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Advances in Brief

Loss of Heterozygosity on the Long Arm of Chromosome 11 in Nasopharyngeal Carcinoma[1]

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Abstract

Loss of heterozygosity (LOH) on the long arm of chromosome 11 had been reported in many types of solid tumors. In this study, we investigated the LOH patterns of chromosome 11 on 52 primary nasopharyngeal carcinomas using 10 microsatellite polymorphic markers. The results revealed that 28 of the 52 cases (53.8%) demonstrated LOH on at least one of the nine 11q microsatellite loci studied. The highest frequencies of LOH were found at the two loci *D11S2000* (36.1%) and *D11S934* (34.5%), both located at 11q22–24. Two distinct regions of deletion were found at 11q, with the first one defined by *INT-2* and *D11S900* at 11q13.3–22, and the second region located between *D11S2000* and *D11S934* at 11q22–24. The two deletion regions overlap with the common areas of deletion reported in other tumor types. This suggests the presence of multiple putative tumor suppressor genes on chromosome 11q that may play a role in the development of nasopharyngeal carcinomas.

Introduction

Tumor suppressor genes have been implicated in the development of several types of solid tumors and have been shown to be related to chromosomal rearrangements, particularly deletions (1). Detection of LOH[3] has been used to identify regions of the genome that may contain tumor suppressor genes in various human cancers. LOH on chromosome 11 is one of the common genetic aberrations in many tumors (2–5). Regions of LOH at chromosome 11q are frequently reported in different types of solid tumors such as breast cancer, ovarian cancer, and adenocarcinoma of the lung (2–4). Moreover, amplification of 11q13 had also been identified in breast cancer (6). NPC is uncommon in western countries but is one of the common cancers among the Southern Chinese. Only a small number of studies have been conducted to investigate the genetic alterations of NPC. Our previous studies have demonstrated frequent allelic loss on the short arm of chromosome 3p and that of chromosome 9, and homozygous deletion of the *p16* gene (7–9). This suggests that inactivation of multiple tumor suppressor genes may play a role in the development of this cancer.

Our previous study on the cytogenetic analysis of NPC xenografts had demonstrated rearrangement of chromosome 11q (10). In this study, we use nine microsatellite polymorphic markers of the long arm of chromosome 11 to study the LOH patterns of 52 NPC tumors. Of the cases studied, 53.8% demonstrated LOH on at least one of the microsatellite loci. Two distinct regions of LOH were found at 11q13.3–22 and 11q22–24. These two regions are the same as the common regions of LOH observed in other tumor types (11–15), suggesting the presence of multiple tumor suppressor genes at the long arm of chromosome 11.

Materials and Methods

Patients and Specimens. Primary NPC biopsies from 52 patients were obtained prior to treatments: 10 from the Cancer Institute, Sun Yat-Sen University of Medical Sciences Cancer Centre (Guangzhou, China) and the rest from the Prince of Wales Hospital, The Chinese University of Hong Kong (Shatin, Hong Kong). Corresponding blood samples of these patients were used as constitutional DNA controls. High molecular weight DNA was extracted from both the tumor and blood samples according to conventional methods (16).

