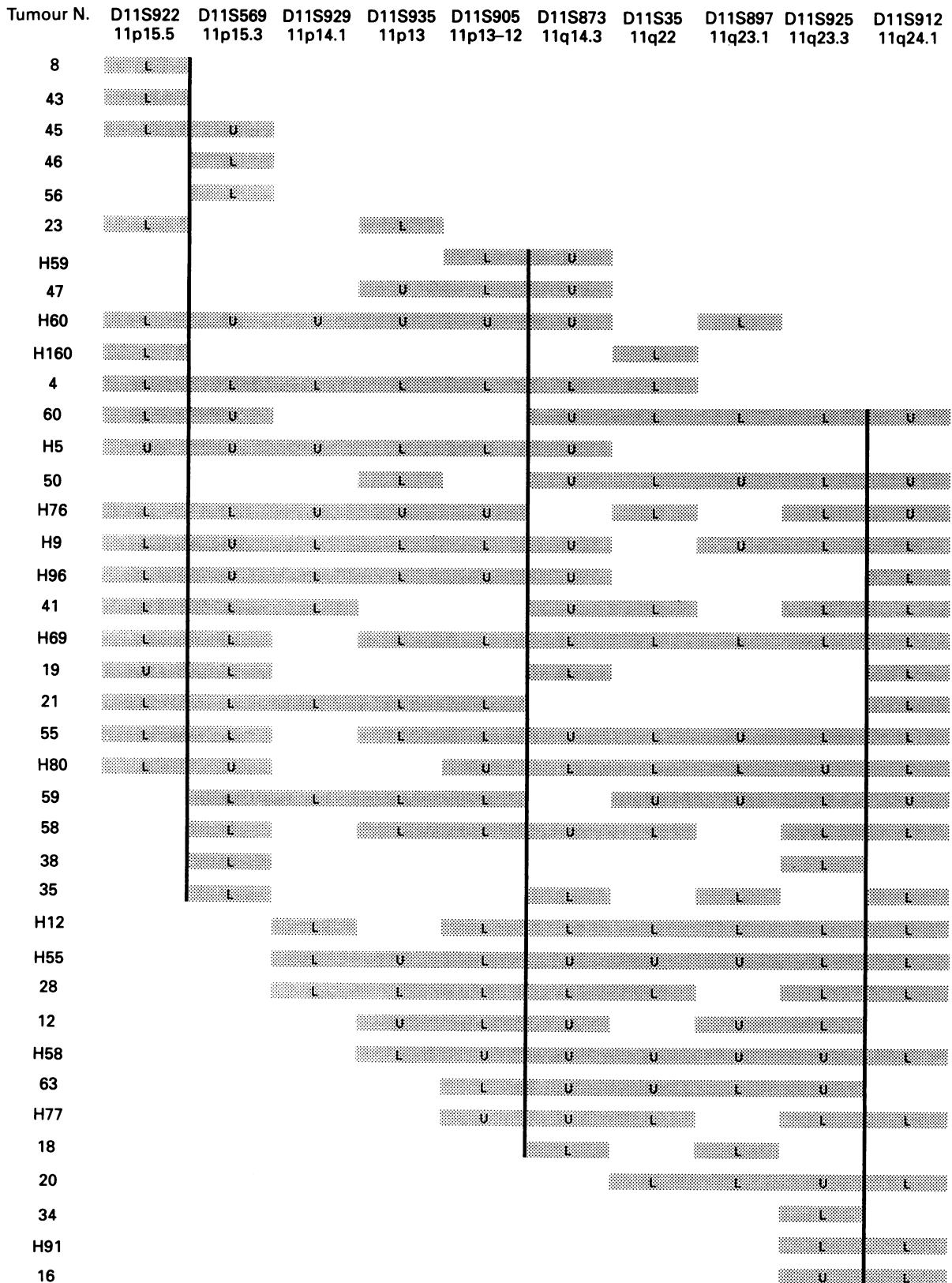

Figure 3 Grey horizontal bars represent the extent of subchromosomal deletions in EOCs. Black vertical lines represent approximate positions of the shortest regions of overlap (SROs); three such regions are apparent. L = constitutively heterozygous with allele loss/imbalance in tumour DNA. U = constitutively homozygous and therefore uninformative at that locus.
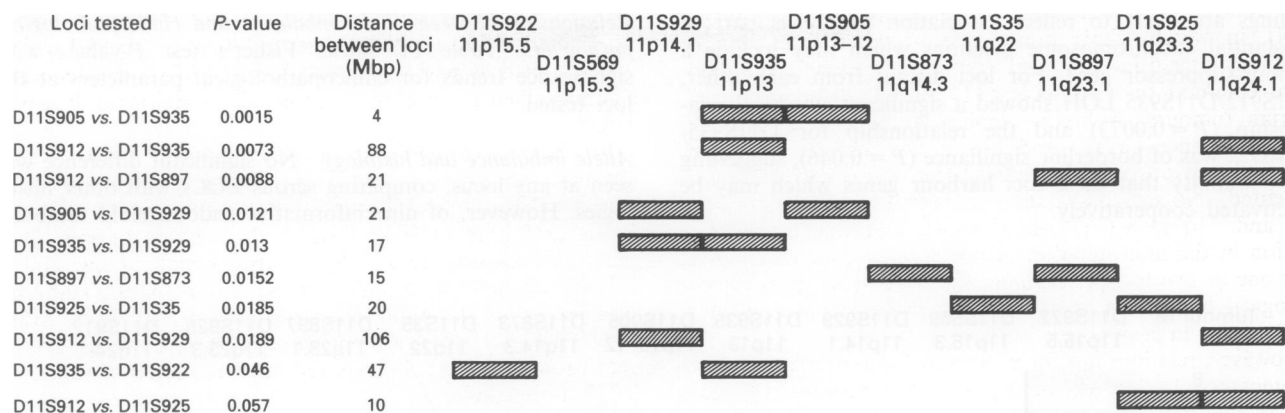
Loci tested	*P*-value	Distance between loci (Mbp)	D11S922 11p15.5	D11S569 11p15.3	D11S929 11p14.1	D11S935 11p13	D11S905 11p13–12	D11S873 11q14.3	D11S35 11q22	D11S897 11q23.1	D11S925 11q23.3	D11S912 11q24.1
D11S905 *vs.* D11S935	0.0015	4				▨	▨					
D11S912 *vs.* D11S935	0.0073	88				▨						▨
D11S912 *vs.* D11S897	0.0088	21								▨		▨
D11S905 *vs.* D11S929	0.0121	21			▨		▨					
D11S935 *vs.* D11S929	0.013	17			▨	▨						
D11S897 *vs.* D11S873	0.0152	15						▨		▨		
D11S925 *vs.* D11S35	0.0185	20							▨		▨	
