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Identification of a 6-cM Minimal Deletion at 11q23.1–23.2 and Exclusion of *PPP2R1B* Gene as a Deletion Target in Cervical Cancer[1]

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ABSTRACT

Previous functional and deletion mapping studies on cervical cancer (CC) have implicated one or more tumor suppressor genes (TSGs) on chromosome 11 at q13 and q22–24 regions. Of these, the 11q22–24 region exhibits frequent allelic deletions in a variety of solid tumor types, suggesting the presence of critical genes for tumor suppression in this region. However, the precise region of deletion on 11q is not clearly defined in CC. In an attempt to accurately map the deleted region, we performed an extensive loss of heterozygosity (LOH) mapping in 58 tumors using 25 polymorphic loci on both the short and long arms. The pattern of LOH identified three sites of deletions, two on 11p (p15.11–p15.3 and p12–13), and one on 11q (q23.1–q23.2). The 11q23.1–q23.2 exhibited highest frequency (60.6%) of deletions, suggesting that this could be the site of a candidate TSG in CC. The minimal deletion at 11q23.1–23.2 was restricted to a 6-cM region between 123.5 and 129.5 cM genetic distance on chromosome 11, identifying the site of a potential TSG important in the pathogenesis of CC. At least five known genes and 28 UniGene clusters were mapped to the present commonly deleted region. In addition, we have excluded a previously known TSG *PPP2R1B* at 11q23 as a deletion target in CC. The definition of the minimal deletion and the availability of expressed sequence resources should facilitate the identification of the candidate TSG.

INTRODUCTION

CC[3] is a substantial public health issue among women worldwide, causing high mortality, and the incidence is rising in certain countries (1). Most invasive CCs are believed to be preceded by distinct preinvasive changes called CINs (CINI–CINIII), which represents a pathological continuum from mild to severe epithelial dysplasias. Biological behavior of cervical precursor lesions vary in which only a fraction of higher-grade dysplastic and preinvasive lesions progress to invasive cancer (2–5). Most precancerous and preinvasive lesions are readily curable, whereas the prognosis of invasive CCs are generally poor. The genetic events that initiate the multistep pathway in cervical tumorigenesis and cause invasion are of considerable importance in understanding the molecular basis of CCs. A large body of evidence has implicated infection of high-risk HPV types as the critical etiological factor in CCs in which HPV E6 and HPV E7 proteins interact with critical cell cycle check point genes *p53* and *pRB*, respectively, resulting in inactivation of these genes (6). However, epidemiological data suggest that only a certain fraction of HPV-infected CIN lesions progress to invasive CCs with variable latency periods (2–4). These data, therefore, suggest that additional genetic alterations may be necessary for the progression of CCs. Delineation of such genetic changes may be of relevance in understanding cervical carcinogenesis and will have implications in early detection and identification of high-risk lesions.

Molecular genetic studies of CCs have identified frequent LOH affecting multiple chromosomal regions such as 3p, 5p, 6p, and 11q (7–10), suggesting the presence of TSGs in these regions. The functional evidence for the presence of a TSG in CC was first identified on chromosome 11 by somatic cell hybrid studies using HeLa cells (11) and subsequent analysis in other CC cell lines (12–14). Pursuing these studies, chromosome 11 has been shown consistently to exhibit LOH at different regions, 11q13 and 11q22–23 (15–17). Although these data provide strong evidence for the presence of a TSG on chromosome 11 relevant to CC, the critical region or the gene involved in the development of this tumor is not yet identified. In the present study, we defined a common minimal deletion at 11q23 that spans a 6-cM genetic distance by LOH analysis and excluded the *PPP2R1B* gene at 11q23 as a target of deletion, which was shown earlier to be mutated in other tumor types (18).

MATERIALS AND METHODS

Tumor and Normal Tissues. A total of 58 tumor biopsies derived from previously untreated primary invasive CCs and the corresponding peripheral blood samples comprised the material for the study. The tissues were ascertained from patients treated at the Instituto Nacional de Cancerologia (Santa Fe de Bogota, Colombia) after appropriate informed consent and the approval of the protocol by the institutional review board. Clinically, the tumors were classified by Fédération International des Gynaecologistes et Obstetristes class 1B ($n = 3$), IIB ($n = 15$), IIIB ($n = 36$), and IV ($n = 4$). Histologically, 56 tumors were classified as squamous cell carcinomas and 2 as adenocarcinomas. The ages of the patients ranged from 28 to 85 years, with a median of 49 years.

