# CHAPTER VI

## Discussion

Allelotype analysis were performed to identify the genomic regions with high frequency of LOH. The analysis revealed frequent LOH on 9 chromosomal arms, including sites of deletions previously identified in other cancers that may be involved in NPC tumours system.

The analysis of the 9 LOH sites were noted at 1p, 3p, 6p, 9p, 11q, 13q, 14q, 17p and 20q. The chromosome arms were divided into 3 groups according to the percentage of LOH : group 1 was considered to be the common sites of LOH with the observed LOH >60% of informative patients. The highly frequent LOH was observed on chromosome 3p (65.20%) and 9p (77%). Group 2 was considered to be moderate frequent of LOH when the observed LOH equaled 30-50%. The moderate frequent LOH was observed on chromosome 11q(36.4%), 13q(38%), 14q(37.4%), and 20q(30%). Group 3 was considered to be less frequent of LOH when the observed LOH equaled 20-30%. The less frequent LOH was observed on chromosome 1p (23%), 6p(27.30%), and 17p(22%). The frequent LOH on 3p and 9p in nasopharyngeal cancer (NPC) has been previously reported by several investigators (Huang et al., 1991, 1994; Choi et al., 1993; Lo et al, 1995). The high frequency of LOH on chromosome 3p demonstrated as a common genetic alteration that occured in NPC (Huang et al., 1991; Choi et al., 1993). The frequent LOH on chromosome 3p has also been reported in several tumour

types, e.g., renal cell carcinoma ( Seizinger et al.,1988; Van der Hout et al., 1988), uterine cervix (Yokota, et al., 1989), malignant mesothelioma (Popeseu et al., 1988; Decker et al., 1990), small cell lung carcinoma (Kok et al., 1987; Brauch et al., 1990; Marshall,1991), head and neck cancer (Latif et al., 1992; El-Naggar et al., 1993; Maestro et al., 1993). These studies indicate that the presence of a putative tumour suppressor genes in several tumour type locate in the short arm of chromosome 3. The loss or inactivation of genetic material may be crucial in the development of cancer (Huang et al., 1991; Marshall, 1991). The result from this study suggesting that the high frequency of LOH on chromosome 3p(65.20%) is a common event in nasopharyngeal cancer. There are at least 3 distinct regions that are likely to harbor tumour suppressor genes on chromosome 3p (Hibi et al.,1992).The VHL tumour suppressor gene on 3 p25-26 has been identified as one of these genes (Seizinger et al.,1988;Van der Hout et al.,1988 ; Latif et al.,1992).The region at 3p13-21.1 has been shown to harbor a tumour suppressor locus commonly involved in carcinoma of uterine cervix (Yokota,et al.,1989 ; Kohno et al.,1993).The region at 3p21-23 has now been identified as *hMLH1* gene that encoded a DNA mismatch repair protein (Bronner et al.,1994 ; Papadopoulos et al.,1994).

The highest frequency of LOH was found on chromosome 9p and was presented in 77% (17 of 22) of the informative specimens. A homozygous deletion region at 9p21-22 between loci D9S161 and D9S162 was found in one of the reported cases of primary NPC tumour (Huang et al., 1994). A common region of deletion on chromosome 9p has been identified in NPC and is localized near the interferon cluster at 9p 21-22 (Olopade et al., 1993 ; Van der Riet

et al., 1994; Merlo et al., 1994). Recent studies had demonstrated a frequent LOH on chromosome 9 in a variety of tumour, e.g., acute lymphoblastic leukemia (Diaz et al., 1990;), bladder cancer (Cairns et al., 1994), glioma (James et al., 1993), malignant mesothelioma (Cheng et al., 1993), melanomas (Coleman et al., 1994), non-small cell lung cancer (Olopade et al., 1993; Merlo et al., 1994), and head and neck cancers (Nawroz et al., 1994; Van der Riet et al., 1994). The observations indicated that this common regions is involved in several tumour types. This might possibly imply the presence of a tumour suppressor gene(s) residing at 9p21-22, and its loss or inactivation may play an important role in the development of cancers (Huang et al., 1994). The frequent LOH was observed on chromosome 9p by using 3 STRP markers (D9S169, 9p21; IF2A, 9p21-22 D9S165, 9p). In a recent study, a putative tumour suppressor gene, pl6/MTS1/CDKN2 was localized to the region 9p21-22 (Kamb et al.,1994; Nobori et al., 1994). The p16 gene encodes a cell cycle regulator protein, which binds to and inhibits the catalytic activity of the CDK4/cyclin D complex. If its function loss would lead to uncontrolled cell growth (Serrano et al., 1933; Lo et al., 1995).

