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Advances in Brief

Definition and Refinement of a Region of Loss of Heterozygosity at 11q23.3-q24.3 in Epithelial Ovarian Cancer Associated with Poor Prognosis

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Abstract

Previous cytogenetic and loss of heterozygosity (LOH) data suggest that disruption of chromosome 11q23-qter occurs frequently in epithelial ovarian cancer and is associated with an adverse clinicopathological phenotype. Ten polymorphic microsatellite repeat loci were analyzed by PCR from the 11q22-q25 region between *D11S35* and *D11S968* in 40 ovarian tumors (including 31 epithelial ovarian cancers). Two distinct regions of loss were detected, suggesting possible sites for genes involved in epithelial ovarian neoplasia: a large centromeric region between *D11S35* and *D11S933* (11q22-q23.3) and a telomeric 8.5-Mb region lying between *D11S934* and *D11S1320* (11q23.3–24.3) not previously defined. LOH of the latter region but not the former one was significantly associated with poor survival, despite all tumors in this study having LOH somewhere on chromosome 11. This analysis provides a starting point for positional cloning.

Introduction

EOC[3] the most common cause of death from gynecological malignancy, is typically a late-presenting disease with poor prognosis. The pathophysiological pathways to this common, aggressive phenotype are determined by multiple genetic lesions accumulated by the ovarian surface epithelial cells. Inactivation of tumor suppressor genes are frequent and important components of these pathways, and regions of the genome containing such genes may be delineated by cytogenetic and LOH analyses. The list of putative tumor suppressor regions is growing rapidly, and many chromosomes appear to have such regions lost at fairly high frequencies in EOC. The 11q23-qter region has received much recent interest as the site of a putative tumor suppressor gene(s) in cancers of the ovary (1, 2), breast (3–5), lung (5), colon (7), cervix (8), and cutaneous malignant melanoma (9, 10). The region was shown to be significantly associated with advanced stage and poor prognosis in cancers of the ovary (2) and breast (11). The recent cloning and localization to chromosome 11q of the ataxia telangiectasia gene (12) (thought to contribute as a modifying gene to perhaps 20% of breast cancer) provides a plausible candidate in this region, which is increasingly described in solid tumors.

The present study refines the LOH map of the 11q22-qter region in EOC using selected tumors known to have loss somewhere on chromosome 11, concentrating on the subregion of LOH previously identified and associated with adverse survival (2).

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[3] The abbreviations used are: EOC, epithelial ovarian cancer; LOH, loss of heterozygosity; SRO, shortest region of overlap; FIGO, International Federation of Gynecology and Obstetrics.

Materials and Methods

Patient Population and Tumor Samples. Fresh primary ovarian tumor tissue from 40 patients with ovarian tumors (31 with epithelial ovarian cancer) was obtained as previously described (2). FIGO staging, histopathology, and differentiation state were determined and reviewed in a standardized fashion at a multidisciplinary combined gynecological oncology clinic. Treatment was planned and delivered in accordance with standard protocols, and follow-up data on this group are complete. Minimum and maximum follow-up on living patients are 33 and 52 months, respectively; all deaths that have occurred have been due to ovarian cancer. Patient characteristics are outlined in Table 1.

Primary tumor and constitutive DNA were extracted using standard methods as previously described (13). In the present study, all tumors were known to have LOH somewhere on chromosome 11, including 11p and proximal 11q in cases H59, G56, G43, H5, G47, H55, G42, G46, and G18, prior to refinement of the telomeric region (2).

LOH Analysis. DNA samples were analyzed as normal/tumor pairs by PCR using primers for the following CA repeat polymorphic microsatellites lying in the region 11q22 to 11q24.3: *D11S35, D11S925, D11S1336, D11S933, D11S934, D11S707, D11S1351, D11S912, D11S1320,* and *D11S968.* Primer sequences were obtained from the Genome Data Base. The PCR products were resolved by electrophoresis on a 6 or 9% denaturing urea/polyacrylamide gel, passively transferred to Hybond nylon and probed with $\gamma-^{32}P$ end-labeled $(CA)_{35}$ oligonucleotide as previously described (14). LOH was initially scored on the basis of independent visual reporting by two observers who registered for clear reduction in intensity of one tumor allele. Computerized densitometric analysis of the autoradiographs was then performed on a high-resolution flat bed scanner using a Bioimage whole band analyzer software system (v3.2.2) run on a SUN SPARC UNIX platform. The relative ratio of alleles was determined, normalized, and compared. Where the tumor allele ratio differed from the normal allele ratio by 30% or more ($r \leq 0.7$), LOH was assigned, as previously described (4).