DNA Isolation and Analysis of LOH. High molecular weight DNA from tumor and peripheral blood samples were isolated using standard procedures of proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. A panel of 25 dinucelotide polymorphic markers were chosen on the basis of their map position and heterozygosity (Table 1; Gene Map 99[4]) and were obtained from Research Genetics (Huntsville, AL). A standard PCR reaction was carried out in a 10-μl reaction volume containing 1.5–2.5 mM $MgCl_2$, 10–15% glycerol, 4 pmol of each primer (one-fifth of one of which was end-labeled with [γ-^{32}P]dATP), 0.2 mM deoxynucleotide triphosphates, 25 ng of DNA, and 0.3 unit of AmpliTaq DNA polymerase (Perkin-Elmer Corp., Branchburg, NJ). The amplification was carried out for 30 cycles at annealing temperatures ranging from 50 to 58°C. The PCR products were denatured in sequence stop buffer containing formamide and electrophoresed on a 6% urea-containing polyacrylamide gel, and the dried gels were autoradiographed for 4–16 h. Criteria applied for scoring LOH was described earlier (19). All autoradiograms were independently scored visually by three investigators (H. A. P., F. M., and V. V. V. S. M.). The definition of minimal region of deletion was based on LOH of the loci that span common deletion in several

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[3] The abbreviations used are: CC, cervical cancer; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; LOH, loss of heterozygosity; TSG, tumor suppressor gene; SSCP, single-strand confirmation polymorphism.

[4] Internet address: http://www.ncbi.nlm.nih.gov/genemap.

Table 1 *Frequency of LOH on chromosome 11 in cervical carcinoma*

Chromosome band	Locus	Genetic position	No. studied/ Informative	LOH (%)
11p15.11	*D11S4189*	20.50	52/39	13 (33.3)
11p14	*D11S4099*	24.90	55/34	10 (29.4)
	D11S4096	24.90	56/33	9 (27.3)
11p12–13	*D11S4200*	46.90	58/50	13 (26.0)
	D11S4083	50.70	57/50	11 (22.0)
11q12–13.1	*D11S4207*	80.00	57/42	11 (26.2)
11q13.2–13.3	*D11S911*	84.20	58/48	7 (14.6)
	D11S937	84.60	56/48	14 (29.2)
11q13.4–13.5	*D11S4147*	92.50	58/43	15 (34.9)
11q14.2–14.3	*D11S4120*	100.90	58/45	12 (26.7)
	D11S898	103.10	58/45	17 (37.8)
	D11S1339	104.80	58/37	10 (27.0)
11q21–22.1	*D11S1343*	106.50	56/22	9 (40.9)
	D11S1347	110.30	55/36	11 (30.6)
11q22.2–22.3	*D11S939*	117.9	56/35	12 (34.3)
	D11S1356	118.60	56/53	23 (43.4)
11q23.1	*D11S925*	123.50	58/44	19 (43.2)
	D11S4167	124.60	58/46	21 (45.7)
11q23.2	*D11S1353*	127.80	56/41	24 (58.5)
	D11S4094	129.00	55/33	20 (60.6)
	D11S4144	129.50	58/47	22 (46.8)
	D11S933	129.50	58/31	14 (45.2)
11q23.3	*D11S934*	131.70	56/35	11 (31.4)
11q24–25	*D11S4131*	143.80	58/40	18 (45.0)
	D11S4085	147.20	58/37	14 (37.8)

tumors and retention of heterozygosity of adjoining markers at both the boundaries in at least two tumors.

