

CHAPTER V

Results

Twenty three samples were obtained from 21 primary tumours and 2 samples from recurrence patients. The clinical staging of the tumours was stage II, III and IV. All biopsy specimens were histopathologically proven to be NPC type II and type III according to the WHO classification. The tumours specimens were analyzed for Epstein-Barr virus (EBV) genome and identified EBV type by using PCR assay (Hording et al., 1993; Rajadurai et al., 1995). The detail of patients data were shown in Table 7.

Table 7 : Patient details ; Age, sex, presence of EBV genome, EBV type, tumour histology type, and TNM stage.

Patient No	Age	Sex	Histological type	EBV/ Type*	TNM stage			
					T	N	M	Stage
11	59	M	II	+/A	3	0	0	III
18	80	F	II	+/A	3	26	0	IV
19	31	F	II	+/A	2	20	0	IV
24	32	M	III	+/A	4	30	0	IV
31	40	F	III	+/A	Recurrence			
34	52	M	III	+/A	4	26	0	IV
35	48	F	III	+/A	2	20	0	IV
38	71	M	II	+/A	2	0	0	II
44	73	M	II	+/A	2	1	0	III
45	38	M	II	+/A	1	26	1	IV
46	62	F	III	+/B	2	0	0	II
47	36	M	III	+/A	Recurrence			
50	42	M	II	+/A	4	0	0	IV
51	63	F	III	+/A	3	3	0	IV
53	65	M	II	+/A	3	1	0	III
56	44	M	II	+/A	3	3	0	IV
57	55	M	III	+/A	1	20	0	IV
67	30	F	II	+/A	2	2	0	IV
70	73	M	III	+/A	4	2	0	IV
72	59	F	II	+/A	3	0	0	III
76	70	M	III	+/A	2	2	0	IV
83	48	M	II	+/B	3	3	0	IV
86	58	M	II	+/A	3	0	0	III

* : Tumour specimens were found to be positive for EBV genome and were identified the EBV type by PCR assay.

T: Primary tumour ; N: Regional lymph nodes ; M:Distant metastasis

Note : Histological typing or Tumour typing is based on the histological scheme of the WHO, Stage is according to the TNM stage by AJCC 1988. The definition of histological type and TNM type are listed in appendix A.

PCR analysis

The twenty-three cases of nasopharyngeal cancer were examined for LOH with STRPs. Using 89 STRPs, all 17 autosomal short arms and 22 autosomal long arms were analyzed with at least 1-3 markers per arm. The results of all loci studied in twenty three nasopharyngeal specimens were shown in details in Table 8, Figure 9. The autoradiographs showing LOH of some loci studied were presented in Figure 10. The autoradiographs of some multiplex PCR analysis were shown in Figure 11.

All chromosome arms were subjected to LOH in at least one tumour. The frequency of LOH for each chromosome arms varied from 0% (2p, 4q, 8p, 10p, 19q, 20p, and 21q), to 77% (9p). Thirteen tumours (57%) exhibited LOH in 1-5 chromosomal arms, while ten tumours (43%) displayed LOH in 6 or more chromosomal arms.

The regions with significant LOH together with the percentage of LOH found in the informative cases were as followed : 3p, 65.20%(15 of 23), 9p,77% (17 of 22) that considered to be a common sites of LOH. LOH at 11q, 36.4% (8 of 23) ; 13q, 38% (8 of 21) ; 14q, 37% (7 of 19) ; and 20q, 30% (6 of 20) were considered to be a moderate frequent of LOH. In addition, several chromosome arms exhibited 20-29% LOH : 1p, 23% (5/22); 6p, 27.30% (6/22); and 17p, 22% (5/23). These arms were considered to be less frequent of LOH.

The regions of LOH on chromosome 3p were analyzed using 7 STRPs (D3S1038, 3p25 VHL; D3S192, 3p25 VHL; D3S1600, 3p14; D3S966, 3p21.3; D3S1480, 3p; D3S1255, 3p25VHL; D3S1217, 3p13-14 ; D3S1481, 3p14 ; and D3S1241, 3p21.3). Fourteen of 23 informative tumour specimens had LOH more than one markers at the VHL loci, 4 of 18 showed LOH at D3S1600, 4 of 6 showed LOH at D3S966, 1 of 5 showed LOH at D3S1217, but there was not any LOH at D3S1480, D3S1481 and D3S1241. There was no LOH presented in six tumours from patients no. 11, 34, 45, 47, 50 and 51 with 9 markers. The typical feature of LOH at 3p VHL loci is shown in Figure 10.

