CHAPTER V

DISCUSSION AND CONCLUSION

Genetic susceptibility of NPC is explicit in people originated from Southern part of China and perhaps in South-East Asia. Knowing these genes and their mutations will enhance the understanding of nasopharyngeal carcinogenesis, leading to effective prevention and control of this disease in Asia. Inheritance of NPC phenotypes display several interesting characteristics. First, NPC can be considered multifactorial inheritance. Although there are few reports from Caucasian families revealed autosomal dominant inheritance, the recurrence risk of endemic NPC in Southern China is not close to autosomal dominant with or without incomplete penetrance. In addition, previous studies and this one demonstrated that there were at least three genes, *HLA*, *CYP2E1* and *pIgR*, and two environmental factors, nitrosamine and EBV, contributed to the disease development. Secondly, the functions of susceptibility genes and mutations may interact with their environmental factor. For example, the role of HLA may regard the immunization to EBV infection and *CYP2E1* is the metabolic enzyme for nitrosamine. Finally, because the much higher frequency of NPC, the mutations of these susceptibility genes should be originated in ancient Chinese and resulted as more frequent variant allele in population in Southern China.

This study used these NPC genetic characteristics as its hypothesis to define the susceptibility genes involved in NPC development. Ideally there are a large number of candidate gene to study linkage disequilibrium. All genes involved in metabolic pathway of nitrosamine and all genes in which their products interacting with EBV proteins directly or indirectly should be studied. Nevertheless, because of limited resources, technology and time, most significant and interesting genes were chosen. Regarding, nitrosamine interaction, we studied *CYP2E1* not only because it is the rate limiting step of nitrosamine metabolism but also shown as a susceptibility gene in Taiwanese population. More importantly, we are interested in asking questions whether gene control EBV entering nasopharyngeal epithelium would be as well susceptibility gene. This process is a unique process separate susceptibility between NPC and endemic

Burkitt lymphoma, frequently occurring in Africa. Consequently, if there is genetic susceptibility of gene playing important role in this process, the mutation should explain not only the frequent in NPC but rare in endemic Burkitt lymphoma in Asia. *CR2* and *pIgR* are the candidate genes chosen for evaluation since number of studies hypothesized that they may have role in epithelial infection. The positive association between one of these genes will not only explain the susceptibility but the mechanism. Nevertheless, negative association may not prove otherwise since there may be other mutation of other genes, such as IgA or tight junction proteins, involved or the polymorphism may not exist in this process.

This study has shown an increased risk of developing NPC associated with the homozygous variant form of the *CYP2E1* gene. This higher RR was demonstrated in both the Thai and Chinese populations in Thailand. This finding was similar to the result reported from Taiwan. However, these results were marginal statistical significance, which may well be due to the small sample size employed in the present study. Thus the *CYP2E1* gene appears to be a susceptibility gene for NPC development regardless of the patient's genetic background. Patients of both Thai and Chinese ethnic origin revealed a higher relative risk from the same allele, despite their distinct ancestry. Thus it is more likely that the *RsaI* negative allele affects the phenotype directly rather than being a consequence of linkage disequilibrium from another mutation or gene. This confirms the previous finding that the polymorphic *RsaI* site was essential for a marked difference in transcriptional activities.[104] A higher level of expression in the variant form would result in larger amounts of procarcinogens being changed into carcinogens, that then produce DNA damage. The affect of the distinct expression level of a metabolic gene should be reduced if the person with abnormal genotype is not exposed to the substrate. For example, Phenylketonuria (PKU) patients would not demonstrate mental retardation if they were prevented completely from exposure to tyrosine.[105] In other words, a mutation can not cause the phenotype without interaction from environmental factors. Regarding NPC development, the role of *CYP2E1* variant may be varied upon the amount of consumed salted fish and/or preserved foods that contain nitrosamine

and nitrosamine precursors. As mentioned briefly, this gene involves the mechanism of xenobiotic. Consequently, the reactive species of xenobiotic produced by metabolism, which bind to cell macromolecules such as DNA, RNA and protein. Thus, the toxic effects of xenobiotic can injure cells as severe enough to result in cell injury, immunologic damage or cancer. This mechanism may be one of cause NPC development because *CYP2E1* is expressed in nasal epithelium.

In addition, the result of this study showed association between candidate genes for EBV entry and NPC development. In contrast to *CR2*, the polymorphism of *pIgR* related to NPC development because the patient group has significant higher RR than normal control. Both total and Chinese sample groups not only had the higher RR value but also statistical significance of both heterozygous (+/-) and variant (+/+) form. Interestingly, the RR value of the total group is lower than Chinese. In addition, the Thai group showed no increase risk associated with their variant alleles. Therefore, *pIgR* may be a candidate susceptibility gene for NPC development especially for Chinese population and, unlike other candidate genes, *HLA* or *CYP2E1*, could explain the much higher incidence of this tumor in Chinese population than native South-East Asia. Furthermore, this data suggests that Chinese population may have a variant allele from one of their ancestor. As mentioned earlier, EBV can infect nasopharyngeal epithelium if there is altering epithelial polarity or some specific domain of *pIgR* was mutated. This mutation can contribute to NPC development by enhancing the mechanism of EBV entering nasopharyngeal epithelium. In other words, one possibility for susceptible people who are Chinese in origin develop their potency for NPC development is increase chances of having nasopharyngeal EBV infected epithelium by their ancient mutated *pIgR* molecules. This hypothesis also agrees with the strong correlation between EBV IgA titer and NPC patients. IgA antibody can combine with virus to form immune complex and endocytosed at basolateral surface by *pIgR*. For person who will develop NPC or become chronic EBV infected nasopharyngeal epithelium, the vesical cannot secret into the lumen. In contrast to less susceptible populations, their epithelial cells can secrete immune complex into the lumen (Figure 12). According to this

hypothesis, the RFLP allele distribution of *pIgR* is found more frequently in NPC patients. This could be a result of linkage disequilibrium between the RFLP and the ancient mutation of the *pIgR* especially from Chinese population. On the contrary, native Thai do not have the same risk due to not having the disease allele. Consequently, their RFLP marker showed negative result. The intermediate result identified in Thai-Chinese population may be the same consequence as mixed total population regarding true frequency of the variant *pIgR* in such mixed population.