Microsatellite Polymorphism Analysis. Nine microsatellite polymorphic markers of the long arm of chromosome 11 were used to examine LOH at the following loci using PCR analyses: *D11S913* (11q13), *INT-2* (11q13.3), *D11S901* (11q14), *D11S873* (11q14.3–21), *D11S900* (11q22–23), *D11S2000* (11q22–23), *D11S934* (11q23.3–24), *D11S912* (11q25), and *D11S968* (11q24.1–25). The sequences of the primers and chromosomal localization were according to the Genome Data Base (The Johns Hopkins University). An additional pair of primers mapping to the short arm of chromosome 11, *D11S926* (11p15.4), was included. These polymorphic markers were purchased from Research Genetics, Inc. (Huntsville, AL). PCR reactions were conducted in a DNA thermal cycler (Perkin Elmer/Cetus, Norwalk, CT), and 30 amplification cycles were performed. Each cycle included denaturation at 94°C for 40 s, annealing at 53–56°C for 30 s, and extension at 72°C for 30 s. The annealing temperature for each pair of primers was optimized to give distinct bands of alleles with minimal background signals. In each reaction, 0.25 pmol of left primer was first end labeled with [γ-^{32}P]ATP (Amersham Corp., Buckinghamshire, United Kingdom) and T4 polynucleotide kinase (Amersham Corp.). PCR was then performed in 5 μl of reaction volume containing the labeled left primer, another 0.25 pmol of right primer, 62.5 μM each of deoxynucleotide triphosphates, 1.5 mM $MgCl_2$, 0.25 units *Taq* polymerase (Perkin Elmer/Cetus), and 50–100 ng extracted DNA in 1× PCR buffer. The PCR products were mixed with 45 μl buffer containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF (Sigma, St. Louis, MO), heated at 95°C for approximately 10 min, and resolved by electrophoresis through 6% denaturing polyacrylamide gel containing 7 M urea. Afterward, gels were transferred onto paper supports followed by exposure to Kodak X-OMAT AR film for 4 h to 1 day at room temperature. In most cases, allelic imbalances were inferred visually when there were visible differences in the ratio of allele radiographic signal intensities in the tumor samples and the corresponding blood samples. For the rest of the cases, ultrascan laser densitometer (Pharmacia LKB, Uppsala, Sweden) was used to detect the intensity variances. Allelic imbalances were assigned when the allelic ratio differences of tumor and blood control were greater than 30%, as described previously (7, 8).

Multiplex PCR Analysis. Multiplex PCR analysis of microsatellite polymorphism was conducted to determine whether the allelic imbalances found were due to allelic amplification or LOH. The DNA samples were investigated for the loci on 11q13.3–23 at *D11S913*, *INT-2*, *D11S901*, *D11S873*, *D11S900*, and *D11S2000* as above. An additional primer pair (either *MPO* on chromosome 17q or *D14S81* on chromosome 14q) was included as an internal control. The control primer was chosen depending on the size of the microsatellite primers used. The PCR products of the tumor sample were serially diluted and

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[3] The abbreviations used are: LOH, loss of heterozygosity; NPC, nasopharyngeal carcinoma.