The most frequently affected region of LOH at chromosome 9p was analysed by using 3 markers D9S169, 9p21 ; D9S165, 9p ; and IFNA, 9p21-22. Seventeen of 23 informative tumour specimens revealed LOH at one or more of these 3 markers. Patient no.46 and 50 appeared to have lost all 3 markers. Figure 10 showed the LOH of patient no.38 at D9S169 locus.

The regions of LOH on chromosome 11q were analyzed using 5 STRPs markers (D11S534, 11q13; D11S956, 11q13; D11S976, 11q23; D11S897, 11q23 ; and INT2, 11q13.3). Eight of 23 informative tumour specimens had shown LOH at one or more loci according to the 5 markers analyzed for 11q arm. Six of 23 tumour specimens showed LOH at 11q23 loci and 3 of 23 informative tumour specimens showed LOH at 11q13 locus(Figure 10). But there was no LOH at locus D11S534 in all the informative tumour specimens studied.

The LOH on chromosome 13q were observed in 8 of 21 (38%) informative cases when analyzed with two STRPs marker :D13S284,13q 13RBI; D13S119, 13q. Eight tumours showed LOH at D13S284(Figure 10), while D13S119 locus did not present any LOH.

The chromosome 14q was analyzed for LOH with two STRPs markers : TCRD, 14q11.2 ; D14S118,14q. Seven of 19 (37%) informative cases showed LOH in more than one markers used. Five of 19 informative cases had shown lost of heterozygosity at the TCRD locus (Figure 10), and 4 of 19 informative cases tumours exhibited lost of heterozygosity at D14S118 locus. Frequent LOH on 20q (D20S17) was noted in 6 out of the 20 (30%) informative cases. Figure 10 presented the LOH on chromosome 20q arm.

Two of the 23 (8%) tumour specimens (no.38, 51) analyzed were characterized by microsatellite instability (MSI) which had been evidenced by the presence of allelic fragments not found in the matched normal DNA. Only tumour no. 38 showed MSI at 25 locus, while tumour no.51 had showed MSI at 1 locus (D11S956). No.38 showed both MSI and LOH at loci D3S1600 (3p), IGF2R (6q), D13S284 (13q), and D16S511 (16q). The appearance of MSI in this study is similar to that described for MSI-positive colorectal tumours (Aaltonen et al., 1993; Thibodeau et al., 1993). Figure 12 presented the feature of MSI found in the nasopharyngeal cancer DNA.

TABLE 8 : Allelotyping results for all tumours at all loci

Chromosome	Arm	Locus	Location																									LOCUS (%LOH)	ARM : %LOH (no.of LOH/no.of INF)
				11	18	19	24	31	34	35	38	44	45	46	47	50	51	53	56	57	67	70	72	76	83	86			
1	1P	D1S243	1p36.1-36.2	0	0	0	•	•	0	0	•	0	u	•	0	0	•	0	0	0	0	0	0	0	0	23%	23 % (5/22)		
	1Q	D1S103	1q31-q32	0	0	•	0	0	0	0	0,i	0	0	0	0	u	•	0	u	•	0	0	0	0	0	14.30%	14.30 % (3/22)		
2	2P	D2S131	2p			0		0	0	0	0,i	0	0		0		0	0	0	0	0	0	0	0	0%	0%			
		D2S405	2p	u	0									0	u										0%				
	2Q	D2S102	2q33-q37	0	0	•	0	•	0	0	0	u,i	•	0	u	0	0	0	0	0	0	0	0	u	0	14.30%	14.30 % (3/21)		
3	3P	D3S1038	3p25VHL	u	•	u	•	•	u	•	•	•	0	u	0	0	0	•	0		0	•	•	•	•	67%			
		D3S192	3p25VHL	u					0							0	•		u	u				•	•	50%			
		D3S1600	3p14				•	0	u	0	•,i		0	•	0	u	0	0	u	0	•	0	0	0	u	29%			
		D3S966	3p21.3	0		•	•		0											•			•			67%			
		D3S1480	3p	0		u				0					0			u								0%	65.20% (15/23)		
		D3S1255	3p25VHL	0		0	0			0	0	u		u	0					0	•	0	0	•		18%			
		D3S1217	3p13-14		0			0		•					0			0								20%			
		D3S1481	3p14		0							0														0%			
		D3S1241	3p21.3												0			0								0%			
	3Q	GLUT2	3q26.1-q26.2	u	0	0		0	0	0	0,i	0	•	0	0	u	0	0	u	u	0	0	0	0	0	6%	4.50% (1/23)		
		D3S1744	3q23-q24	0			0								0			0	0							0%			
4	4P	D4S174	4p11-p15	0	0	0	0	0	0	0	0,i	0	0	0	0	0	0	0	0	0	0	0	0	u	0	0%	9 % (2/23)		
		D4S1599	4p	0	0	u	0	•	0	0	u,i	0	0	0	0	u	0		•	0	u	0	u	0		12.50%			

