

High Frequency of Chromosome 3p Deletion in Histologically Normal Nasopharyngeal Epithelia from Southern Chinese¹

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Abstract

We have examined the presence of loss of heterozygosity (LOH) on chromosome 3p in histologically normal nasopharyngeal epithelia (NP), dysplastic lesions, and carcinoma of the nasopharynx from different ethnic and geographic regions. Microdissected normal NP from noncancerous individuals and nasopharyngeal carcinoma (NPC) samples from both the high-risk group (southern Chinese in Hong Kong) and two low-risk groups for NPC (central/northern Chinese in Anhui/Beijing and Caucasians in Toronto) were included. All NPC samples showed high incidence of 3p deletion (81–100%). High frequencies of LOH on 3p were also detected in normal NP (73.9%) and dysplastic lesions (75%) from the southern Chinese. Significant lower frequency of LOH on 3p was noted in normal NP from the low-risk groups (20%) than those from high-risk groups ($P = 0.0003$). The presence of such genetic alterations in the histologically normal NP and dysplastic lesions suggests that it is an early event in tumor development. The higher frequency of 3p LOH found in normal NP from southern Chinese compared with those from low-risk groups may be related to the distinct cancer incidence among these populations.

Introduction

NPC³ is a rare cancer in most parts of the world, and the incidence of this cancer in Caucasians is <1 per 100,000 persons/year. The highest incidence of the disease is observed in Cantonese who lives in south China including Hong Kong. Males in this high-risk region exhibit an incidence of over 23 per 100,000 persons/year. In females, the incidence is about 8.7 per 100,000 per year. For Chinese in the central and northern part of China, the incidence rate drops to 1–4 per 100,000 per year (1, 2). Geographic and ethnic distribution of NPC suggests that the disease is associated with specific genetic and environmental factors. Etiological studies have also suggested the disease might be acquired by inheritance (1, 2). Individuals who have specific HLA haplotypes were found to have a higher risk of NPC (3). It was reported that individuals homozygous for the C2 allele of the *CYP2E1* gene have increased risks of NPC (4). *CYP2E1* is involved in the metabolic activation of various procarcinogens including nitrosamines. Case control studies in Hong Kong and Guangzhou both demonstrated that the consumption of traditional diets of southern

Chinese such as salted fish increases the risk, the consumption of which in childhood is the major determinant of future cancer risk of NPC (5, 6). In addition, studies demonstrated that malignant nasal cavity tumors were inducible in rats fed with salted fish (7). It was suggested that the volatile nitrosamines identified in this diet might be the major carcinogens affecting the southern Chinese population (1). Other environmental factors such as tobacco smoke may also increase the risk of NPC (8). Previous studies have demonstrated that the development of NPC may involve EBV latent infection and multiple genetic changes including deletion of chromosomes 3p, 9p, 11q, 13q, and 14q (2, 9–14). The highest frequency of 3p LOH suggested that the inactivation of tumor suppressor gene(s) in this chromosome might be an early event in the tumorigenesis of NPC. By the monochromosome transfer study in NPC cells, a growth-suppressive role of gene(s) on chromosome 3p in NPC tumorigenesis has been identified (15). The loss of 3p seems to play a crucial role in the development of this cancer. In the present study, we aimed to investigate the frequency of molecular abnormalities in the NP and NPCs from different geographic regions. We have examined the LOH of 3p in various archived specimens of the nasopharynx including normal NP from noncancerous individuals in the high- and low-risk regions.

Materials and Methods

Tissue Samples. One hundred and thirteen specimens were collected from three different geographic regions. In Hong Kong (a high-risk region), 62 archival specimens were obtained from the Pathology Tissue Bank in the Department of Anatomical and Cellular Pathology at the Prince of Wales Hospital. They include 10 histologically normal NP from aborted fetuses, 23 NP epithelia, 8 dysplastic lesions, and 21 undifferentiated NPC. The dysplastic lesions consisted of 4 low-grade dysplasias and 4 high-grade dysplasias. Fifty-one nasopharyngeal samples were collected from the low-risk regions. Sixteen normal NP from noncancerous individuals and 12 undifferentiated NPCs were collected from the Department of Pathology, Anhui Provincial Hospital, Anhui, and from the Department of Pathology, Chinese PLA General Hospital, Beijing, China. Fourteen normal NP and 9 undifferentiated NPCs were collected from the Department of Laboratory Medicine-Pathology, St. Michael's Hospital, University of Toronto. All of the specimens were subjected to histological diagnosis by a pathologist (K. F. T.).