The LOH at chromosome 11q has been sporadically reported in insulinoma (Patel et al.,1990), ovarian cancer (Foulkes et al.,1993 ; Osborne and Leech,1994), head and neck cancer (Ah-See et al.,1994), cervical cancer (Srivatsan et al.,1991a) and neuroblastoma (Srivatsan et al.,1991b).The long arm of chromosome 11 suppresses tumourigenicity of HeLa cells suggesting that a tumour suppressor gene is presented on chromosome 11q (Misra and Srivatsan,1989). One of these gene is the multiple endocrine neoplasia type 1 (MEN1) gene which map to 11q13 (Larsson et al.,1988 ; Fujimori et al.,1992). In

1991, Lamine and collegue had demonstrated a link between 11q13 regions and bcl-1/ prad-1/ cyclin D locus of which the bcl-1 linked gene may be important in the 11q13 amplification (Rosenberg et al.,1991). The adult high affinity folate receptor (FOLRI) gene is maps to 11q13.3-13.5 (Ragoussis et al., 1992). It is amplified in a limited number of epithelial tumours but lost of heterozygosity at this locus is very rare (Lamine and Peters,1991).The ataxia telangiectasia (AT) genes are mapped to the 11q22-23 region (Gatti et al.,1988). Epidemiological studies suggested that heterozygous AT carriers may be predisposed to several cancer (Swift et al.,1991). The risk of breast cancer has increased up 5 folds in AT carriers (Swift et al.,1991). Both cytological and LOH studies have described the presence of aberrations on chromosome 11q22-23 in breast cancer (Ferti-Passantonopoulou et al.,1991 ; Carter et al.,1994). In this thesis, the frequency of LOH was presented in 36.4% (8/23) when analyzed with five STRP markers : D11S534 , 11q13 ; D11S956, 11q13 ; D11S976, 11q23 ; D11S897,11q23 ; INT2 ,11q13.3. Six of 23 informative tumour specimens showed frequent LOH at 11q23 locus (D11S976, D11S897), and 2 of 23 informative tumour specimens showed LOH at 11q13(D11S956), 11q13.3(INT-2). These observation suggesting that a putative tumour suppressor gene(s) on 11q might be involved in the development or progression of NPC.

The LOH on chromosome 13q were observed in 8 of 21 (38%) informative cases when analyzed with two STRP markers: D13S284, 13q13 RBI; D13S119, 13q. Similar frequent of LOH on chromosome 13q has previously been observed in retinoblastoma (Benedict et al.,1987), small cell carcinomas of the lung (Brauch et al.,1990; Naylor et al.,1987), hepatocellular carcinoma

(Buetow et al.,1992), Osteosarcomas (Yamaguchi etal.,1992),colorectal cancer (Cawkwell et al.,1994). The 13q13RBI regions of chromosome 13 is cloned and characterized to be the sites of tumour suppressor gene (Marshall,1991; Sager,1989). However, recently another candidate tumour supressor gene has been identified on chromosome 13 by linkage analysis, the breast cancer susceptibility gene BRCA2 that also resided in this region (Cleton-Jansen et al.,1995; Gudmundsson et al.,1995). It is possible that LOH on chromosome 13q may involve in the development of NPC. On the other hand, retinoblastoma gene (Rb) mutation analysis in NPC was negative results.

LOH was observed in 7 of 19 informative case (37%) on chromosome 14q by testing STRP markers at two loci : TCRD, 14q 11.2 and D14S118, 14q. Five of 19 informative tumours showed LOH at TCRD locus, while homozygous or retained heterozygosity was found at D14S118. On the other hand, 2 of 19 informative tumours showed LOH at D14S118 locus with homozygosities at TCRD. This pattern of LOH suggested that there may be more than one genes which involved in the tumourigenesis of NPC localized on 14q. Frequent LOH on 14q has been previously reported in different types of cancers, including renal cell carcinoma with 45% LOH at 14q22ter by cytogenetic studies (Kovacs et al., 1989). Suzuki and collegue have observed a highly LOH at 14q in 6 of 12 (50%) tumour specimens of neuroblastoma (Suzuki et al., 1989) and 46% in ovarian cancer (Osborne and Leech, 1994). In endometrial carcinoma, LOH was found on 14q (TCRD locus) 19.1% (9 of 47) and defined a minimal region of deletion at 14q32 (Fujino et al., 1994). In 1995, Chang and collegue demonstrate that 14q loss (14q12 and 14q32.1-32.2 ) is common in

invasive bladder cancer (Chang et al.,1995). However additional markers are needed to be studied and the mapping on 14q are needed to identify the common region of LOH and the site of the candidate tumour suppressor gene.