TABLE 8 : Allelotyping results for all tumours at all loci (continue)

Chromosome	Arm	Locus	Location	11	18	19	24	31	34	35	38	44	45	46	47	50	51	53	56	57	67	70	72	76	83	86	LOCUS (%LOH)	ARM : %LOH (no.of LOH/no.of INF)	
4	4Q	D4S1554	4q11-q35		u			0	0	0	u,i	0	0		0		0	u	0	0	0	0	0	0	u	0	0	0%	0%
		D4S1625	4q	u	0	u					0			0	u		0							0			0%		
5	5P	D5S392	5p	0	0			0				0	u	0		0				0	0				•	0	0	9.10%	10%(2/21)
		D5S819	5p	u	u	0		•	0	0	0	u	u		0		0	0	0	u	u	0	0	u		u	9.10%		
	5Q	D5S82	5q14-21	•	0	0	u	•	0	0	0	0	u	0	u	u	u	0	0	0	•	0	0	0	0	0	0	18%	15%(3/20)
		D5S346	5q21-q22		0		u	0	0	0	0,i		0		0	u		0	0				0	0		0	0%		
6	6P	D6S309	6p	•	0	0	•	0	0	u	u,i	•	0	0	0	0	•	u	0	u	u	0	u	0	u	u	27%	27.30%(6/22)	
		D6S477	6p	•	0	0		u	0	u	0	•	0	0	0	u	u	0	0	0	•	0	0	0	0	•	22%		
	6Q	IGF2R	6q27	u	0	u	u	0	u	u	•,i	0	u		0		0	0	0					u	u		13%		
		D6S503	6q	0		u			0	0			0	u		u		u			0	0	u	u	u	0	0%	5%(1/20)	
		D6S292	6q			0									0		0						0	0			0%		
7	7P	D7S517	7p	0	•	0	0	0	0	0	•	•	0	0	0	0	0	•	0	0	0	0	u	0	0	0	u	19%	18%(4/22)
		D7S460	7p											0								0			0		0%		
	7Q	D7S486	7q31	0	0	0	0	0	0	0	0	•	0	0	0	0	0	u	u	0	0	0	0	0	0	0	0	5.30%	4.35%(1/23)
		D7S821	7q											0				0	0						0		0%		
8	8P	NEFL	8p	u	u	0	u	0	0	u	0	0	0	0	0	u	u	0	0	0	u	0	0	0		u	0%		
		D8S87	8p21.3-p22	0	0					0					0	u					u				0	u	0%	0%	
		D8S110	8p	0												0					0					u	0%		

TABLE 8 : Allelotyping results for all tumours at all loci (continue)

Chromosome	Arm	Locus	Location	T U M O U R S N O																							LOCUS (%LOH)	ARM : %LOH (no.of LOH/no.of INF)
				11	18	19	24	31	34	35	38	44	45	46	47	50	51	53	56	57	67	70	72	76	83	86		
8	8Q	D8S88	8q22	0	0	•	0	0	0	0	0,i	0	0	0	u	0	0	0	0	0	0	0	0	u	0	5%	5%(1/21)	
		MCC	8q24	u		u					u	0	u		0	u										0%		
9	9P	D9S169	9p21	•	•	•	•	•	0	u	•	•	u	•	•	•	0	•	u	0		u	•	u		80%	77%(17/22)	
		D9S165	9p											•		•	0	u	0					u	•	60%		
		IFNA	9p22						0	0				•	•	u	•	0	•	•	0	•	u	u	•	0		u
	9Q	D9S51	9q	0	0	0	0	u		u	u,i	0	0	0	•	u	0		u	0	0		u	u	0	0	8%	5%(1/20)
		D9S290	9q34						u	0						0		u	0			0	u	0	0		0%	
		ABL1	9q34					u	0		u					u		0	u			u	u	u	u	0	0%	
10	10P	D10S89	10p11.2-pter	0				0				0	0	0	0	0									0		0%	0%
		D10S249	10p		0	0		0	u	0	0	u	u		u		0	0	0	0	0	0	0	0	0	0		
	10Q	D10S169	10q11.2-qter	u	0	0	u	u	0	u	0,i	0	u	u	0	0	u	u	u	u	0	u	0	u	0	•	9%	5%(1/22)
		D10S677	10q	0				0		0						0	0	0	0		0		0				0%	
11	11P	WT1	11p13	u	u	0		0	0	0	u	u	0	u	0	u	0	0	0	u	0	0	0	u		0	0%	9%(1/22)
		D11S554	11p	0	0						•	0	0	0		0				0					0	0	20%	
	11Q	D11S534	11q13	u	0			0	0	0	0	0	0		u	u	0	u	0	0	0	u	u	0	0	0	0%	36.4%(8/23)
		D11S956	11q13	u	0	u	u		0	0			0	0	0	0	0,i	•			u	0	0	0		0	15.40%	
		D11S976	11q23							•	•							u		0					0	66.66%		