The gender and age of the above patients are listed in Table 1. The age ranges of the NPC patients from Hong Kong, Anhui/Beijing, and Toronto were 31–73 (mean, 50.9), 25–67 (mean, 49.3), and 35–74 (mean, 54.6), respectively. For the normal NP from the three different geographic regions, the ages of noncancerous healthy individuals were 11–94 (mean, 45.9), 18–50 (mean, 35.3), and 27–80 (mean, 57.4), respectively. The male:female ratios of NPC and normal NP samples from Hong Kong, Anhui/Beijing, and Toronto are shown in Table 1.

DNA Extraction. Five- μ m sections were lightly stained with hematoxylin and then microdissected using a fine needle under a dissection microscope. The adjacent stromal cells, lymphocytes, or glandular tissues were also collected as a source of normal DNA. At least 1×10^3 cells were isolated from each microdissected sample. The microdissected tissues were suspended in 40 μ l of

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³ The abbreviations used are: NPC, nasopharyngeal carcinoma; NP, nasopharyngeal epithelia; LOH, loss of heterozygosity; EBER, EBV-encoded RNA; ISH, *in situ* hybridization.

Table 1 Chromosome 3p LOH and distribution of sex and age in neoplastic and nonneoplastic nasopharyngeal tissues from high-risk and low-risk regions for NPC

	Normal NP ^a				NPC ^c			
	Case no.	Gender	Age	3p LOH ^b	Case no.	Gender	Age	3p LOH ^b
High-risk region								
Hong Kong (southern Chinese)	S-NP1	F	27	+	S-NPC1	M	45	+
	S-NP2	M	41	-	S-NPC2	M	35	+
	S-NP3	F	11	+	S-NPC3	M	45	+
	S-NP4	F	17	+	S-NPC4	M	35	+
	S-NP5	M	15	+	S-NPC5	F	73	+
	S-NP6	F	16	+	S-NPC6	M	35	+
	S-NP7	M	19	+	S-NPC7	M	70	+
	S-NP8	F	26	-	S-NPC8	M	43	+
	S-NP9	M	44	+	S-NPC9	M	31	+
	S-NP10	M	38	+	S-NPC10	M	50	+
	S-NP11	F	33	+	S-NPC11	M	41	+
	S-NP12	M	39	-	S-NPC12	M	65	+
	S-NP13	M	55	+	S-NPC13	M	36	+
	S-NP14	F	79	-	S-NPC14	F	71	+
	S-NP15	M	79	+	S-NPC15	M	43	+
	S-NP16	M	68	-	S-NPC16	M	66	+
	S-NP17	F	42	+	S-NPC17	F	51	+
	S-NP18	M	94	+	S-NPC18	M	69	+
	S-NP19	M	85	+	S-NPC19	M	52	+
	S-NP20	M	46	+	S-NPC20	M	69	+
	S-NP21	M	69	+	S-NPC21	M	44	+
	S-NP22	M	41	-				
	S-NP23	F	72	+				
		M:F, 14:9	Mean, 45.9	17/23 (73.9%)		M:F, 18:3	Mean, 50.9	21/21 (100%)
Low-risk region								
Anhui/Beijing (northern Chinese)	N-NP1	M	28	-	N-NPC1	M	47	+
	N-NP2	M	50	-	N-NPC2	M	67	+
	N-NP3	F	30	-	N-NPC3	M	40	+
	N-NP4	F	36	+	N-NPC4	M	53	+
	N-NP5	M	46	-	N-NPC5	M	35	+
	N-NP6	F	34	-	N-NPC6	M	39	+
	N-NP7	M	34	+	N-NPC7	F	50	+
	N-NP8	M	18	-	N-NPC8	M	25	-
	N-NP9	M	11	-	N-NPC9	M	60	+
	N-NP10	M	42	-	N-NPC10	M	58	-
	N-NP11	M	76	-	N-NPC11	M	62	+
	N-NP12	M	54	-	N-NPC12	M	56	+
	N-NP13	M	53	+				
	N-NP14	M	55	-				
	N-NP15	M	45	-				
	N-NP16	M	54	-				
Toronto Canada (Caucasians)	C-NP1	F	76	-	C-NPC1	M	37	+
	C-NP2	M	66	-	C-NPC2	M	74	+
	C-NP3	F	69	-	C-NPC3	M	63	+
	C-NP4	M	55	+	C-NPC4	M	59	+
	C-NP5	F	68	-	C-NPC5	M	48	+
	C-NP6	F	64	-	C-NPC6	M	55	+
	C-NP7	F	61	-	C-NPC7	F	66	+
	C-NP8	M	80	-	C-NPC8	F	54	-
	C-NP9	F	57	-	C-NPC9	M	35	-
	C-NP10	M	31	-				
	C-NP11	M	41	+				
	C-NP12	F	27	-				
	C-NP13	F	67	+				
	C-NP14	F	42	-				
		M:F, 18:12	Mean, 49	6/30 (20%)		M:F, 18:3	Mean, 51.6	17/21 (81%)