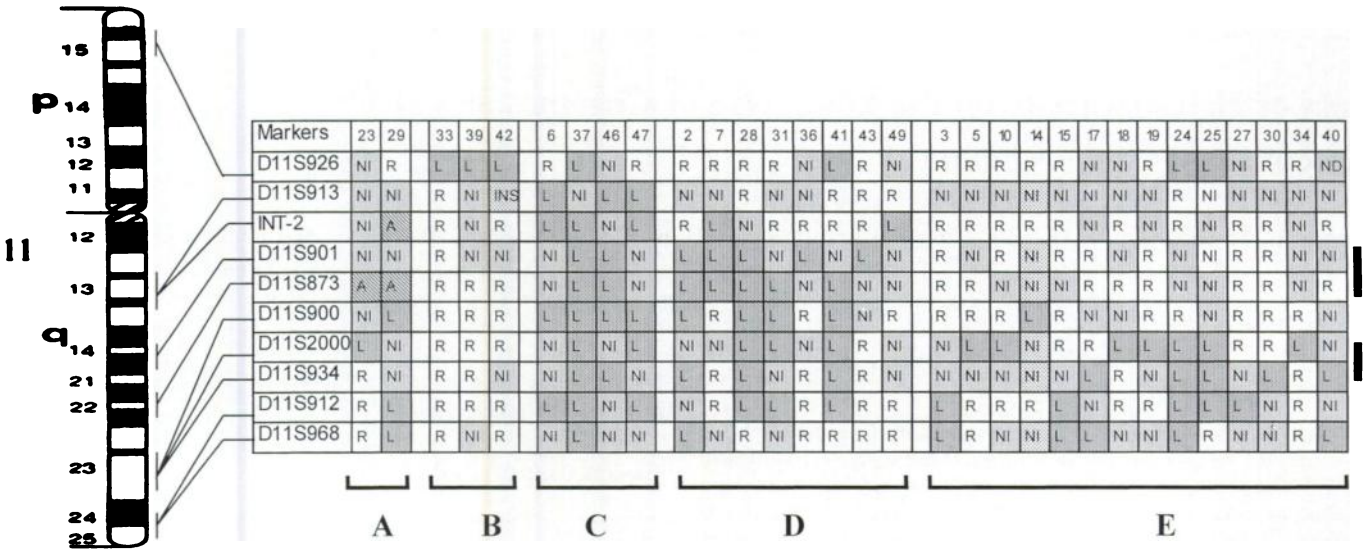

Fig. 1. Results of LOH analysis on 31 primary NPC samples demonstrated allelic imbalances on chromosome 11. *Top*, case numbers. *Left*, studied microsatellite polymorphic markers. *Bars on the right*, regions of deletion. Group A, tumors with amplification; group B, tumors with LOH on 11p only; group C, tumors showed allelic loss of all informative loci of 11q; group D, tumors defined the first deletion region; group E, tumors defined the second deletion region. *A*, amplification; *NI*, noninformative; *INS*, instability; *L*, LOH; *R*, retention of heterozygosity; *ND*, not determined.

resolved using electrophoresis with the corresponding PCR products of normal DNA. An optimal dilution was chosen when the intensities of the internal control marker in both tumor and normal samples were identical and normalized for the comparison of the allele intensities of a target marker in tumor DNA *versus* normal DNA.

Results

DNAs from 52 undifferentiated primary NPCs and corresponding blood samples were assessed for LOH at chromosome 11. Nine microsatellite polymorphic markers mapping to the long arm and one to the short arm were included in the study. The results of LOH analysis on the tumor samples are shown in Fig. 1. Twenty-eight of the 52 cases (53.8%) showed allelic imbalances on at least one of the loci at the long arm, and 7 cases showed allelic imbalances at loci *D11S926* of the short arm. Amplifications were found at 2 (cases 23 and 29) of the 52 cases (3.8%) at the locus *INT-2* or *D11S873*. One case of instability (case 42) was found in the locus *D11S913*. Table 1 shows the frequencies of LOH at different loci. The highest frequencies of LOH were found at region 11q22–24, with 36.1% at *D11S2000* and 34.5% at *D11S934*.

Multiplex PCRs with the internal control primers *MPO* or *D14S81* were performed at loci *D11S913*, *INT-2*, *D11S901*, *D11S873*, *D11S900*, and *D11S2000* to determine the regions of amplification. Case 29 demonstrated amplification at *INT-2* and *D11S873*, but LOH on the locus *D11S900* (group A, Figs. 1 and 2). In case 23, amplification was shown at locus *D11S873*, whereas LOH was found at *D11S2000* (Figs. 1 and 2). The rest of the allelic imbalances were confirmed to be LOH.

Table 1 *Frequencies of LOH of all of the studied tumors in the chromosome 11 polymorphic loci*

Markers	Location	No. of tested cases	No. of informative cases	No. of LOH	% of LOH
D11S926	11p15.4	49	37	7	18.9
D11S913	11q13	52	17	3	17.6
INT-2	11q13.3	52	38	5	13.2
D11S901	11q14	52	26	7	26.9
D11S873	11q14.3–21	52	36	7	19.4
D11S900	11q22–23	52	40	10	25.0
D11S2000	11q22–23	51	36	13	36.1
D11S934	11q23.3–24	52	29	10	34.5
D11S912	11q25	52	44	12	27.3
D11S968	11q24.1–25	52	30	8	27.7