Furthermore, this hypothesis can not only explain the individual contribution of *pIgR* allele but also population distribution. Interestingly unlike *CYP2E1*, only one variant *pIgR* allele, showing AD like pattern, can be responsible for NPC susceptibility. In general autosomal recessive disorder genes produce enzymes but autosomal dominant medical disorders are structural proteins. For example, PKU causes by homozygous mutation of tyrosinase and familial hypercholesterolemia, an AD disorder, has heterozygous LDL receptor mutation. This is mainly because enzyme defects depend on their dosage to express phenotype. In case of *pIgR,* defect of one out of two alleles in each cell can increase chances of transcytosis failure and consequently prone to be infected with EBV. Regarding population contribution, the higher frequency of mutated ancient *pIgR* allele in Chinese population may be explainable by selective advantage. Zhang and colleague[106] found that *pIgR* was a nasopharyngeal epithelial lumeninal pneumococci receptor. This is tempting to hypothesize that the Chinese *pIgR* mutation led to failure of locating the protein at the luminal surface. Consequently, those people would resist from pneumococcal nasopharyngitis and septicemia, which would be a significant cause of death in the past. On the contrary, though NPC is a fatal disease, the onset is beyond NPC patients' active reproductive phase. As a result, the NPC phenotype was not selected against but nasopharyngitis was the frequency of this allele and was selectively high in Chinese ancient population.

In conclusion, this study demonstrated the feasibility of candidate gene approach to study complex disease by searching for important candidate genes in which their proteins interact with the disease's environmental factor. This study confirms

the role of *CYP2E1* for NPC development in Thai population both Chinese and Thai in origin. More importantly, we demonstrated the first evidence of variant *pIgR*, which a possible function as EBV epithelial entry, might explain the highest incidence of NPC in Chinese population and also suggest the mechanism of EBV entering nasopharyngeal epithelium. However, our experiment studied polymorphism in intron. Thus the searching for mutation on exons and study their function must be done to prove the hypothesis propose by this thesis. Finally, the knowledge gain from this study will lead not only to understand the mechanism of NPC development but also may be an important knowledge for future NPC screening, diagnosis and treatment.

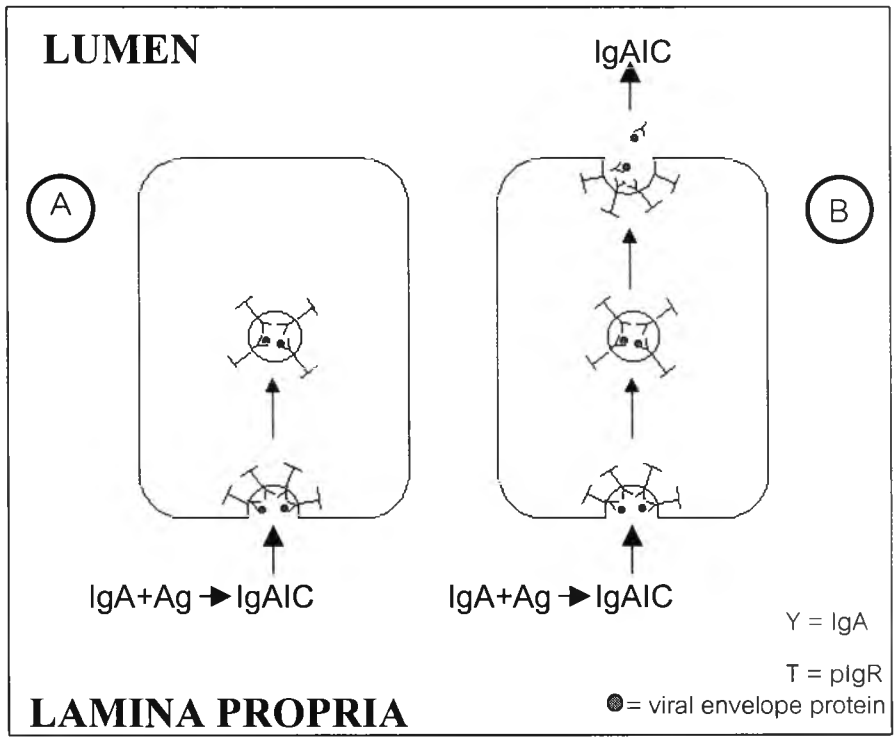

Figure12 Hypothesis of EBV entering nasopharyngeal epithelium and NPC development. *Cell A* shows that IgA antibody can combine with virus (**Ag**, antigen) to form immune complexes (**IgAIC**), which can be endocytosed at basolateral surface by pIgR receptor but not secreted into the lumen due to altering epithelial polarity or mutant pIgR. Thus, *cell A* may become cancer cell. Whereas, the vesicle can be secreted into the lumen in *cell B* as normal cell.