^a High-risk region, *n* = 23; Low-risk region, *n* = 30.

^b +, LOH on at least one microsatellite marker on chromosome 3p; -, no LOH at all informative loci on chromosome 3p.

^c High-risk region, *n* = 21; Low-risk region, *n* = 21.

1× TK buffer [10× contains 5% Tween 20, 0.5 M Tris/HCl (pH 8.9), 20 mM EDTA, 10 mM NaCl, and 2 mg/ml proteinase K]. The samples were then incubated at 55°C for 3 days, followed by heating at 95°C for 10 min to inactivate the enzyme. Samples were briefly centrifuged, and 1 μl of supernatant was used for PCR-based microsatellite analysis.

Microsatellite Analysis. A total of five microsatellite polymorphic markers were analyzed at loci on chromosomes 3. These include *D3S659*, *D3S1228*, *D3S1067*, *D3S1076*, and *D3S1038* mapping to the regions 3p13, 3p14.1–14.3, 3p14.3–21.1, 3p21.1, and 3p25, respectively. The sequences of the primers and chromosomal localization were obtained from the Genome Database (The John Hopkins University, Baltimore, MD). Prior to PCR, 0.25 pmol of the forward primers were end-labeled with [γ -³²P]ATP (Amersham Corp., Buckinghamshire, United Kingdom) and T4 polynucleotide kinase (Amersham Corp.). One μl of the DNA was amplified by PCR in a final reaction volume of 5 μl

containing 62.5 μM each deoxynucleotide triphosphate, 3 μM MgCl₂, 1× PCR buffer (Applied Biosystems, Foster City, CA), and 0.25 unit AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). An initial enzyme activation step of 95°C for 12 min was followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 50–60°C for 1 min, extension at 72°C for 1 min, and then a final extension at 72°C for 7 min. The annealing temperature for each pair of primers was optimized to give distinct patterns of alleles with minimal nonspecific signal. The products were resolved by electrophoresis through a 6% denaturing polyacrylamide gel containing 7 M urea and exposed to Kodak X-OMATAR film for 12–36 h. LOH was initially scored by visual inspection when there was a 50% decrease in band intensity when comparing with the normal alleles. In some cases, the assessment of LOH was confirmed by densitometric analysis.