TABLE 8 : Allelotyping results for all tumours at all loci (continue)

Chromosome	Arm	Locus	Location	T U M O U R S N O																								LOCUS (%LOH)	ARM : %LOH (no.of LOH/no.of INF)
				11	18	19	24	31	34	35	38	44	45	46	47	50	51	53	56	57	67	70	72	76	83	86			
11	11Q	D11S897	11q23	0	0	0	0	•	0	u		•	u	u	0	u	0	u	•	u	•	0	0	0	0	0	24%		
		INT2	11q13.3	0		0		•		u		0						0	u							20%			
12	12P	D12S341	12p	0	0	0	0	0	0	0	•	u	0	0	0	0	0	0	0	0	0	u		0		0	5.00%	5%(1/23)	
		D12S62	12p								0										0	0		0		0%			
	12Q	MFD133	12q	u	0	u	0	0	0	0	0,i	•	u	0	u	•	0	u	•	0	0	0	u	0	0	0	18%	13%(3/23)	
		D12S86	12q			0							0		0			0					0			0%			
13	13Q	D13S284	13qRBI	•	u	•	•	0	0	0	•,i	0	0	0	u	0	•	•	•	0	•	0	0	0	0	0	38%	38%(8/21)	
		D13S119	13q	0	u	0		0	0		0	0		0	u		0	0	0	0	0		0	0	0	0%			
14	14Q	TCRD	14q11.2	0	u	u	u	•	u	u	u	u	u	u	0	0	•	0	0	•	0	•	u	0	0	0	•	36%	37%(7/19)
		D14S118	14q	0	•	u		0	0	0	u	•	u	0	0	u	0	u	u	u	u	u	0	0	u	0	0	15%	
15	15Q	GABRB3	15q11-13	0	0	0		u	u	0	u,i	u	0	u	0	0	u	0	0	•	u	0	0	0	0	0	7%		
		D15S131	15q					0	0			0		0						0						0%	5%(1/22)		
		D15S123	15q					0	u			0		0						0						0%			
16	16P	D16S287	16p13.11	0	u	0	0	•	0	0	0	0	0	0	0	0	•	0	u	0	0	u	0	u	u	0	0	11.11%	10%(2/21)
		D16S748	16p13.2-13.13		u													u			0		0	0		0%			
	16Q	D16S511	16q22-24	0	0	0	0	u	0	0	•,i	•	u	0	u	0	0	u	0	0	0	0	0	0	0	u	0	11.11%	11%(2/19)
		D16S539	16q24.2-q24.3					u					u		u			0								0%			

TABLE 8 : Allelotyping results for all tumours at all loci (continue)