Statistical Analysis. Two-tailed Fisher's exact test was used to look for associations between LOH regions and clinicopathological parameters. Kaplan-Meier analysis and the log rank test were used to analyze overall

Table 1 *Clinicopathological characteristics of the study cohort*

Number of patients	40
Previously known to have chromosome 11 LOH	40
Ovarian adenocarcinoma (EOC)	31
Histology	
Serous	16
Endometrioid	10
Mucinous	4
Clear cell	1
Differentiation	
Well	2
Moderate	12
Poor	17
Stage	
I/II	11
III/IV	19
Not known	1
Benign adenofibroma	3
Borderline Malignant potential	3
Granulosa tumor	2
Teratoma	1

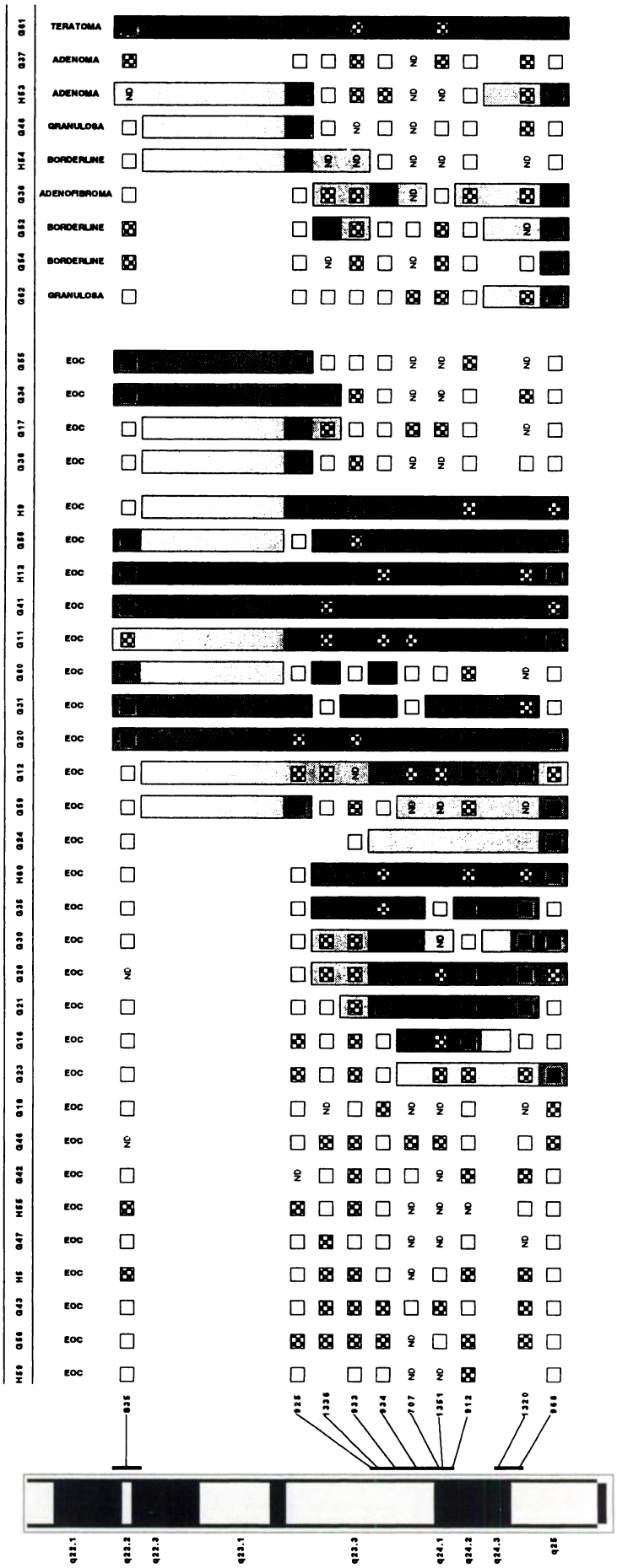

Fig. 1. Schematic representation of regions of LOH in all of the tumor samples. Case numbers are shown at the *top* with histological diagnosis below. Microsatellite loci, used to detect LOH, are at the *left* of the diagram, and their approximate position is indicated on the chromosome 11q idiogram. *Shaded areas*, regions of LOH. *Dark shadowing*, a region of secure LOH; *light shadowing*, a region of uncertainty; *unshadowed*, heterozygosity. □, loci maintaining heterozygosity without LOH; ▩, uninformative (homozygous) loci; ■, LOH. *ND*, not determinable.