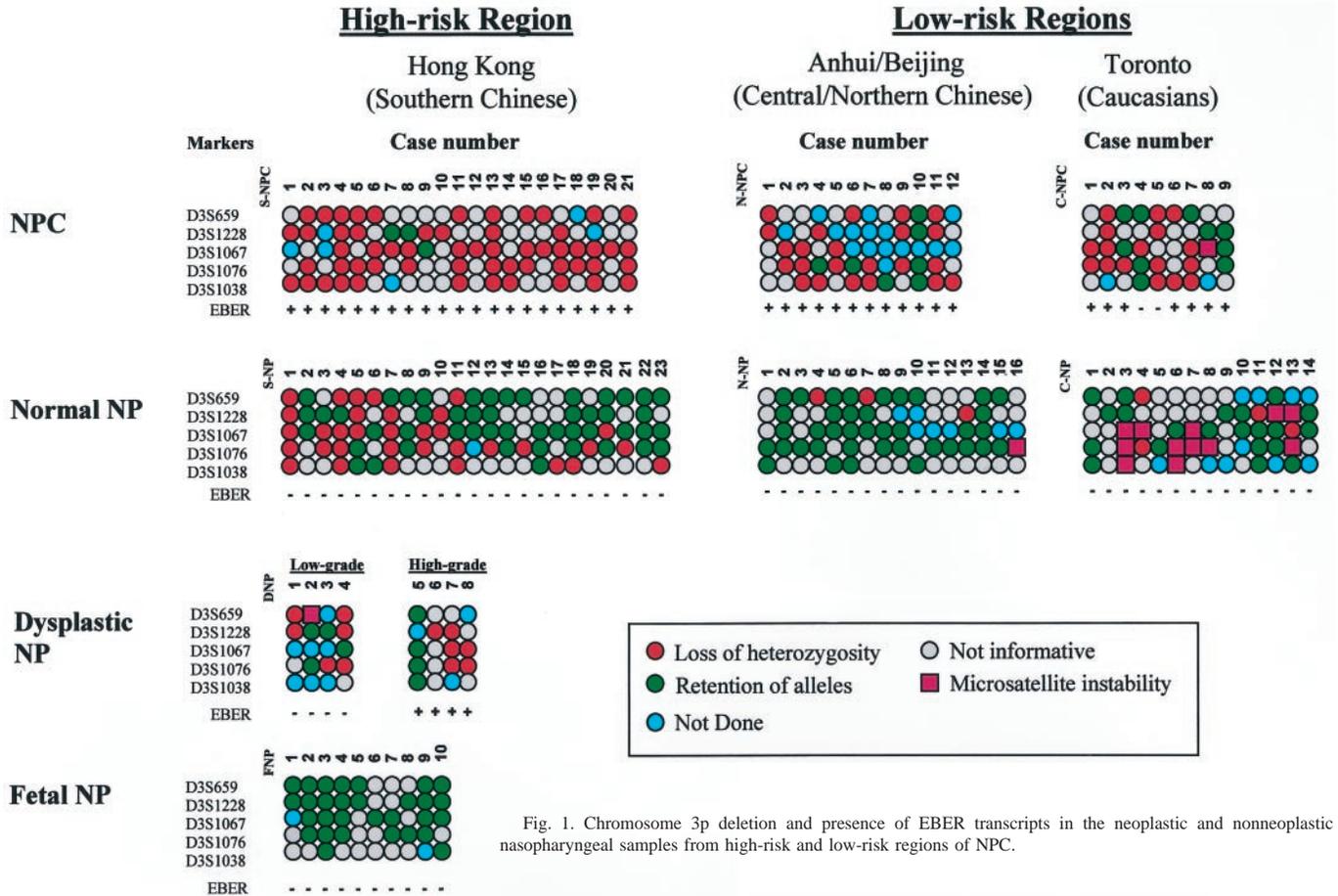


Fig. 1. Chromosome 3p deletion and presence of EBER transcripts in the neoplastic and nonneoplastic nasopharyngeal samples from high-risk and low-risk regions of NPC.

The data were examined for all categories by the use of two-tailed Fisher's exact test with a significant level of 0.05.

EBER in Situ Hybridization. Four of the serial formalin-fixed, paraffin sections of each nasopharyngeal tissue was used for H&E stain and ISH. Detection of EBERs was carried out with an EBV probe ISH kit (Novocastra, Newcastle, United Kingdom) according to the manufacturer's instructions. The tissue sections were deparaffinized, rehydrated, and hybridized to a fluorescein-conjugated oligonucleotide probe, which consists of a mixture of the two EBERs, EBER-1 and EBER-2. Hybridization was detected by incubation with rabbit anti-fluorescein antibody tagged with alkaline phosphatase, followed by reaction with the substrate 5-bromo-4-chloro-3-indoyle phosphatase and the colorimetric indicator nitroblue tetrazolium. The sections were counterstained with 1% methyl-green.