Chromosome	Arm	Locus	Location	T U M O U R S N O																								LOCUS (%LOH)	ARM : %LOH (no.of LOH/no.of INF)
				11	18	19	24	31	34	35	38	44	45	46	47	50	51	53	56	57	67	70	72	76	83	86			
17	17P	D17S520	17p12	0	0	0	0	0	0	0	u	0	•	0	u	0	0	0	0	0	u	0	0	0	u	u	6%		
		D17S945	17p13								0,i				0											0%	22%(5/23)		
		D17S1176	17p	•	0	0	0	0	0	0	0	0	•	0	0	0	0	•	•	0	•	0	0	0	0	22%			
	17Q	KRT9	17q21NME1	•	u	0	0	0	u	0	u,i	0	0	0	0	0	0	0	u	0	u	0	0	0	u	6.30%	5%(1/22)		
		NFI	17q11.2		u				0	0								0	0				0	0	0%				
18	18P	D18S59	18pter-p11.22	u	0	u	0	0	0	0	0	•	0	0	•	0	0	u	0	0	0	0	0	0	0	10%	10%(2/20)		
	18Q	D18S35	18q21.2-q21.3	u	u	0	u	0	0	0	•	0	0	0	0	u	0		u	u	u	0	u	0	0	8%			
		DCC	18q21.1	u	0	u		0	0	0	0,i	u	0		0	•	0	0	0	0	u	0	u		0	7%	10%(2/21)		
		D18S535	18q	0																0		0		u	0%				
19	19P	D19S221	19p	0	0	0	0	0	0	0	0,i	•	0	0	0	0	0	•	0	0	0	0	0	0	0	9%	9%(2/23)		
	19Q	D19S412	19q	u	0	0		u	0	0	0,i	0	u	0	0	u	u	0	0	0	0	0	0	0	0	0%	0%		
		D19S246	19q	u				0					u			0	0							0	0%				
20	20P	D20S470	20p	0	0	0		0	u	u	0,i	0	0	0			0	0	0	0	0	0	0	0	0	0%	0%		
		D20S27	20p12						u	0					0	u										0%			
	20Q	D20S17	20q12-q13.1	0	0	u	•	0	0	0	0	•	•	u	0	•	0	0	0	•	•	0	0	u	0	0	30%	30%(6/20)	
21	21Q	D21S258	21q	u	u	u	u	0	0	0	0	0	0	u	0	0	0		0	0	0	u	u	0	0	0	0%	0%	
		D21S11	21q21	0	0	0								0				0				u	0				0%		

TABLE 8 : Allelotyping results for all tumours at all loci (continue)

Chromosome	Arm	Locus	Location	T U M O U R S N O																		LOCUS (%LOH)	ARM : %LOH (no.of LOH/no.of INF)					
				11	18	19	24	31	34	35	38	44	45	46	47	50	51	53	56	57	67			70	72	76	83	86
22	22Q	D22S446	22q11NF2	0		0		•	0							u	0	u		u		u		0	0	25%	18%(3/18)	
		IL2RB	22q	u	0			u	•	0	0,i	0	0	0	0	0	u	u	u	0	u	0	u	0	u	u	15.40%	

NOTE

- ; Loss of heterozygosity
- 0 ; Retaintion of heterozygosity
- u ; Uninformative
- i ; Microsatellite instability
- blank ; Failed or no detect