D11S912 *vs.* D11S929	0.0189	106			▨							▨
D11S935 *vs.* D11S922	0.046	47	▨			▨						
D11S912 *vs.* D11S925	0.057	10									▨	▨

Figure 4 Fisher's exact test analysis of co-loss between markers on chromosome 11.

Table IV Fisher's exact test comparing ovarian adenocarcinoma clinicopathological groups at chromosome 11 loci

| Marker LOH	Segregation parameter	P-*value*
D11S912	Dead (*vs* alive) patients with 24 months minimum follow-up	0.0067
D11S912	Dead (*vs* alive) patients at 24 months	0.067
D11S912	Late (*vs* early) FIGO stage tumours	0.035
D11S35	Non-endometrioid (*vs* endometrioid) histology	0.04
D11S873	Well (*vs* poorly) differentiated tumours	0.07
D11S912	Non-debulked (*vs* debulked) tumours post surgery	0.075
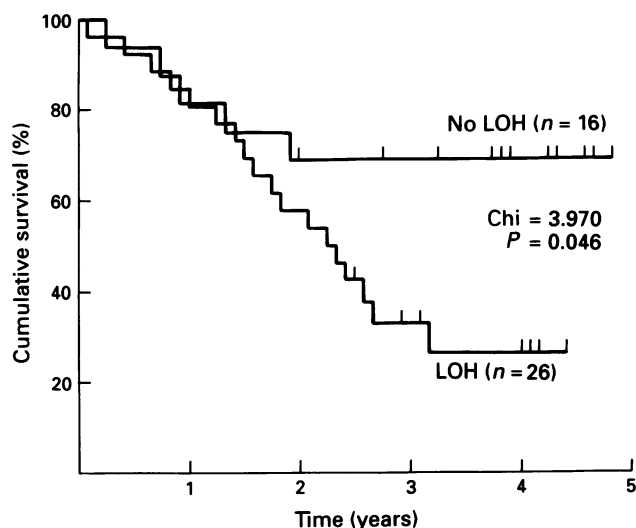
Figure 5 Kaplan–Meier survival curve with log-rank analysis for subtelomeric 11q allele imbalance status at presentation.
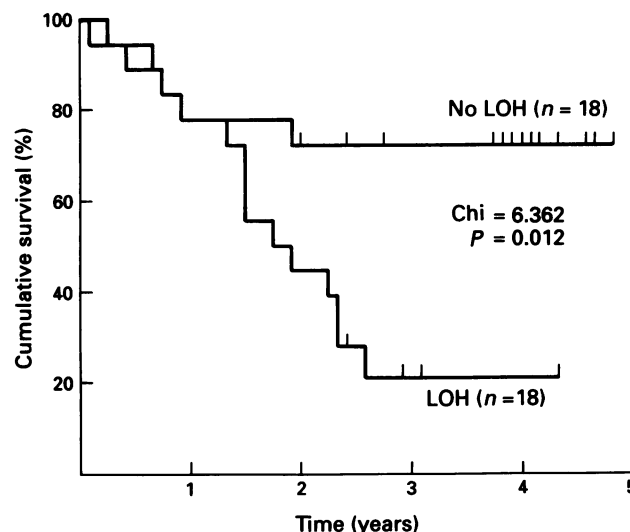