Results

We have examined the genetic changes in the neoplastic and nonneoplastic lesions of the nasopharynx from the high-risk and

low-risk regions. In the high-risk region, samples were collected from southern Chinese in Hong Kong. For the low-risk regions, specimens were retrieved from central/northern Chinese (Anhui and Beijing) and from Caucasians (Toronto, Ontario, Canada). The percentages of informative loci on chromosome 3p in all cohorts were 48–72%. All samples were analyzed in duplicates. LOH was scored when consistent allelic deletions were observed in two separate reactions.

NPC and Dysplastic Lesions from a High-Risk Region (Hong Kong). All of the 21 (100%) undifferentiated NPCs of southern Chinese from Hong Kong showed allelic loss on one or more loci on the short arm of chromosome 3 (Fig. 1; Table 2). In the dysplastic lesions, 6 of 8 (75.0%) showed chromosome 3p LOH on one or more loci examined. In the 8 dysplasias, 3 of 4 (75%) low-grade and 3 of 4 (75%) high-grade dysplastic lesions showed 3p LOH. EBV infection was detected in 21 of 21 (100%) NPCs and 4 of 8 (50%) dysplastic lesions (Fig. 1; Table 2). All of the EBER-positive cases were found

Table 2 The association of LOH on chromosome 3p and presence of EBER transcripts in the neoplastic and nonneoplastic nasopharyngeal samples from high- and low-risk regions of NPC

	High-risk region		Low-risk regions					
	Southern Chinese (Hong Kong)		Total of all regions		Central/Northern Chinese (Anhui/Beijing)		Caucasians (Toronto, Canada)	
	3p LOH	EBER	3p LOH	EBER	3p LOH	EBER	3p LOH	EBER
NPC	100% (21/21)	100% (21/21)	81% (17/21)	90.5% (19/21)	83.3% (10/12)	100% (12/12)	77.8% (7/9)	77.8% (7/9)
Normal NP ^a	73.9% (17/23)	0% (0/23)	20% (6/30)	0% (0/30)	18.8% (3/16)	0% (0/16)	21.4% (3/14)	0% (0/14)
Fetal NP	0% (0/10)	0% (0/10)	NA	NA	NA	NA	NA	NA
Dysplastic NP (Total)	75.0% (6/8)	50.0% (4/8)	NA	NA	NA	NA	NA	NA
High-grade	75.0% (3/4)	100% (4/4)						
Low-grade	75.0% (3/4)	0% (0/4)						

^a NP, histologically normal nasopharyngeal epithelia; NA, not available.

in high-grade (cases 5–8) but not in low-grade dysplasias (cases 1–4), and 3 of 4 (75%) EBV-positive cases contained 3p LOH (cases 6–8; Fig. 1). Examples of EBER-ISH in high-grade lesions and NPCs are shown in Fig. 2, B and C.

NPCs from Low-Risk Regions (Anhui/Beijing and Toronto). Ten of 12 (83.3%) undifferentiated NPCs from central/northern Chinese (Anhui/Beijing) and 7 of 9 (77.8%) undifferentiated NPCs from Caucasians (Toronto) showed 3p LOH on one or more loci examined (Fig. 1; Table 2). No difference was found between these two groups ($P = 1.0$). Collectively, 17 of 21 (81%) undifferentiated NPCs from the low-risk regions showed 3p LOH. EBV was detected in all NPCs from central/northern Chinese (100%) and 7 of 9 (77.8%) NPCs from Caucasians (Table 2; Fig. 1). In the samples from Caucasians, EBERs were positive in 2 of 2 cases (C-NPC 8 and 9, Fig. 1) without 3p LOH. The other 2 Caucasians' samples (C-NPC 4 and 5, Fig. 1) without EBV latent infection showed LOH on 3p.

NP from a High-Risk Region. Among the histologically normal NP from noncancer individuals in Hong Kong, 17 of 23 (73.9%) showed 3p LOH on one or more loci examined (Fig. 1; Table 2). To obtain a better insight into the correlation of genetic lesions and chronic exposure to our environment, we had therefore examined the status of 3p LOH in the NP from 10 aborted fetuses. Our results demonstrated that there was no LOH on 3p detected in any of the nasopharyngeal epithelia from the aborted fetuses (0 of 10; 0%). All of the above NP samples were diagnosed by a pathologist (K. F. T.) and were classified into either respiratory (Fig. 2A, Cases 1 and 11) or squamous types (Fig. 2A, case 4). The presence of the EBV latent transcripts, EBERs, was also examined in all NPs from southern Chinese using ISH. No EBER transcripts were detected in the epithelial cells of these specimens (Table 2).