Mutation Analysis. SSCP analysis was performed on all 15 exons of the *PPP2R1B* gene using primers flanking intronic sequences (18). PCR was performed as described above except that 0.5 μCi of α [^{32}P]dCTP was included in the reaction instead of labeled primers. The PCR products were diluted in 0.1% SDS/10 mM EDTA, denatured in sequencing stop buffer, and run overnight in 6% nondenaturing polyacrylamide gels containing 10% glycerol at room temperature. Dried gels were autoradiographed and examined for conformational changes. Automated sequencing was performed on exons that showed suggestive mutations using purified PCR products.

RESULTS

In the present study, a panel of 58 paired normal–tumor DNAs from CCs were assayed for LOH using 25 polymorphic sequenced-tagged sites (STSs) mapped to chromosome 11 (5 on 11p and 20 on 11q; Table 1). The analysis revealed deletions in at least one locus in 46 (79%) tumors. Of these, 4 tumors (T-46, T-48, T-52, and T-114) showed loss of one allele at all informative loci, suggesting genetic monosomy of chromosome 11. The remaining 42 tumors showed LOH at one or more loci while retaining heterozygosity at the remaining loci, suggesting regional losses. These tumors with regional losses were used to identify a pattern of minimal deletions. The frequency of LOH varied among the markers from 14.6% (*D11S911* at 11q13) to 60.6% (*D11S4094* at 11q23.2; Table 1). The pattern of LOH identified multiple regions of discrete deletions both on the 11p and 11q. The frequency of LOH did not show any correlation with age, tumor stage, size, or histology.

Deletions on 11p. Twenty-three (39.7%) tumors showed deletions on the 11p, and 8 (4 with complete monosomy of chromosome 11) of these showed LOH at all informative loci, suggesting monosomy of 11p. The remaining 15 tumors exhibited partial deletions. The patterns of LOH identified two regions of minimal deletion, one at 11p15.11 and the other at 11p12–13. The 11p15 deletion spanned by the locus *D11S4189* showed LOH in 33.3% of informative cases where tumors T-29 and T-79 exhibited deletions while retaining heterozygosity at a proximal marker *D11S4099*. The second site of deletion at 11p12–13 spanned by the markers *D11S4200* and *D11S4083* exhibited LOH in 26 and 22%, respectively. This deletion was defined by tumors T-117 and T-75, with distal marker *D11S4096* retaining heterozygosity, whereas 3 other tumors (T-92, T-28 and T-29) exhibited LOH at *D11S4083* and retained heterozygosity at *D11S4200*. Thus, the patterns of LOH on 11p identified two discrete regions of deletions (Fig. 1).

Identification of 11q23 Minimal Deletion. The 11q LOH was observed in 40 of 58 (69%) tumors studied. In addition to 4 tumors with complete loss of chromosome 11, two others (T-105 and T-116) showed LOH at all informative loci on 11q, suggesting monosomy. The pattern of LOH in the remaining 34 tumors with interstitial allelic deletions on 11q identified one common region of loss at 11q23.1–q23.2 (Fig. 2). Of the 34 tumors with partial 11q LOH, 24 tumors exhibited deletions at 11q23.1–23.2, and the remaining 10 showed scattered deletions outside the 11q23 region (Fig. 2). The common deleted region at 11q23.1–q23.2 spanned the loci *D11S4167* (45.7% LOH), *D11S1353* (58.5% LOH), *D11S4094* (60.6% LOH), and *D11S4144* (46.8% LOH). Boundaries of this deletion flanked by the marker *D11S925* proximally with retention of heterozygosity in 4 tumors (T-16, T-28, T-40, and T-112) and *D11S933* distally with retention of heterozygosity in two tumors (T-110 and T-76; Fig. 3).