Figure 6 Kaplan–Meier survival curve with log-rank analysis for D11S912 (11q24.1) allele imbalance status at presentation.

none had LOH at D11S35, and comparing this group with other histologies a trend towards significance was observed for LOH of this marker with non-endometrioid histology ($P = 0.04$).

Allele imbalance and FIGO stage The only observed trend towards significance was for the association of LOH at D11S912 with FIGO stage III/IV EOCs ($P = 0.035$).

Allele imbalance and differentiation grade The only apparent trend towards significance was between D11S873 LOH and well/moderately differentiated tumours ($P = 0.07$).

Survival D11S912 loss of heterozygosity at 11q23.3–24.1 in primary tumours at diagnosis was associated with adverse survival of patients with adenocarcinoma ($P = 0.0067$) with minimum follow-up of 24 months.

Kaplan–Meier survival analysis Telomeric 11q LOH (D11S925 and D11S912) was significantly associated with adverse survival for patients with ovarian adenocarcinoma (Figure 5), and significance was increased when D11S912 LOH was considered alone (Figure 6). Actuarial survival of those without LOH shows 70% survival at 4 years *vs.* 20% for patients who had lost a D11S912 allele in their primary tumour at diagnosis.

Discussion

With a panel of ten highly informative, well-distributed, accurately mapped microsatellite polymorphisms (MSPs), significant levels of chromosome 11 allele imbalance were detected in our population of 60 ovarian tumours. Although the term allele imbalance is used interchangeably with LOH in this paper, we have opted to use this term rather than LOH since imbalance can also be a consequence of allele-specific amplification and need not necessarily imply deletion of a region of DNA. Furthermore, amplification of a region of DNA is not mutually exclusive with loss of function at a tumour-suppressor locus; loss of a chromosome or sub-chromosomal region may occur with reduplication of the other allele/chromosome, and amplification of a region of DNA is not necessarily associated with gain of function if accompanying inactivating mutations are involved.

In contrast to findings with chromosome 17 (Steel et al., 1994), in which whole homologue or whole arm loss is common, interstitial and small terminal deletions are in fact more common in chromosome 11 in this same group of ovarian tumours. A highly significant association between allele imbalance on 17p and 17q in this material has been observed previously (Fisher's exact test, $P = 0.0007$; data not presented). No such association is observed for imbalance on 11p and 11q ($P = 0.65$) as a whole, and this argues for caution in the interpretation of allelotyping data that utilise only one or two loci per chromosome arm where no previous biological hypothesis associates that chromosome arm with involvement in neoplasia.

However, relationships for allele imbalance between distant chromosome 11 loci do occur; not only between adjacent sites (which are likely to reflect larger deletions). Significant associations do occur between two distant loci while intervening loci are excluded from the relationship, as shown for example by D11S912/D11S935 and D11S935/D11S922 (at borderline significance). These pairs of loci may harbour genes which are cooperatively inactivated as part of a multistep process. Consensus analysis of those EOCs with partial deletion suggests at least three distinct regions of allele imbalance, at 11p15.5–p15.3, 11q23.3–24.1 and 11p12–q14.3.