NP from Low-Risk Regions. In the low-risk regions, histologically normal epithelia were collected for the investigation of genetic changes on 3p. Only 3 of 16 (18.8%) NPs from central/northern Chinese (Anhui/Beijing) and 3 of 14 (21.4%) NPs from Caucasians (Toronto) showed LOH on one or more loci on 3p (Fig. 1; Table 2). No significant difference was found between these two groups ($P = 1.0$). In these low-risk regions, the combined frequency of 3p LOH was determined to be 6 of 30 (20%). Of note, microsatellite instability was observed in the 7 of 14 (50%) NP samples of Caucasians and only 1 of 16 (6.3%) of those from the central/northern Chinese (Fig. 1) but none of the NPs from the southern Chinese (Fig. 1). All histologically normal epithelia were found to be EBER negative (Table 2).

Statistical Analysis. The frequencies of LOH on chromosome 3p in NPC and histologically normal NPs from the high- and low-risk regions were analyzed using the two-tailed Fisher's exact test. For NPC specimens, no significant difference was found between the NPC from southern Chinese and those from central/northern Chinese ($P = 0.13$) or from Caucasians ($P = 0.08$). When comparing the NPCs from the high-risk region (Hong Kong) and the low-risk regions (Anhui/Beijing and Toronto), no significant difference of frequencies of LOH on 3p was found ($P = 0.1$). For the histologically normal NPs, the frequency of LOH on 3p in the samples from Hong Kong was significantly higher than that from Anhui/Beijing and ($P = 0.002$) and that from Toronto ($P = 0.005$). There was no significant difference between the NP from the central/northern Chinese (Anhui/Beijing) and those from the Caucasians (Toronto; $P = 1.0$). When comparing the NPs from the high-risk region (Hong Kong) and low-risk regions (Anhui/Beijing and Toronto), the difference in the frequency of 3p LOH was found to be highly significant ($P = 0.0003$).

Discussion

Previous molecular genetic studies of NPCs from southern Chinese have suggested that deletion of chromosome 3p is a common genetic event in the tumorigenesis of this cancer (9, 10). The consistent loss of genetic materials on 3p strongly suggests the presence of NPC-related tumor suppressor gene(s) residing on this chromosome arm. In the present study, a high frequency of LOH on 3p was found in tumor samples from both high-risk (100%) and low-risk (81%) regions. No significant difference of frequency in 3p deletion was detected between these regions ($P = 0.1$). As compared with previous reports (Lo *et al.*, 67%; Hu *et al.*, 70%), LOH on chromosome 3p occurred in higher frequency in the present study, indicating that microdissection could increase the sensitivity of detection for genetic changes in NPCs (9, 10). Our findings further confirmed the crucial role of this specific genetic aberration in the tumorigenesis of NPCs, irrespective of geographic and ethnic origin.

The identification of chromosome 3p LOH in histologically normal NP from noncancerous, healthy individuals in the endemic region for NPC indicated the presence of genetically abnormal clones in the nasopharynx in our population, revealed for the first time. Notably, lower frequencies of LOH on 3p were observed in the normal NP from central/northern Chinese and Caucasians in the low-risk regions when compared with those from southern Chinese. Moreover, statistical significant frequencies of LOH on 3p in normal nasopharyngeal epithelia were detected between the high-risk and low-risk groups (73.9% versus 20%; $P = 0.0003$). The high frequency of genetic damage found in the normal NP from southern Chinese might reflect differences in chronic exposure to different environmental factors and genetic susceptibility. The significant difference in the incidence of LOH on 3p between different ethnic and geographic regions may be correlated to the distinct risk for cancer development. Of note, microsatellite instability was observed in the normal NP tissues from the Caucasians but not in those analyzed for southern Chinese and rarely for central/northern Chinese. The reason for such observation is unclear and remains to be investigated in future studies.