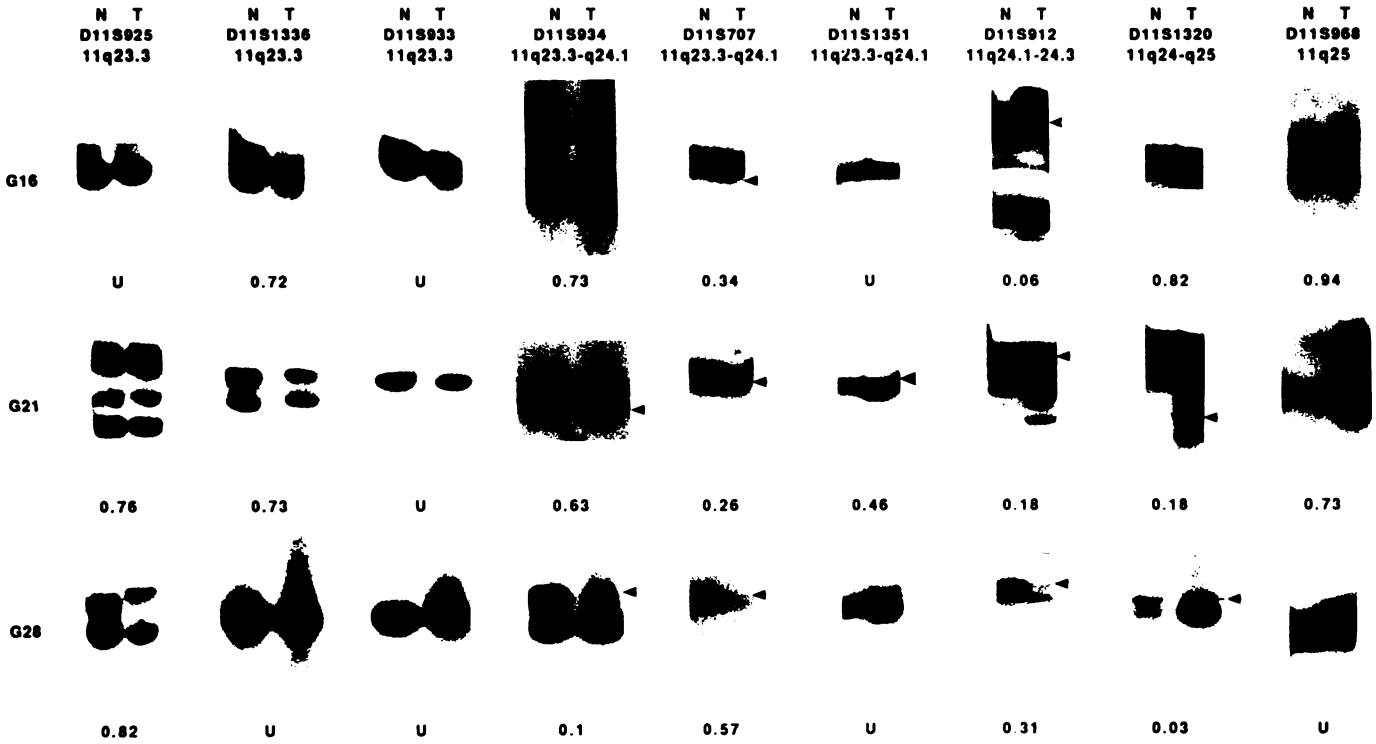

Fig. 2. Primary LOH data from three cases critical to the definition of the 11q23.3–q24 locus. *N*, normal DNA; , tumor DNA shown at *top* from patients G16, G21, and G28 (*left*). Microsatellite loci are shown form centromeric (*top left*) to telomeric (*top right*). *Arrows*, alleles showing LOH (allele imbalance). Densitometric ratios of allele intensity were calculated (*shown at bottom*), and values between 0.0 and 0.7 are taken to indicate LOH. *U*, uninformative (homozygous). Note microsatellite instability at *D11S925* for patient G26.

survival (diagnosis to death) for those patients with *versus* those without LOH in defined regions of chromosome 11q.