The other chromosomal site with allelic loss was 20q. The frequency of LOH at 20q (D20S17, 20q12-q13.1) was 30% (6 of 20) of the informative cases. There are few studies of LOH at 20q. Osborne and Leech, in 1994 found 28% LOH at 20q in ovarian cancer cases. Hollings and collegue, in 1995 reported the cytogenetic deletion at the long arm of chromosome 20 that associated with Philadelphia chromosome positive acute lymphoblastic leukemia. Similarly in this study, there has been evidence shown that the 20q was the possible target site of LOH that played an important role in the development of NPC.

This study presented the phenomenon of microsatellite instability (MSI) in two of 23 (9%) NPC tumours, no.38 and 51.The results of MSI distribution on individual arms are demonstrated in table 8. The microsatellite instability : MSI or replication-error-positive : $RER^{+}$ is a genetic alterations with defect in the DNA mismatch repair gene found in bacteria and yeast (Modrich,1991; Strand et al., 1993). The implication is that mutations in these mismatch repair genes decrease the ability of that system to correct the errors created during DNA replication, particularly those error located in the repetitive nucleotide sequences (Loeb,1994). The instability in the microsatellite loci consists of an expansion and-/or contraction of DNA within the simple repeated sequences of tumour DNA as compared to normal DNA from the same individual (Loeb,1994 ; Nakashima

et al.,1995). The MSI has been observed in various types of cancer as well as in hereditary nonpolyposis colorectal cancer (HNPCC : Lynch syndrome) (Aaltonen et al.,1993,1994; Han et al.,1993; Ionov et al.,1993; Lothe et al.,1993; Risinger et al.,1993; Thibodeau et al.,1993). The mutation in these mismatch repair genes have been linked to loci on chromosomes 2p15-16, 2q31-33, 3p21-23, and 7p22 *(hMSH2, hMLH1, hPMS1, and hPMS2)* in HNPCC (Peltomaki et al.,1993; Nicolaides et al.,1994; Papadopoulos et al.,1994). Mutations in *hMSH2* or *HML1* genes are homologous with either mut*S* or mut*L* which have been established as the major inherited defect in HNPCC and have been associated with variability of repetitive nucleotide sequences in DNA from these tumours (Leach et al.,1993). Similarly, MSI was described in many tumours : breast cancer ( Yee et al.,1994), Barrett's associated esophageal adenocarcinomas (Meltzer et al.,1994), multiple primary cancers (Horii et al., 1994), endometrial carcinomas (Peiffer,1995), head and neck carcinoma (Field et al.,1995), esophageal carcinoma (Nakashima et al.,1995), prostate cancer (Watanabe et al.,1995), lung cancer (Ryberg et al., 1995). These observations suggest that the occurrence of MSI is common and may play an important role in the pathogenesis of many types of cancers (Loeb,1994). But this study, only two of 23 (9%) NPC tumour tissues (no.38,51) show evidence of MSI (Table 8, Figure 12) which indicating that MSI is infrequently found in NPC.

Interestingly, the prevalence of LOH on 3 chromosome arms: 1p, 23% (5/22); 6p, 27.30% (6/22); and 17p, 22% (5/23) indicates that these loci are possibly the significant target sites of LOH for future study of NPC. LOH on chromosome 1p, 6p and 17p has been reported in several other types. LOH at

chromosome 1p had been reported to responsible for the development in neuroblastoma (Suzuki et al.,1989). Aldaz et al.,1995 demonstrated that LOH at 1p appears to be the late events in breast cancer. The LOH on chromosome 6p is a common phenomenon in ovarian cancer (Foulkes et al., 1993). Merlo and Collegue, 1994 revealed 46% LOH on 6p in small cell lung cancer while Mitra et al.,1994 had found 28% LOH of the same locus in cervical carcinoma.