PCR analysis on another locus (*D11S926*) of the short arm of chromosome 11 was also performed to investigate whether the region of loss is only restricted to the long arm. Among the 27 informative cases, only 7 cases (18.9%) showed LOH, as referred to in Fig. 1. Four of them (cases 24, 25, 37, and 41) had LOH on both the p and q arms, and the other three cases (cases 33, 39, and 42) had LOH on the p arm only (Fig. 1, group B). Cases in group C in Fig. 1 showed allelic losses on all of the informative loci of 11q. Case 37 demonstrated allelic loss on both the short arm locus and all informative loci of the long arm. Hence, chromosome 11 of case 37 is suspected to be completely lost.

Our study of LOH on chromosome 11 defined two distinct deletion regions. The first common region of loss was defined by the eight cases in group D in Fig. 1. The region of allelic loss in case 2 included the loci *D11S901*, *D11S873*, and *D11S900*, whereas cases 36 and 43 showed LOH on the locus *D11S901* (Figs. 1 and 3). All three of these cases retained heterozygosity at *INT-2*, which defined the proximal margin of the region of loss. Case 7 showed LOH at *INT-2*, *D11S901*, and *D11S873* but retained heterozygosity at the locus *D11S900* (Figs. 1 and 3). This suggested that the deletion region is located at 11q13.3–22 and lies between *INT-2* (11q13.3) and *D11S900* (11q22–23). It includes loci *D11S901* and *D11S873*. Nine of the 28 cases (32.1%) demonstrated loss at either one or both loci *D11S901* and *D11S873*. LOH frequencies at these two loci are 26.9% for *D11S901* and 19.4% for *D11S873*.

The other distinct region of deletion is located at the telomeric part of the long arm. This region of loss is defined by the 14 cases in group E in Fig. 1. Both cases 17 and 30 showed LOH at the locus *D11S934* (11q23–24) but retained heterozygosity at *D11S2000*. On the other hand, cases 18, 23, and 34 showed LOH at *D11S2000* (11q22–23) and retained heterozygosity at locus *D11S934*. Hence, this second deletion region is located between *D11S2000* and *D11S934* at 11q22–24 as indicated in Figs. 1 and 3. Eighteen of the 52 cases (34.6%) showed loss at one or both loci *D11S2000* and *D11S934*. LOH frequencies at *D11S2000* and *D11S934* were 36.1% and 34.5%, respectively.

Discussion

We have investigated the LOH pattern along the long arm of chromosome 11 of 52 primary NPC tumors with nine polymorphic microsatellite markers. LOH was observed in 53.8% of the 52 NPC tumor samples. Two distinct regions of deletion at 11q13.3–22 and 11q22–24 were found. The loci *INT-2* and *D11S900* defined the first deletion region. The second region lies between loci *D11S2000* and *D11S934*. Multiple independent LOH regions had been previously reported in breast cancer and adenocarcinoma of the lung (3, 12). Our results demonstrated that NPCs share with these solid tumors of similar multiple genetic alterations on chromosome 11q.

The first region of loss is defined by *INT-2* and *D11S900* at 11q13.3–22, consisting of loci *D11S901* and *D11S873*. This region is within the 11q13–23.3 deletion region identified in the carcinoma of bladder (17) as in Fig. 4. Furthermore, transfer of the normal chromosome region 11q13-q23 into the MCF-7 breast cancer cell line had been shown to decrease the tumorigenicity of that cancer cell line in nude mice significantly (18). A putative tumor suppressor gene may thus be located in this common deletion region and play a role in the development of NPC as in other human cancers. LOH on chromosomal 11q13 (at the locus *INT-2*) was more frequently reported in multiple endocrine neoplasia type 1-associated tumor, *in situ* and invasive carcinoma of the female breast, and in association with progression of male and female breast cancer (5, 13, 14). No previous study had been conducted to investigate this deletion region in detail. Hence, LOH studies on more cases of primary NPC can be held to confirm the boundaries of the region of loss in NPCs. Previous cytogenetic studies of an undifferentiated NPC xenograft showed the rearrangement of 11q13-qter in a NPC xenograft (10). Cytogenetic data on a newly established NPC cell line in our laboratory (cell-666) demonstrated consistent loss of the long arm of chromosome 11, starting from around the 11q13 region to qter.[4] These data further support that deletion of 11q is common in NPC.