Several studies have also demonstrated the presence of genetic changes in the normal-looking epithelia, such as breast terminal ductal-lobular units and bronchial epithelium. Genetically abnormal clones were found in normal breast tissues and in normal bronchial epithelia (16–19). Moreover, in the study by Wistuba *et al.* (19), a significant difference in frequencies of chromosomes 3p/9p deletion was found between the normal bronchial epithelia of smokers and nonsmokers. Mao *et al.* (18) suggested that such genetic damages might be a consequence of chronic exposure to carcinogenic agents present in cigarette smoke. The high frequency of genetic changes found in bronchial epithelia from former smokers indicates that such damages may persist for years after smoking cessation. Similarly, the nasopharynx may also be exposed to various carcinogenic agents that are suspected to be present in the environment, such as dietary factors and smoking. Smoking may also contribute as one of the environmental factors to NPC development (8). Unfortunately, the smoking histories of the noncancerous individuals in the present study were not available. Chronic exposure to various carcinogens may lead to genetic instability/damage of the epithelial cells in the nasopharynx and may give rise to the development of multiple abnormal cell clones in the epithelium. Differences of frequencies in 3p deletion in the normal nasopharyngeal epithelia among different ethnic and geographic groups may indicate deviated exposure of environmental carcinogens pertaining to different lifestyles. Several studies have reported that the incidence of NPC declined between the first and the third generations after migration. These observations readily reflect that the environmental factors, living habits, and intermarriage may be the important