Mutation Analysis of the *PPP2R1B* Gene. Mutations in the *PPP2R1B* gene at 11q23 were earlier reported in lung and colon carcinomas as a target of deletions in this region (18). To determine whether the *PPP2R1B* gene is the target of 11q23 deletions in CCs, we performed mutation analysis of the entire coding region on a panel of 30 tumor DNAs that exhibited LOH on 11q23. All suspected SSCP variants were sequenced in both orientations to identify the nature of the mutations. This analysis identified three different types of sequence alterations in three tumors: the G → C transversion that changes Ala_{601} → Pro (T-98); G → A transition that changes Arg_{411} → Gly (T-28, T-92); and G → A transition that changes Gly_{90} → Asp (T-98). The Arg_{411} → Gly and Gly_{90} → Asp alterations in both

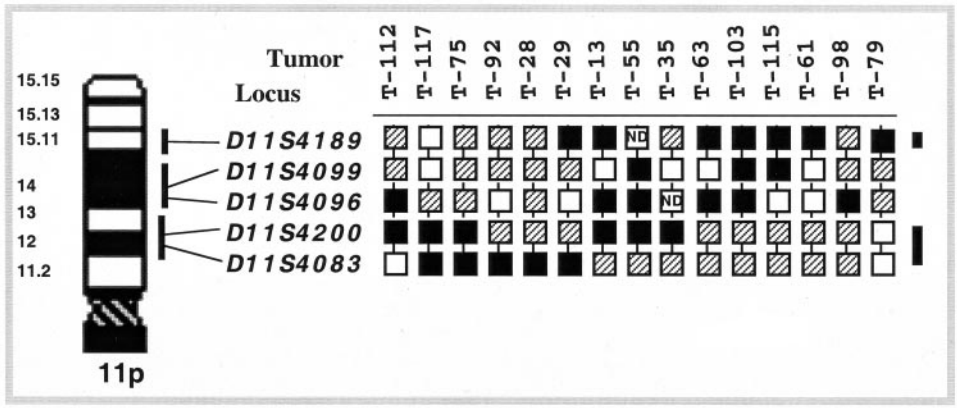

Fig. 1. Patterns of LOH on 11p in cervical carcinoma. Fifteen tumors exhibiting partial deletions are represented. *Left*, G-banded ideogram; *right* of *ideogram*, corresponding loci. Patterns of LOH are shown in *squared boxes* below tumor numbers. ■, LOH; ▨, retention of heterozygosity; □, homozygous and uninformative; *ND*, not done. *Black vertical bars* on the *right*, regions of minimal deletion.