The proto-oncogene *INT-2* and genes *PYGM*, *cyclin D1*, and *FOLR1* are located on region 11q13, which demonstrates amplification in many tumors (5, 13). It was also reported that amplification of INT-2/FGF3 and CCND1 in 11q13 might play an important role in

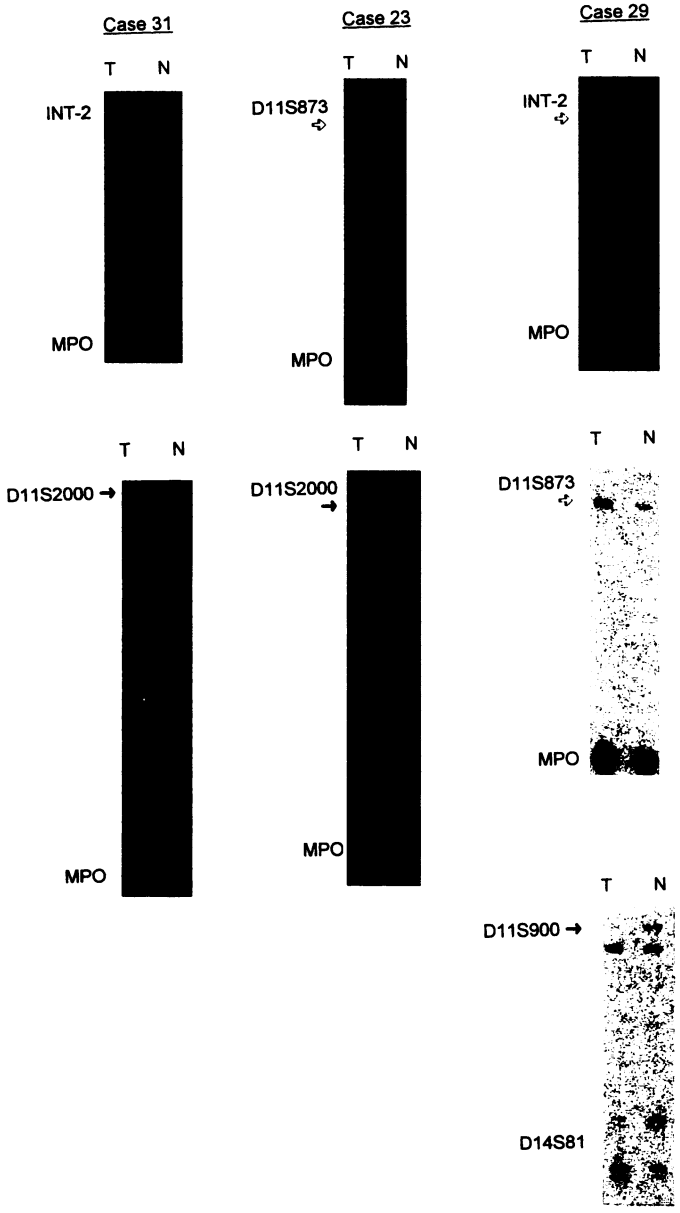

Fig. 2. Illustration of the multiplex PCR results. *Top*, tumor sample numbers. *Left*, studied microsatellite polymorphic markers. ⇨, amplification; →, LOH. *T*, tumor; *N*, normal.