In contrast to previous reports (Zheng et al., 1991; Kiechleschwarz et al., 1993), we found no significant association between differentiation grade and allele imbalance at the 11p15 region (despite 67% LOH) in our sample of EOCs, and this may reflect the lack of uniformity in ovarian cancer grading methodology; nor could we confirm an association between 11p15 LOH and advanced stage disease (Viel et al., 1992), although there appeared to be a non-significant trend for both these parameters (Figure 2). D11S922 LOH (11p15.5) did correlate at a borderline level of significance ($P = 0.046$) with LOH at D11S935. (Significant LOH rates have been detected in several studies at 11p13, near the site of WT1, although direct analysis of the WT1 locus suggests that it is not the gene involved; Bruening et al., 1993; Viel et al., 1994.) There was also evidence of significant LOH at D11S922 in benign and borderline tumours. With this high level of loss in EOCs, and without significant correlations with advanced disease/poor prognosis subgroups, the likelihood is that an 18.6 Mb interval within 11p15 houses a gene involved at an early stage in ovarian carcinogenesis, occurring as part of the development of benign and borderline tumours and also detectable at roughly similar rates in adenocarcinoma. Allele loss at D11S569 (11p15.3) is low (12.5%) in benign and borderline tumours, but is much higher in carcinomas, and there is no difference in LOH rate

between early and advanced FIGO stage adenocarcinomas. This raises the possibility of a second locus at 11p15.3 which is inactivated as part of the development of frank adenocarcinoma (albeit at an early stage of adenocarcinoma development).

We have confirmed and extended (in both numbers and chromosomal position) the recent finding of extensive allele loss distal to 11q23.3 (Foulkes et al., 1993), with 67% of EOCs exhibiting LOH at 11q23.3–24.1 in our sample. Our proximal marker (D11S925) in this region maps about 1.2 Mb telomeric to the most distal marker in the study of Foulkes et al. Loss of heterozygosity at the distal MSP (D11S912) at 11q24.1 is significantly associated with adverse survival and advanced stage, although the latter P-value, at 0.035, is borderline.

No borderline tumours exhibited allele imbalance at D11S912. Allele imbalance at the subtelomeric region at D11S912 showed significant correlation with D11S897 (11q23.1) and D11S935 (11p13 in the region of WT1). These findings suggest that a TSG acting primarily as a 'progression suppressor' may be located at 11q23.3–24.1 (or telomeric), and that its inactivation may be a significant late event in the pathogenesis of epithelial ovarian cancer.