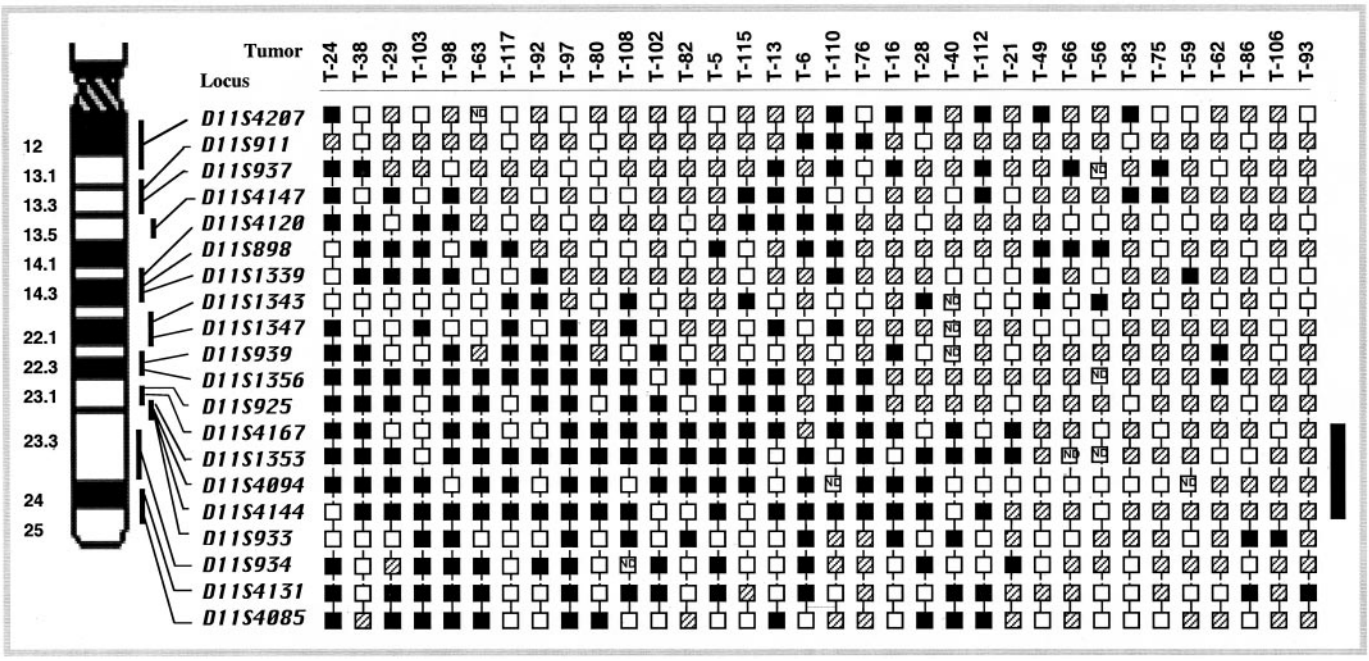

Fig. 2. Patterns of LOH on 11q in cervical carcinoma. Thirty-four tumors exhibiting partial deletions are represented. *Left*, G-banded ideogram; *right* of *ideogram*, corresponding polymorphic loci. Patterns of deletions shown in *squared boxes* below the tumor numbers. ■, LOH; ▨, retention of heterozygosity; □, homozygous and uninformative; *ND*, not done. *Black vertical bar* on the *right*, region of minimal deletion.

tumors were found to be homozygous, whereas the $Ala_{601} \rightarrow Pro$ was heterozygous. The changes $Ala_{601} \rightarrow Pro$ and $Arg_{411} \rightarrow Gly$ were found to be population variants because the similar alterations were also seen in the corresponding control and unrelated normal individuals (data not shown). The $Gly_{90} \rightarrow Asp$ change was also found to be present in the corresponding normal DNA from the patient T-98. The sequence analysis suggests that the wild-type (normal) allele have been deleted in the T-98 tumor DNA, consistent with the observation of LOH at 11q23 (Fig. 2). The residual GGC_{90} signals seen in T-98 DNA may represent contaminating normal cells in the tumor tissue compared with normal DNA from blood from the same patient (Fig. 4). Thus, the normal allele of the *PPP2R1B* is deleted by LOH in T-98, and the variant allele is retained in this tumor.

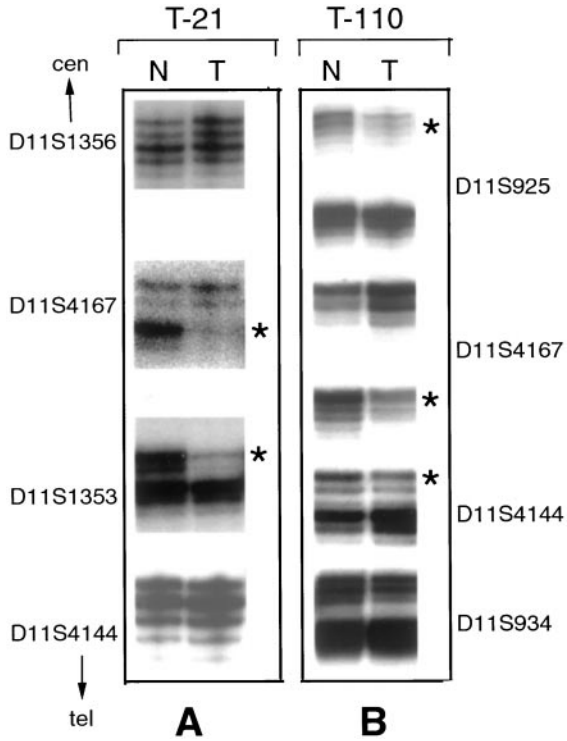

Fig. 3. Illustration of a common region of deletion at 11q23 by LOH analysis. Two cases, T-21 (*A*) and T-110 (*B*), are represented. Tumor T-21 showing LOH at *D11S4167* and *D11S1353*, while retaining the heterozygosity at a proximal *D11S1356* and distal *D11S4144* markers. Tumor T-110 shows LOH at all markers except the distal *D11S934*. *N*, normal; *T*, tumor; ∗, LOH; *cen*, centromere; *tel*, telomere. Tumor numbers are shown on *top* of each *panel*, and markers are shown on the *sides*.