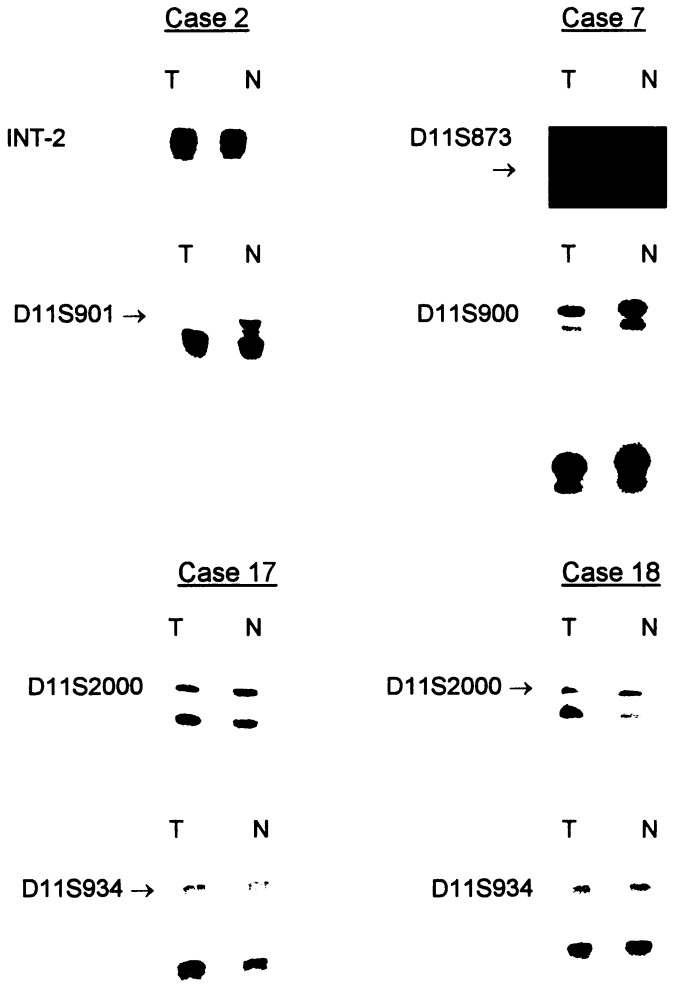

Fig. 3. Illustration of the LOH pattern on chromosome 11 in representative tumor samples. *Top*, case numbers; *left*, studied loci. →, allelic loss in tumor samples. *T*, tumor; *N*, normal.

[4] Unpublished data.