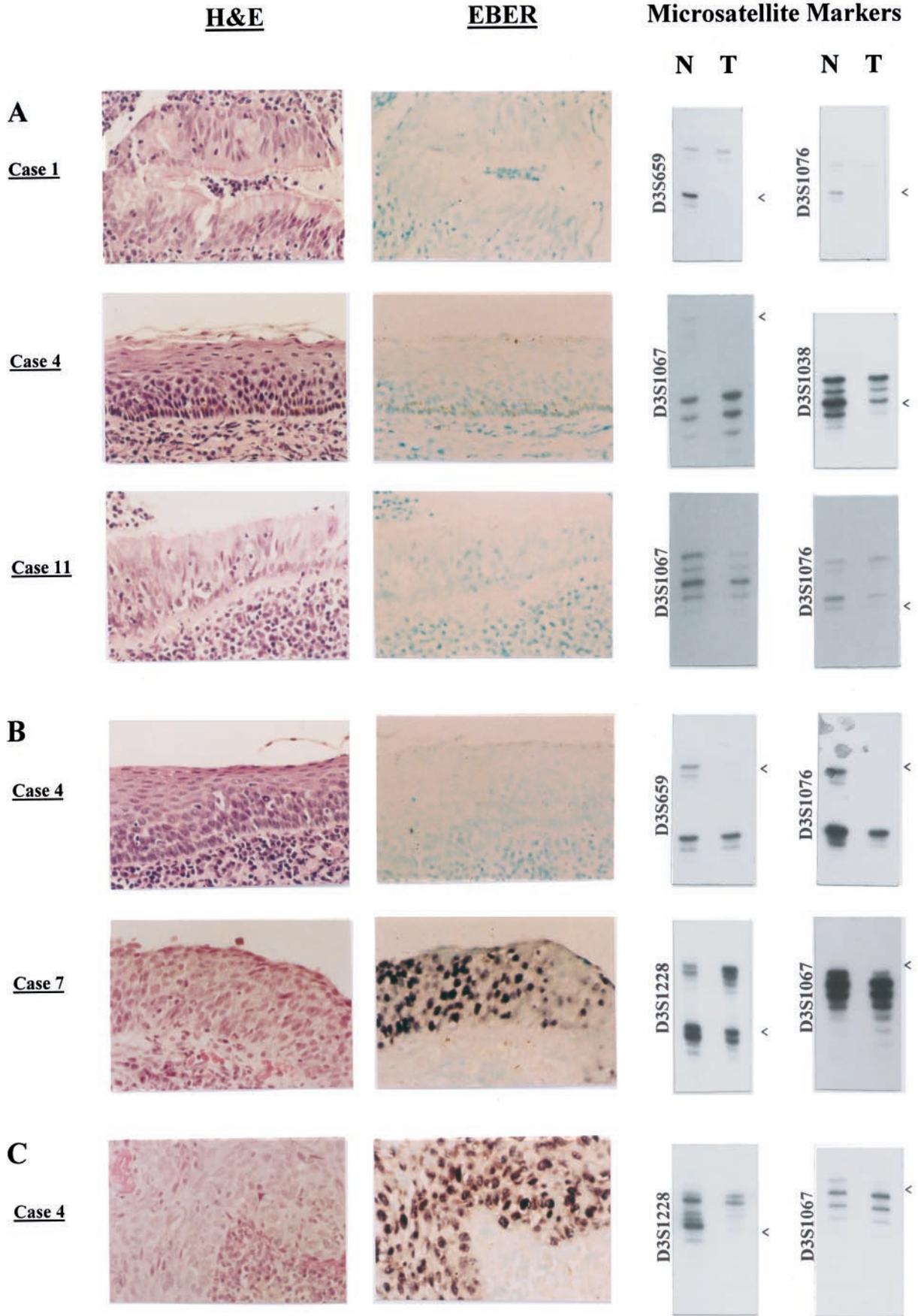


Fig. 2. Examples of chromosome 3p deletion and EBER-ISH in neoplastic and nonneoplastic nasopharyngeal samples from southern Chinese. *A* (Case 1, S-NP 1; Case 4, S-NP 4; Case 11, S-NP-11), histologically normal nasopharyngeal epithelia. *B* (Case 4, DNP-4; Case 7, DNP-7), dysplastic nasopharyngeal epithelia (Case 4, low-grade dysplastic lesion; Case 7, high-grade dysplastic lesion). *C* (case 4, S-NPC 4), undifferentiated NPC. H&E, $\times 200$. EBER-ISH, ISH for EBER transcripts ($\times 200$). N, normal tissue; T, tumor. \wedge , LOH.

determinants of cancer risk. Traditional diet, such as early age of exposure to salted fish, has been suggested as one of the environmental factors and major risk factor for NPC in the southern Chinese population for years (1, 5, 6). However, genetic susceptibility, for example, genetic polymorphism of the *CYP2E1* gene, may also be implicated (4). Our preliminary results suggest that molecular epidemiological studies on the association of the genetic changes in normal NP, environmental factors, and genetic factors in the endemic and nonendemic regions will provide a clearer picture of the carcinogenesis of NPC.

High frequencies of 3p deletion were detected in the normal-looking NP epithelia and precancerous lesions from the southern Chinese. No significant difference of LOH on 3p was found between these two types of samples ($P = 1.0$). It is suspected that this genetic abnormality may occur at a very early stage before any phenotypic change commences. The definition of "normal NP" may be worth extending to the molecular level. Our pilot study on the aborted fetus NP epithelia showed us that the above genetic abnormality was not inherited but rather acquired after birth. Chronic exposure to specific environmental carcinogens may give rise to the development of genetically abnormal clones in the NP.

We have also investigated the presence of EBV latent infection in the normal epithelia, precancerous lesions, and invasive carcinoma of nasopharynx. By EBER ISH, EBV latent infection was detected in all NPC and high-grade precancerous lesions but not in the low-grade lesions and normal epithelia from southern Chinese. Similar findings were reported previously by Pathmanathan *et al.* (20) Their study also demonstrated the clonal EBV genome in both NPC and precancerous lesions. Of note, we illustrated in the present study that EBV latent infection of the nasopharyngeal epithelial cells may occur during progression of low-grade to high-grade precancerous lesions. These findings suggested that the clonal expansion of the EBV latent infected NP cells is an early event in NPC development.

In summary, the present study demonstrated that 3p deletion is common among NPCs from high- and low-risk geographic regions. The presence of such genetic alteration in the histologically normal NP and the dysplastic lesions suggests that it may be an early event. We also showed that the EBV latent infection occurred in the NPC and high-grade precancerous lesions but not in the low-grade lesion and normal NP. Our results suggested that EBV latent infection, together with abnormal genetic changes, might play crucial roles in NPC tumorigenesis. The higher frequency of 3p LOH found in the normal NP from southern Chinese compared with other low-risk groups may be related to the distinct incidence rate of this cancer among these populations. On the basis of the above findings, it may be worthwhile to carry out a large-scale molecular epidemiological survey by the inclusion of other geographic regions/ethnic groups and correlation to their respective environmental factors and genetic susceptibility to NPC.

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