DISCUSSION

Functional studies have identified that chromosome 11 carries a gene(s) responsible for tumor suppression in CC (11–14). Allelotype studies have further identified high frequency of LOH on 11q, consistent with the presence of one or more TSGs on this chromosomal arm (7–10, 15–16, 20). These studies, however, suffer from lack of extensive and systematic deletion mapping analysis in delineating the exact location of the TSG. In CC, only a few previous studies have performed detailed deletion mapping in an attempt to identify the critical regions of deletion. These studies identified at least two putative TSG sites at 11q13 and 11q22–24 (15, 17, 21). Hampton *et al.* (17) using 16 polymorphic markers on chromosome 11 in 32 patients have identified a minimal deleted region to a 35-cM genetic distance at 11q23. More recently, Mugica-Van Herckenrode *et al.* (21) have further restricted the region of deletion to a 19-cM genetic distance at the 11q23 region using only four markers at this region. These studies, thus, identified candidate TSG sites at 11q23 that may be critical for the tumor formation in cervix uteri.

To further define the critical regions of deletion on chromosome 11, we performed a systematic and high-density LOH mapping. The pattern of allelic losses in the present study identified three discrete regions of minimal deletions, two on 11p and one on 11q. The 11p deletions were localized at p15.11 (13 of 39 informative cases; 33.3% LOH) and p12–13 (17 of 52 informative cases; 32.7% LOH). The 11q deletion was localized at 11q23.1–23.2 (30 of 57 informative cases;

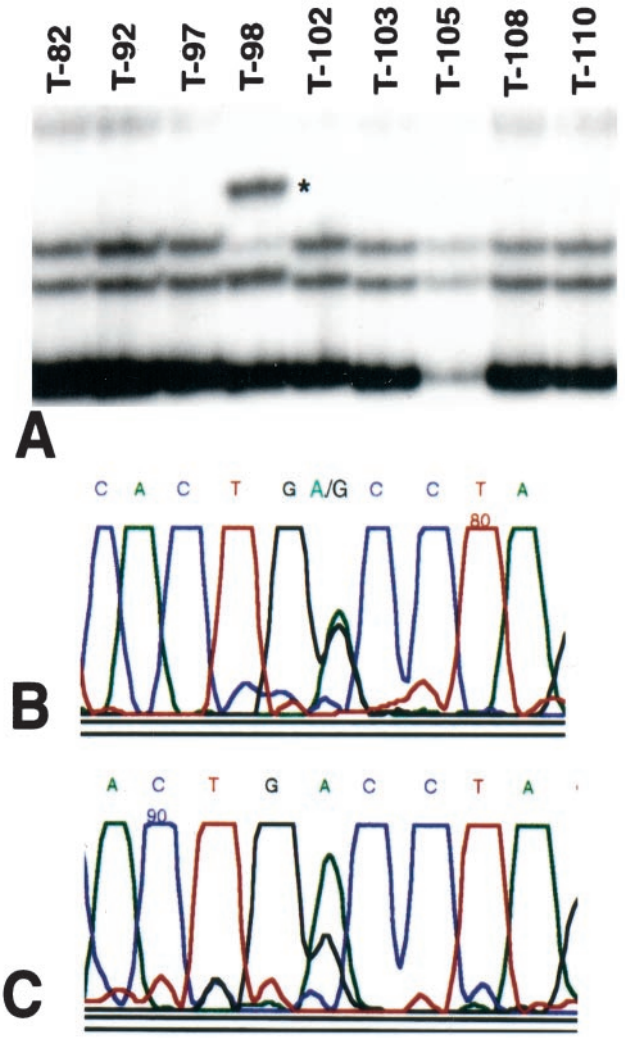

Fig. 4. Mutation analysis of the *PPP2R1B* gene in cervical carcinoma. *A*, PCR SSCP analysis of exon 3. Tumor numbers are indicated on *top*. ∗, conformational change. *B* and *C*, PCR sequence analysis of T-98. Heterozygosity of A/G at amino acid 90 in normal DNA (*B*) and $Gly_{90} \rightarrow$ Asp alteration in tumor (*C*) are shown.