The abnormality of p53 tumour suppressor gene localized at 17p13 was commonly found in several tumour types ( Yokota et al.,1987 ; Mori et al.,1989 ; Sager, 1989 ; Somers et al.,1992). The incidence of LOH at 17p(17 p13) detected in this study (22%) was similar to those reported in head and neck cancer (31%) (Ah-See et al., 1994). However the incidence of LOH at 17 p13 is extremely high (71%) in oral cancer (Largey et al.,1993). However, further studies focused on chromosome 1p, 6p, 17p and NPC should be explored.

In this study, the EBV genome was present in 100% of all NPC tumour tissues (23 cases). Twenty-one of NPC tumour tissues were positive for EBV type A (EBV-A) and two patient (no.46, 83) were positive for EBV type B (EBV-B). These results suggesting that EBV infection is closely associated with NPC type II and type III (Hildesheim et al.,1992 ; Choi et al.,1993; Zheng et al.,1994; Abderrahim and Esteban, 1995; Rajadurai,1995). EBV-A is the predominant EBV type found in Thai NPC patients, while EBV-B appeares to be variation in the prevalence of EBV virus. The specific EBV type found in tumour tissue can

be used to identify the metastatic lesions of NPC (Chan et al.,1989 ; Choi et al.,1993).

Allelotype studies have been the source of much informations in the analysis of genomic regions with high frequency of LOH(Vogelstein et al.,1989; Ah-See et al.,1994; Fujino et al.,1994; Knowes et al.,1994; Mitra et al.,1994; Osborne and Leech,1994). Allelotype analyses in which all chromosomes were examined for LOH have been described for several types of human cancers such as : colorectal carcinomas (Volgelstein et al., 1989), astrocytoma carcinomas (Fults et al., 1990), breast cancer (Sato et al., 1990), renal cell carcinoma (Morita R et at., 1991), hepatocellular carcinoma (Fujimori et al., 1991), osteosarcomas cancer (Yamaguchi et al., 1992), non-small cell lung carcinoma (Tsuchiya et al., 1992), bladder cancer (Knowles et al., 1994), cervical carcinoma (Mitra et al., 1994), endometrial cancer (Fujino et al., 1994), head and neck cancer (Ah-see et al., 1994) and ovarian cancer (Osborne et al., 1994). The studying techniques are much easier in recent years by using restriction fragment length polymorphisms (RFLPs) and more recently short tandem repeat polymorphic markers (STRPs) (Weber and May,1989). STRPs are chosen for this study because of their high degree of polymorphism, randomly distribution throughout the human genome,simple and quick to analyze (Weber and May,1989). The technique of PCR-analyzed STRPs for studying LOH represents a significant advance over RFLP analyzed by southern blotting (Huang et al.,1993). Allelotyping using RFLPs is limited by the low informativity of many loci, the limited number of RFLPs available and the requirement for relatively large amounts of tumours DNA(Sato et al.,1991; Cliby et al.,1993). However, the PCR

technique requires smaller quantity of sample than those for RFLP analysis. The typing of STRPs by PCR is rapid and efficient, particularly when a multiplex PCR approach can be developed, allowing simultaneous analysis of multiple STRPs loci in a single tube (Huang et al.,1993). The main disadvantage of STRPs are the production of "Shadow" bands which caused by the failure of *Taq* polymerase in reading through the repeat region thereby generating the smaller fragments (Litt,1991). These shadow bands in the autoradiographs occasionally are difficult to interpret and the identification of true allele is disturbed (Cawkwell et al., 1993).

The failure to detect LOH in samples may be due to (1) the tumours may not be characterized by chromosome loss; (2) the loss regions may be undetected due to constitutional homozygosity; or (3) the tumours samples may have been contaminated with large amounts of normal cellular (nontumourous) DNA (Peiffer et al.,1995). To resolve the contaminated normal DNA from tumour DNA, the samples are evaluated histologically by cryostat sectioning to obtain > 70% tumour cell specimen for DNA extraction.

In conclusion, these studies suggest that at least 9 chromosome arms 1p, 3p, 6p, 9p, 11q, 13q, 14q, 17p and 20q are possibly involved in the tumourigenesis of NPC. This allelotype studies can form a basis for more detailed dissection of the genetic alterations in NPC which will further our understanding of the disease and leading to novel therapeutic approaches.