The 11p12–q14.3 region (which is a large region containing the centromeric half of 11q), although exhibiting high levels of loss, does not appear to segregate significantly with any particular parameter, although there is a non-significant trend towares LOH in association with better prognosis tumours. Allele imbalance in this region does, however, segregate significantly with imbalance of the 11q subtelomeric region. D11S873 LOH at 11q14–q22 seems to correlate with favourable clinicopathological parameters: higher LOH rates are observed in those with mucinous histology, early FIGO stage and well/moderately differentiated tumours. Higher rates of allele loss at D11S873 are seen in those patients remaining alive (also seen at the neighbouring locus, D11S905 at 11p13–12). These findings suggest the possibility that some well-differentiated, early FIGO stage carcinomas may belong to a genetically distinct subcategory of EOC rather than being simply precursors of aggressive late-stage disease (Figure 7), and that allele loss in the 11p12–q22 region may confer changes incompatible with rapid progression of the disease, e.g. deletion of an oncogene locus essential for tumour progression. It is possible that LOH detected in this region could reflect amplification of the 11q13 region similar to that observed in breast cancer (Karlseder et al., 1994), and we have not ruled out this possibility in the present study, although previous studies of this region in ovarian cancer (Foulkes et al., 1993) suggest that
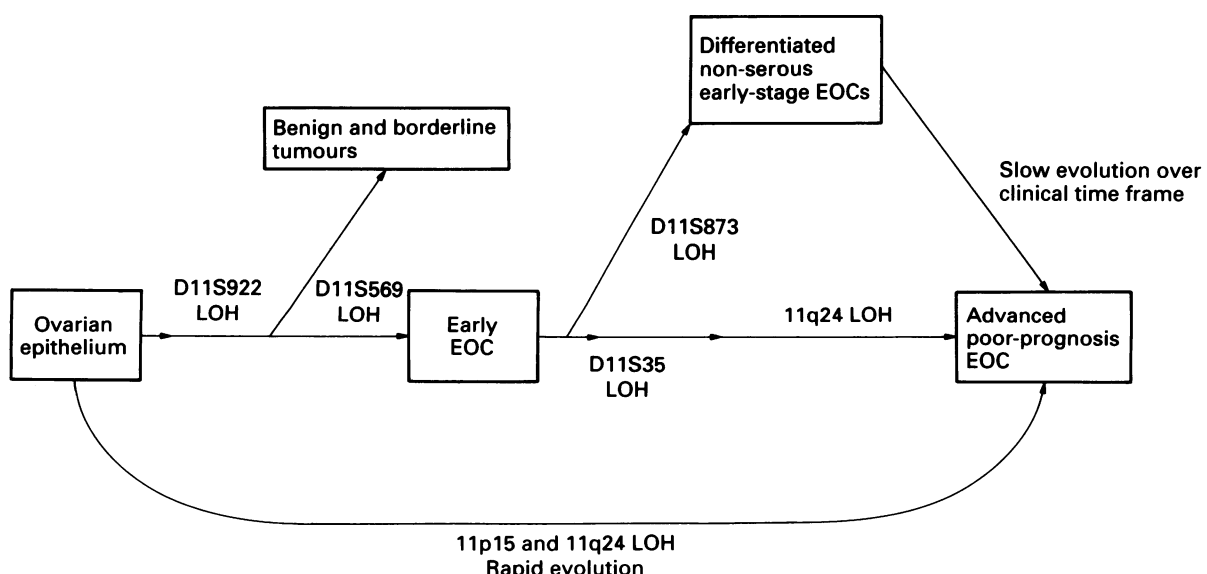

Figure 7 Hypothesis for chromosome 11 involvement in ovarian carcinogenesis. For abbreviations, see text.

amplification occurs infrequently. However, the explanation for our findings of better prognosis associated with allele loss at this locus remains obscure and requires further work.

The absence of LOH at D11S873 and D11S35 specifically in endometrioid adenocarcinoma is of considerable interest, though the finding approached statistical significance only for the latter MSP ($P = 0.04$). D11S35 lies about 160 kb from the site of the progesterone receptor (PgR) gene. At least six studies have reported that endometrioid tumours contain PgR levels that are elevated relative to other histological types (Slotman and Rao, 1988). Furthermore, there is evidence that, in breast cancer, gene dosage, although secondary to regulatory change, plays a significant role in determining hormone receptor levels: tumours that are cytogenetically $6q-/11q-$ have half as many oestrogen receptors (ER) and PgRs as tumours without losses on 6q or 11q (Magdelenat *et al.*, 1994). We would therefore speculate that there may be a role for the PgR gene in the regulation of histological subtypes of ovarian cancer, and possibly that its structural disruption contributes to the generation of adverse histological and prognostic subtypes at a relatively early stage in the development of ovarian cancer.

The findings in this study extend the previous observations of distinctive patterns of aneusomy or molecular aberrations in ovarian cancers belonging to different clinicopathological subgroups. They do not imply that LOH at each of the defined regions of chromosome 11 represents independent prognostic factors, although 11q subtelomere imbalance should perhaps be subjected to a large prospective study.

Of more immediate relevance is the application of the observed correlations synthesis of a multistep model of ovarian carcinogenesis (Figure 7). In this model, there is not only multistep, but also multipath, progression of ovarian cancer, postulating, for example, the involvement of q14.3–q22 imbalance in a biologically distinct subpopulation of early EOC.

Extensive chromosome 17 microsatellite and restriction fragment length polymorphism (RFLP) analysis of this same cohort of patients has been performed in our laboratory (Eccles *et al.*, 1992b; Steel *et al.*, 1994), and we are currently extending our analysis to correlations between regions of LOH on chromosomes 11 and 17 and their relationship to clinicopathological parameters. In addition, more detailed mapping of LOH within the 11q23.3–qter region to better define the imbalance peak is in progress. This will also determine if there are regions telomeric to 11q24 with low rates of LOH and address the possibility that these observed losses are simply due to non-specific telomeric high-frequency breakages as a consequence of neoplasia rather than putative tumour-suppressor genes at these sites.

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