52.6% LOH). The 11q23 region has been implicated previously in CC, whereas the 11p deletions have not been reported earlier. Although the frequencies of allelic deletions at 11p15 and 11p12–13 regions were low, the 11q23.1–23.2 region exhibited high frequency of LOH.

The short arm of chromosome 11 between 11p11 and 11p15 has been shown to contain genes controlling the regulation of HPV-16 viral early promoter (22). Whether the LOH seen at 11p in the present study plays any role in HPV-induced transformation remains to be seen. The 11p15 and 11p13 regions also exhibit genetic alterations in a variety of tumor systems (23). At present, the significance of the low frequency of LOH in these regions is not clear, and additional studies are required to understand the importance of these genetic alterations.

In the present study, the 11q23 region exhibited the highest frequency of LOH (60.6% LOH at *D11S4094*), suggesting that this region contains a TSG critical for CC development. A number of previous studies have also identified frequent LOH at 11q22–24 regions in CC (9–10, 17, 20–21).

The 11q22-q24 region has also been implicated in a variety of other solid tumor types including carcinomas of breast (24–29), lung (30, 31), colon (32), ovary (33, 34), and oral (35, 36) carcinomas, malignant melanoma (37, 38), and neuroblastoma (39). The chromosome 11q deletion maps generated by these studies have been reviewed, and the results are summarized in Fig. 5. That 11q22–23 is the most frequent target of LOH in solid tumors suggests that this region contains one or more TSGs. Furthermore, review of the previous studies revealed at least three distinct regions of deletions at 11q22–23, a proximal region targeting between 106.5 and 116 cM, a second region between 121 and 127 cM, and the distal region between 137 and 145 cM. The proximal deletion is frequently involved in breast, lung, and malignant melanomas, and the middle region is a target in cervical and breast carcinomas (Fig. 5). The third and the distal regions between 137 and 145 cM may be a target in colon, ovarian, and oral carcinomas. These data, thus, suggest that there are at least three TSGs that may be the target of 11q23 deletions in various solid tumor types.

The *PPP2R1B* gene, which maps to the proximal 11q22–23 region between 106.5 and 116 cM, encodes the β-isoform subunit of serine/threonine protein phosphatase 2A and was found to be mutated in 15% of lung and colon carcinomas (18). This region has also been implicated previously in CC (17, 21). To find out whether *PPP2R1B* is a candidate TSG for CC, we performed mutation analysis of the entire gene and found four cases with a single nucleotide alteration. Three of the four sequence alterations were confirmed as polymorphisms because they were also seen in matched normal DNAs and in unrelated controls. The fourth case with codon $GGA_{90} \rightarrow$ GAC alteration was also present in matched-normal DNA but not in 100 additional normal DNAs (data not shown), suggesting that this may represent a germ-line mutation. LOH analysis on this patient tumor DNA showed loss of a normal wild-type allele, suggesting that this may represent a pathogenic alteration. To see whether this change is inherited, we studied four members of the family (son, daughter, sister, and nephew) and found that the son and daughter inherited the same variant but not by the sister and nephew. The *PPP2R1B* $Gly_{90} \rightarrow$ Asp alteration was reported earlier in three tumors of non-small cell lung carcinomas as pathogenic alteration (18). In another recent study of ovarian carcinomas by Campbell and Manolitsas (40), this change was reported as a nonpathological polymorphism. Our data, however, support the view that the *PPP2R1B* $Gly_{90} \rightarrow$ Asp alteration may represent a germ-line change, and individuals carrying this change may be susceptible to developing cervical or other types of solid tumors. This, however, remains to be tested in families with inherited cancer. The present data, however, conclusively exclude *PPP2R1B* as a target of 11q23 somatic deletions in CC.