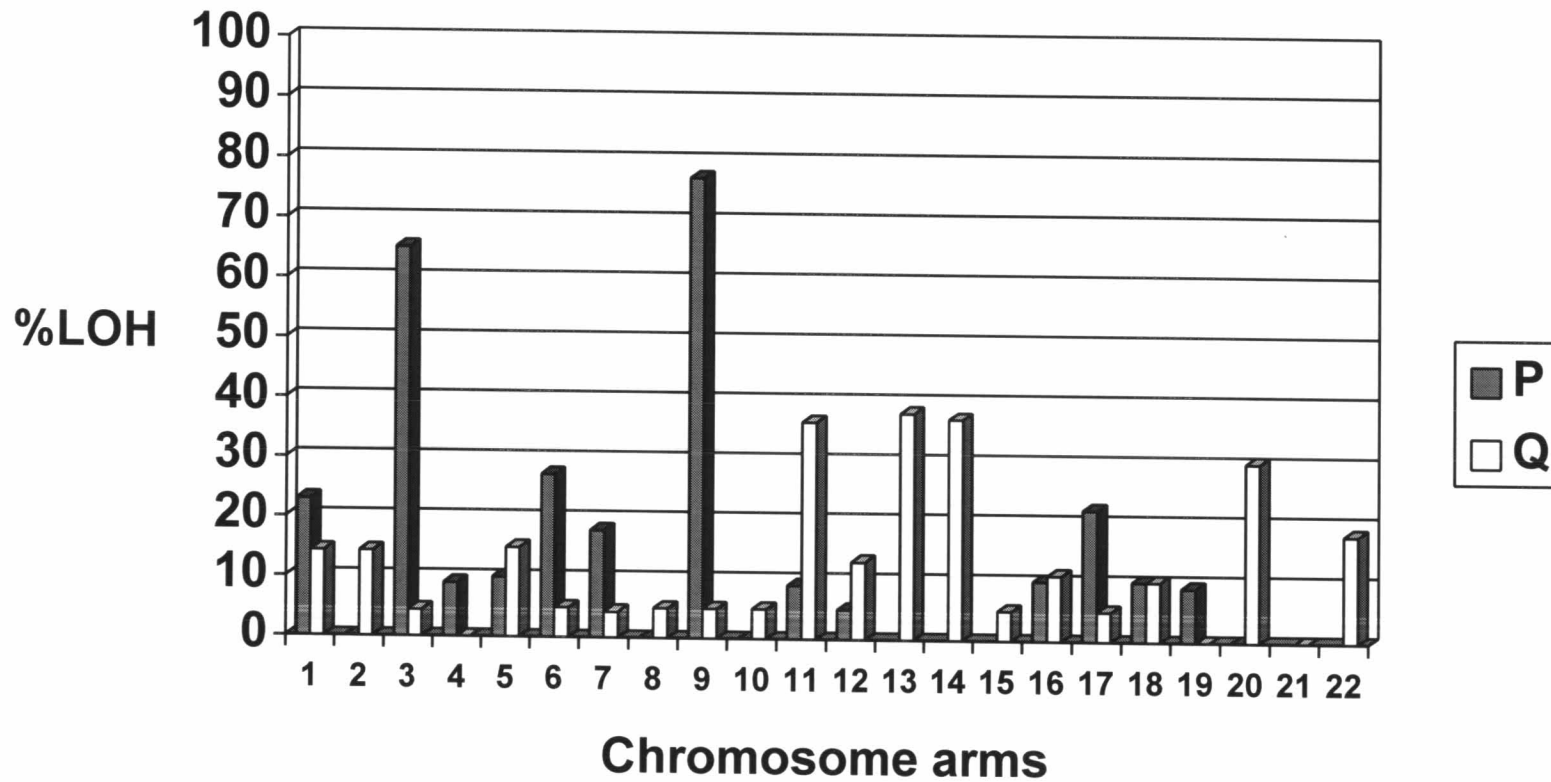


Figure 9 : Percentage of loss of heterozygosity on individual chromosome arms

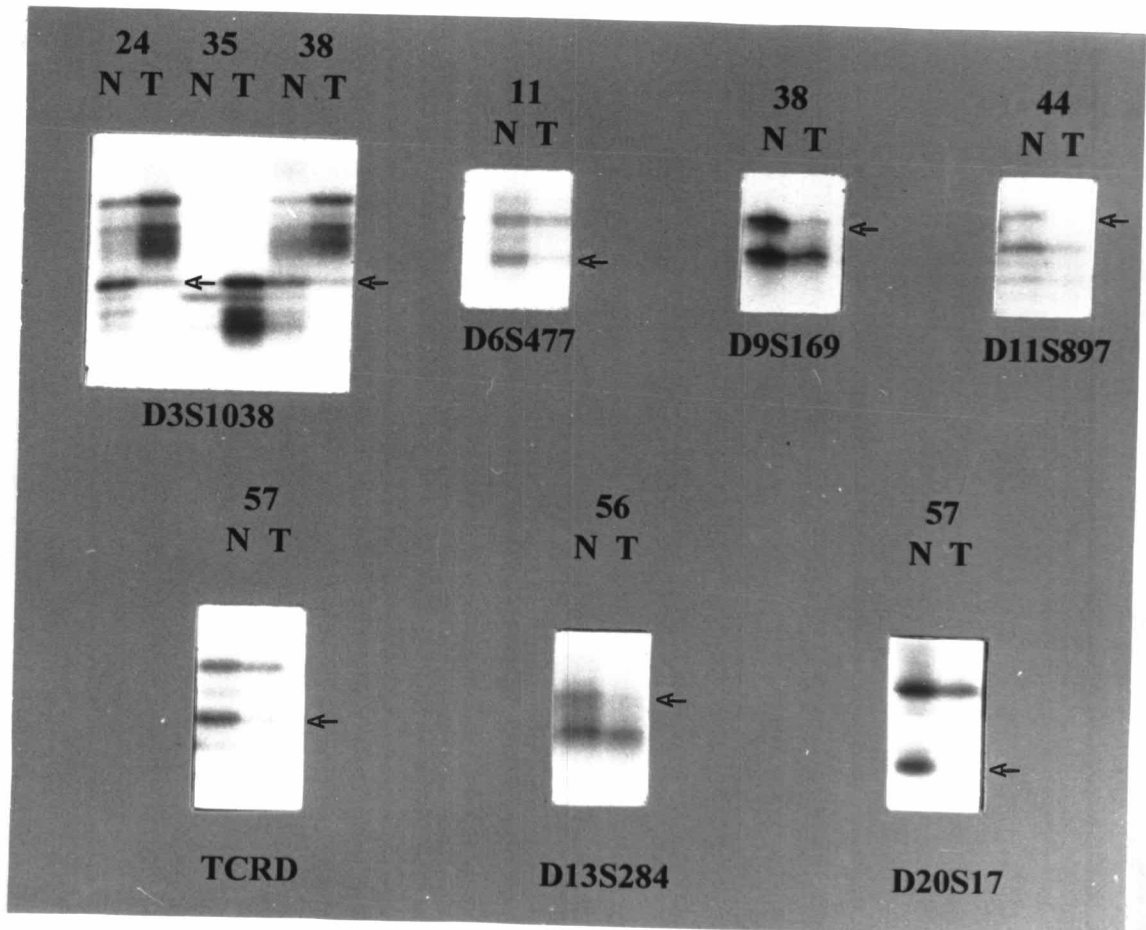


Figure 10 : Autoradiographs of PCR products separated by polyacrylamide gel electrophoresis, showing examples of LOH in tumour specimens N, normal lymphocyte DNA; T, tumour DNA; ➔, loss of heterozygosity.