Results

Molecular Analysis. Clinical and pathological characteristics of the cohort are outlined in Table 1. LOH was detected somewhere on chromosome 11 in all 40 tumors in this series (selected for detailed analysis from our previous study). H59, G56, G43, H5, G47, H55, G42, G46, and G18 all had been shown to have chromosome 11 LOH outside the 11q22–q25 region from a previous analysis (Ref. 2; data not shown). Data from *D11S35, D11S925,* and *D11S912* have been included from this previous report for most of the tumors, although the latter two microsatellite PCR reactions were repeated for this study.

Fig. 1 shows that two separate regions of LOH are identified within the 11q22–q25: the minimum extent of the telomeric region of LOH (11123.3–24.3) is defined by tumor G16. The centromeric extent of this region is defined by *D11S934,* and the telomeric extent is defined by *D11S1320* (Figs. 1 and 2). The size suggested for this region from a recently published radiation hybrid map is 169 centirays (about 8.5 Mb; Ref. 15). Within this region, the frequency of LOH at *D11S912* was 11 (58%) of 19 EOCs and 0 of 5 benign/borderline; at *D11S707* it was 9 (69%) of 13 in EOCs and 0 of 1 borderline. LOH involving this distal deletion unit occurred in 18 (58%) of 31 EOCs.

The large centromeric region of LOH lies between *D11S35* (11q22) and *D11S925* (11q23.3) as defined by tumors G36, G17, G34, and G55 (Fig. 1). LOH involving this centromeric deletion unit occurred in 13 (42%) of 31 EOCs.

Only a minority of tumors exhibited LOH at either 11q22–q23.3 (4/31, 13%) or 11q23.3–24.3 (8/31, 26%) alone. Nearly 30% of the cases simultaneously exhibited LOH at both 11q22–q23.3 and 11q23.3–q24.3 (fig. 1).

Statistical Analysis. Fisher's exact test was used to determine whether loss from these two regions was significantly associated with the clinicopathological features of ovarian cancer. No significant correlations were seen between regional losses and FIGO stage ($P = 0.15$), histological type, or differentiation grade. The centromeric 11q22–q23.3 region showed no statistical association with survival for regional LOH alone or in combination with other regions. However, LOH of the 11q23.3–q24.3 region, either alone or in combination with LOH in other regions, showed a strongly significant correlation with survival by Fisher's exact test ($P = 0.004$; alive *versus* dead patients with 33-month minimum follow-up), despite all of the tumors in the series exhibiting LOH somewhere on chromosome 11, confirming our previous finding for *D11S912* (2).

Kaplan-Meier/log rank analysis showed no survival difference ($P = 0.632$) for the centromeric region of LOH (Figure. 3*A*) but the distal LOH region (11q23.3–q24.3) between *D11S934* and *D11S1320* correlated signifficantly with adverse actuarial survival (log rank test, $p = 0.011$; Figure 3*B*). However, the log rank analysis was not significant when only advanced FIGO stage cancers were considered in relation to 11q23.3–q24.3 LOH status ($p = 0.09$).

Other Tumor Types. We have included data from a benign ovarian teratoma, regarded as haploid parthenogenetic, demonstrating LOH at all informative chromosome 11 loci. This tumor also had clear LOH at all informative chromosome 17 loci tested (data not shown) and provides a useful positive LOH control.

Benign and borderline epithelial tumors showed no case with secure LOH involving the *D11S934–D11S1320* distal SRO. The two granulosa cell tumors had minimal LOH located outside the critical region.

Discussion

This study demonstrates that two regions of LOH within 11q22–q25 can be defined in ovarian tumors. As has been shown from previous studies (1, 5, 16), LOH in this region appears to correlate with genetic loss rather than amplification.