In the present study, we defined the minimal region of deletion at 11q23 in CC to span a 6-cM genetic distance between 123.4 and 129.5 cM interval (Fig. 5). This region is currently known to contain five genes. These include U90916 mRNA of unknown function, zinc finger protein 202 (*ZNF202*), LOH 11 chromosomal region 2 (*LOH11CR2A*), similar to heat shock cognate M_r 70,000 protein 10 (*HSC71*), and a protein kinase C substrate RC3 (neurogranin). Of these, the genes *LOH11CR2A* and *ZNF202* have been examined as 11q23 deletion targets in breast and lung carcinomas. Both the *LOH11CR2A* and *ZNF202* genes were identified in an effort to find a TSG in the region between loci *D11S1345* and *D11S1328* deleted in breast carcinoma (24, 41). However, no genetic alterations in the support of the tumorigenic potential of these genes could be established in breast carcinoma (24, 41). In addition to these known genes, at least 28 UniGene clusters were mapped to this interval of minimal deletion (Gene Map'99[5]).

In the present study, we have defined a minimal region of deletion

[5] Internet address: http://www.ncbi.nlm.nih.gov/genemap/map.cgi?CHR=1.

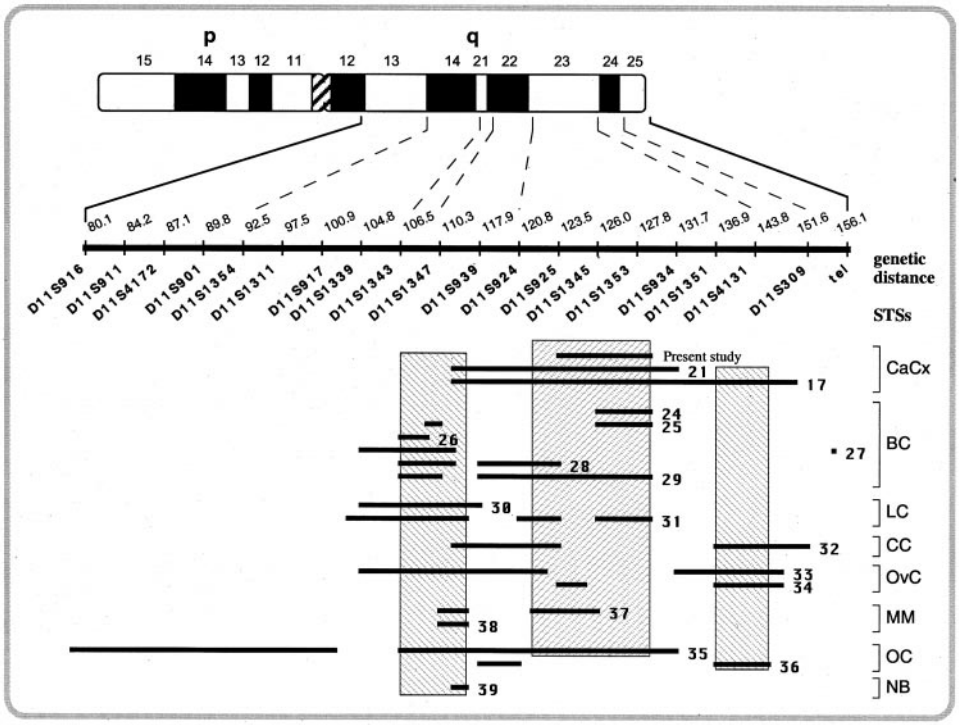

Fig. 5. Summary of 11q LOH studies identifying minimal deleted regions in solid tumors. The chromosome 11 ideogram is shown on *top*, a genetic map of the long arm along with landmark markers is shown *below* it, and minimal regions derived from various studies (indicated by *horizontal bars* and the corresponding reference on the *right*) are shown *below* the genetic map. *CaCx*; cervical cancer; *BC*, breast cancer; *LC*, lung carcinoma; *CC*, colon cancer; *OvC*, ovarian carcinoma; *MM*, malignant melanoma; *OC*, oral carcinoma; *NB*, neuroblastoma. Three common regions of deletions are shown in *shadowed vertical boxes*.

at 11q23 in CC that may contain the critical TSG and excluded a previously known tumor suppressor *PPP2R1B* as a target of 11q23 deletions in CC. The current definition of minimal deletion with the available genomic resources in this region should aid in the identification of the candidate TSG.

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