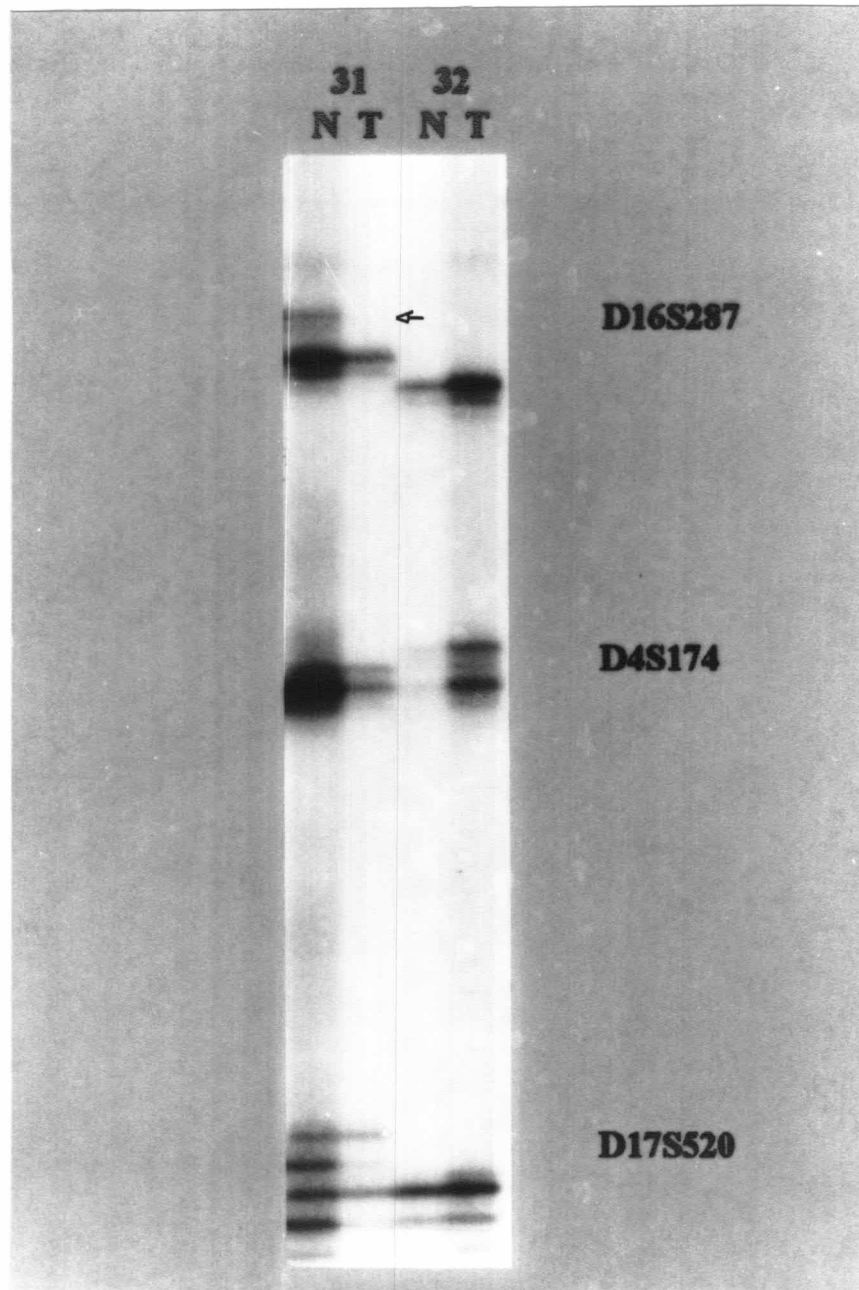


Figure 11 : An example of multiplex PCR at loci D4S174, D16S287, D17S520 in tumour no.31, 32. N, normal lymphocyte DNA; T, tumour DNA; \blackrightarrow , loss of heterozygosity (LOH). In tumour no. 31 showed LOH at locus D16S287.

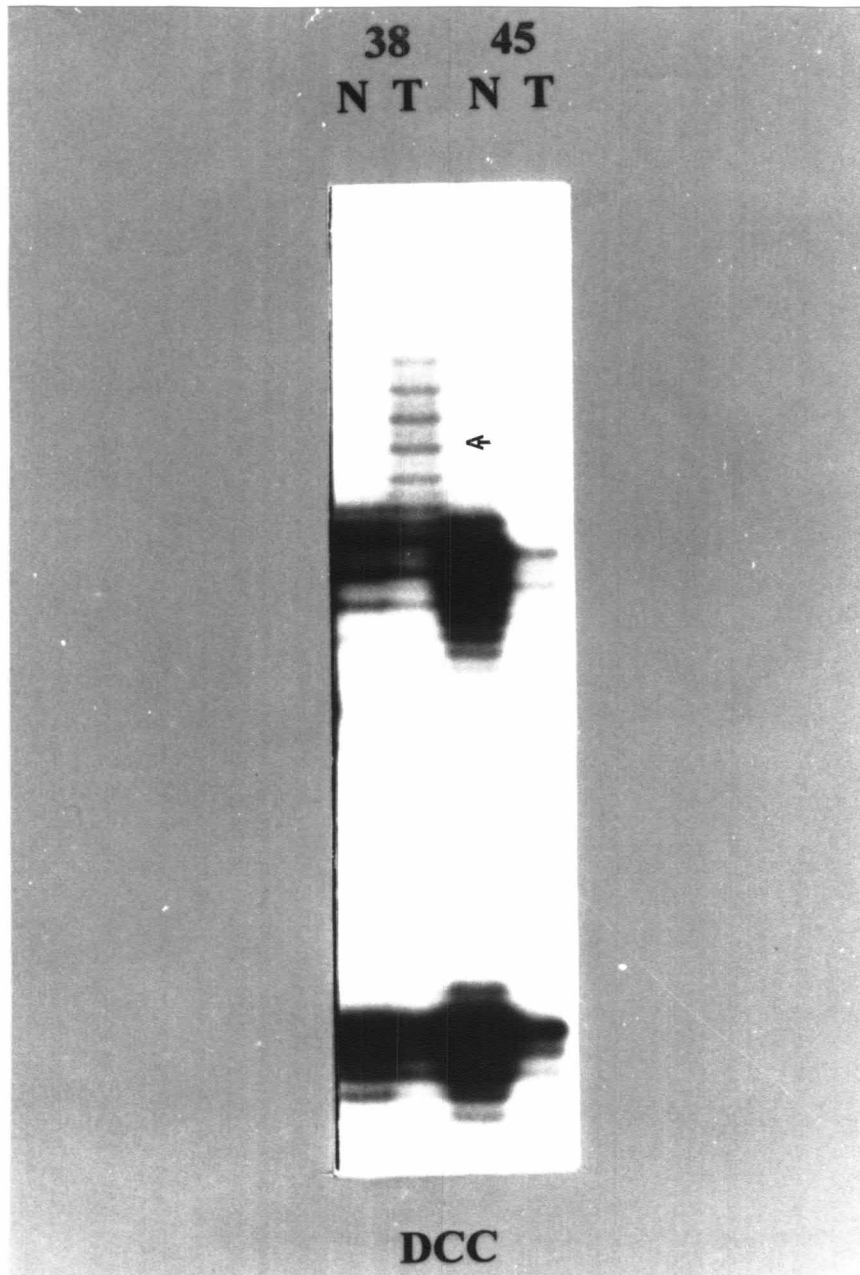


Figure 12 : Microsatellite instability (MSI) observed in tumour no.38 at DCC locus. N, normal lymphocyte DNA; T, tumour DNA; ➔, MSI.