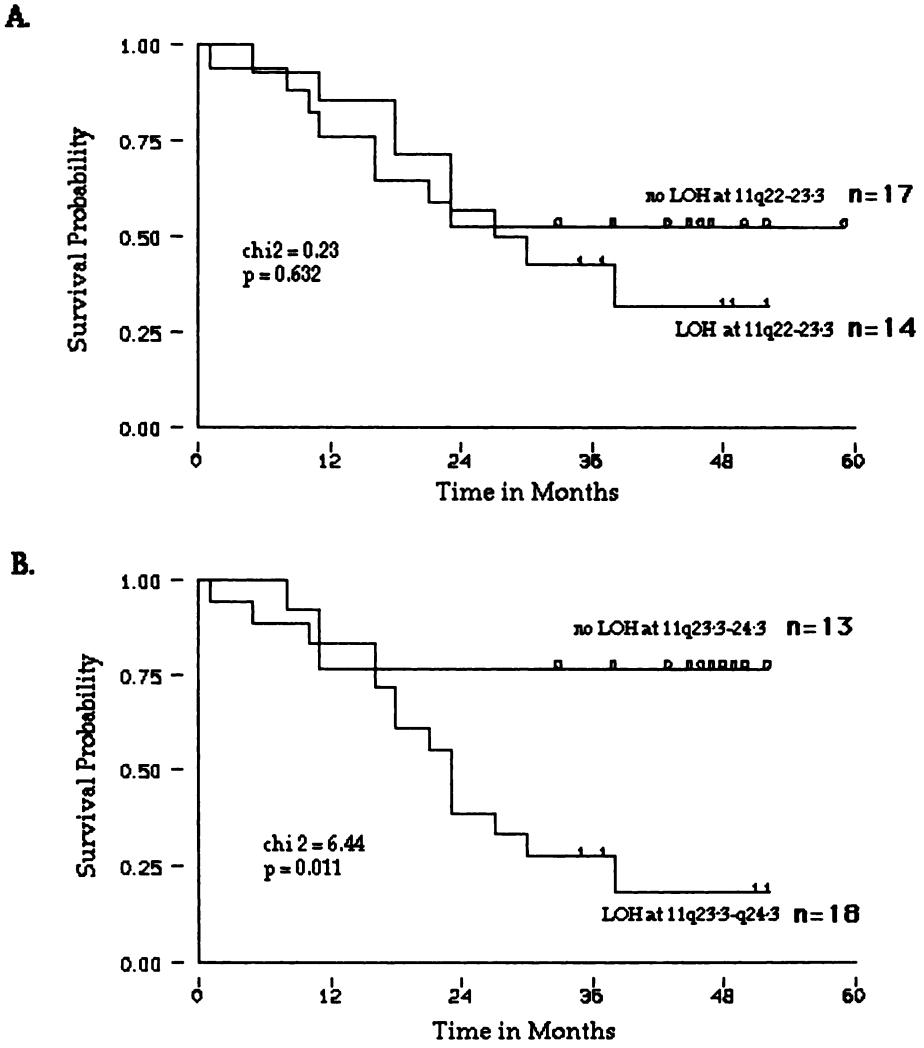

Fig. 3 Kaplan-Meier survival curves comparing survival after a diagnosis of ovarian cancer in those with or without LOH at 11q22–q23.3 (*A*) and in those with or without LOH at 11q23.3–q24.3 (*B*). LOH at 11q23.3–q24.3 shows significantly reduced actuarial survival ($P = 0.011$, log rank test), indicating an aggressive disease course. *Tick marks*, census survival times.

The proximal SRO (which is a large region defined by only two microsatellites in our study) contains the ataxia telangiectasia and progesterone receptor genes and is lost frequently (in 40% of cases), but does not crudely correlate with survival or other adverse prognostic features in EOCs, although it is associated with low tumoral progesterone receptor content (17). This proximal region broadly corresponds to a region that has recently been identified in breast cancer (3–5).

The telomeric 11q23.3–q24.3 region, which we concentrated on due to previous association of *D11S912* LOH with poor prognosis, excludes the *ATM* locus and the region recently mapped in breast cancer. The SRO has been narrowed by our analysis to 8.5 Mb lying between *D11S934* and *D11S912* at 11q23.3–q24.3 based on recently published physical maps (15, 18). Kaplan-Meier survival analysis revealed that LOH involving this 8.5-Mb region is significantly associated with poor survival in EOCs. However, when only the advanced stage tumors were considered with this telomeric locus, the log rank test did not reach significance, although Kaplan-Meier analysis showed a trend toward worse survival in those with LOH of the distal region. This suggests that a late-acting progression-suppressor may be located within this interval. LOH within this region should be subjected to a larger prospective analysis to assess its role as an independent prognostic factor and its value in assessing survival prospectively. The distal deletion interval currently contains five candidate genes: (from centromeric to telomeric) *SRPR, ETS-1, FLI1, ZNF1,* and *NFRKB* (15). The possibility that high-frequency nonspecific genome instability might be responsible for the observed regional losses was controlled by selecting tumors with chromosome 11 loss preferentially, making it unlikely that the observed survival association in this population could be attributed to random high-frequency genetic alteration. Additionally, the retention of heterozygosity telomeric to the 11q23.3–q24.3 LOH region suggests that LOH at this distal unit is not simply due to high-frequency non specific telomeric breaks.

The development of comparative genomic and expression techniques as they impact on positional and functional cloning strategies should allow identification of genes from these critical regions.

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