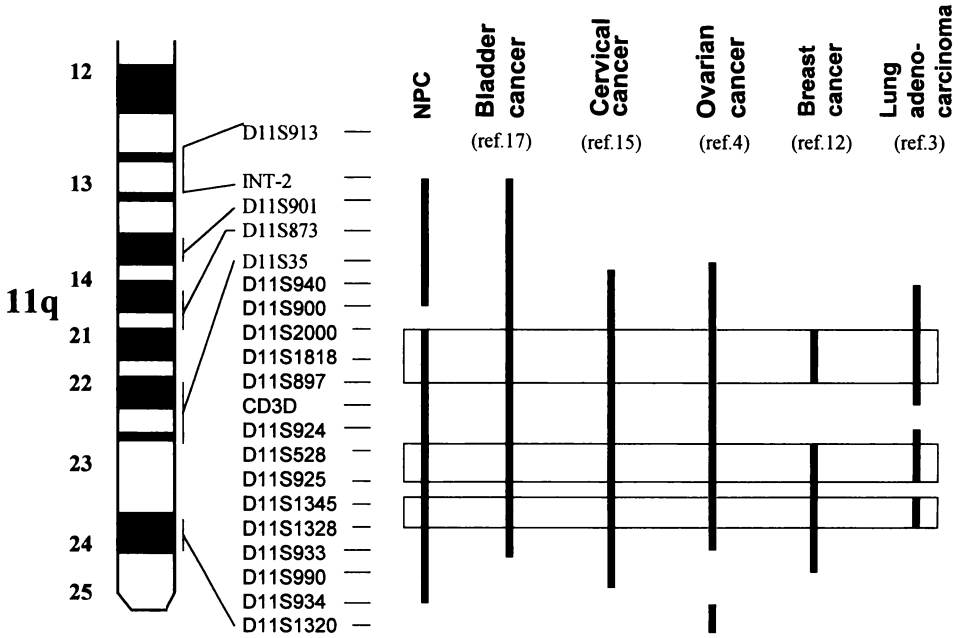

Fig. 4. Deletion pattern of chromosome 11q in various solid tumors. The order of the microsatellite markers is according to Refs. 3 and 12 and the Genome Data Base (The Johns Hopkins University). *Numbers in parentheses*, quoted publications. *Dark black lines*, regions of deletion. The *three rectangles* define shared deletion regions (*D11S2000-D11S897*, *D11S528-D11S925*, and *D11S1345-D11S1328*, respectively).

local relapse of breast cancer (6). The gene of the adult high-affinity folate receptor was found to map to region 11q13.3–13.5 and reported to be amplified in ovarian cancer (5). Amplification at chromosome 11q13 had been reported as one of the main genetic abnormalities in breast tumors (6). This 11q13 region is thought to contain oncogenes involved in the development of many tumors. However, amplification of the 11q13 region appears to be a rare event in NPC. Hence, more studies are required to further define the significance of amplification of this region in NPC tumorigenesis.

The frequency of LOH in the telomeric region of the long arm of chromosome 11 is much higher than that of the q13–14 region in NPC. In our study, a distinct deletion region was found at 11q22–24 between the loci *D11S2000* and *D11S934*. The 11q22–24 region showed the highest frequencies of LOH among the nine microsatellite polymorphic loci in the NPC tumors. This deletion region overlapped with that of bladder cancer (17). LOH on 11q22–24 has been identified in cervical carcinoma (15) and ovarian cancer (4). Deletion mapping of breast cancer defined two distinct deletion regions within 11q22–23 (12). Moreover, three independent deletion regions at 11q22–24 were reported in lung adenocarcinoma (3). Fig. 4 compared the deletion pattern at 11q of NPC and the aforementioned solid tumors. Three common deletion regions at 11q22–24 were summarized from these solid tumors. The first one lay between *D11S2000* and *D11S897*, as indicated by one of the deletion regions in breast tumors (12). The second deletion region has been defined between *D11S528* and *D11S925* from the studies of breast and lung cancers (3, 12). The more telomeric one has been bracketed by the loci *D11S1345* and *D11S1328*. It overlapped with one of the regions that showed LOH in lung adenocarcinoma (3). Since all three of these common deletion regions are overlapped with that of NPC, multiple putative tumor suppressor genes which locate between *D11S2000* and *D11S934* are suggested to be involved in the development of NPC.

The common deletion region (11q22–23) was found to coincide with the genomic location of the *ATM* gene that is involved in the cancer-predisposing, DNA repair deficiency syndrome AT (1, 19). Ataxia telangiectasia heterozygotes are suggested to have an increased risk of cancer (12, 19). Hence, the *ATM* gene is located between the loci *D11S2000* and *D11S934*, which showed the highest frequencies of LOH in our NPC samples. Hence, the *ATM* gene may be one of the candidate tumor suppressor genes on chromosome 11q that is involved in the development of NPC. It is proposed to use more microsatellite polymorphic markers and NPC samples to refine the mapping of this LOH. Moreover, the alteration of the *ATM* gene in NPC will be investigated.

Our study demonstrated two distinct common regions of LOH at the long arm of chromosome 11 at q13.3–22 and q22–24 in primary NPC. This suggests the presence of multiple putative tumor suppressor genes on chromosome 11q, which may act together with other genetic alterations such as chromosomal deletions of 3p and 9p and homozygous deletion of the *p16* gene in a multistep process leading to the development of this cancer.

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