VI. SUMMARY

To investigate the linkage of class II HLA genes with the pathogenesis of Graves' disease, polymorphism of the HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes was analyzed in one hundred and seventy-eight unrelated patients (108 females and 70 males) from northern Thailand by using in vitro enzymatic DNA amplification followed by dot-blot hybridization with sequence-specific oligonucleotide probes (PCR-SSO). Seven, thirteen and nineteen alleles of each locus were found. The HLA-DQA1*0102, HLA-DQB1*0502 and HLA-DRB1*09012 alleles were the most common alleles. The allele frequency of alleles HLA-DQA1*0601 and DRB1*1202 were significantly reduced (RR = 0.21, p_c = 0.00007 and RR = 0.23, p_c = 0.00006, respectively) in both male and female patient groups. The preventive fraction (PF) confered by the alleles HLA-DQA1*0601 and HLA-DRB1*1202 were 9 % and 11 %, respectively. When patients were divided according to the age of onset, the HLA-DQB1*03032 allele was significantly associated with Graves' disease in a subgroup of patients with the estimated age of disease onset below 31 years (early onset group).

When subdivided according to other less common manifestation such as exophthalmos and relapse/exacerbation, the HLA allele distribution in patients with and without exophthalmos was comparable to the combined Graves' patient group. Interestingly, the HLA-DQB1*03032 allele was significantly increased in the early-onset group (RR = 2.28, *pc* = 0.03). The etiologic fraction (EF) confered by the allele HLA-DQB1*03032 was 13.3 %. Similarly, the HLA-DQB1*03032 and DRB1*09012 alleles was significantly increased in patients with relapse/exacerbation clinical courses (RR = 2.33, *pc* = 0.02, EF = 13.8 %; RR = 2.20, *pc* = 0.05, EF = 13.6 %, respectively). Within the early onset group, the HLA-DQB1*03032 allele was associated with Graves' disease with the laboratory proven group (RR =2.98, *pc* = 0.03, EF = 19.1 %) and the exacerbated group (RR = 3.48, *pc* = 0.05, EF = 22.9 %). The distribution of HLA alleles in Graves' disease patients with uncommon manifestations; periodic paralysis, myasthenia gravis and myopathy, tended to be quite similar to those of regular Graves' disease patients.

Two uncommon haplotypes HLA-DRB1*1303-DQA1*0501-DQB1*0301 and DRB1*1202-DQA1*0601-DQB1*0301 were significantly increased in Graves' disease patients. However, they were detected in only 4.3% and 2.9%, respectively, of all patients.

Among different allelic forms of the HLA-DQA1 molecules, there are at least 6 polymorphic positions which line the peptide binding groove. Two of these positions, a tyrosine residue at the position 25 of the alleles HLA-DQA1*0501, *0301, *0101 and *0102 and a leucine residue at the position 69 of the HLA-DQA1*0501, *0301 and *0201 alleles were significantly increased in patients with Graves' disease in this population (RR = 4.11, $p = 0.0000007$, EF = 71.9 %; RR = 1.48, $p = 0.028$, EF = 12.8, respectively).

These findings indicated that the HLA-DQA1*0601 and HLA-DRB1*1202 alleles are associated with resistance to the development of Graves' disease. Most interestingly, the HLA-DQA1 molecules with tyrosine at the position 25 or leucine at the position 69 appear to increase the susceptibility to Graves' disease in this northern Thai population. This finding demonstrated that there was no positive association between Graves' disease and HLA allele in the gene level but the association was clearly seen in the amino acid level. It reflected the role of the relevant functional molecules. On the contrary, the HLA-DQB1*03032 and HLA-DRB1*09012 alleles conferred susceptibility for the development of exophthalmos and the relapse/exacerbated clinical courses in Graves' disease patients who were affected early in life.

However, the associations between Graves' disease and HLA-DQA1, HLA-DQB1 and HLA-DRB1 genes were not strong in this population and there were many associated HLA allele were seen in other Oriental populations. The study in the amino acid level may find the cause of disease. Thus, further studies of the finding of the TSHR antigen and the interaction between the TSHR peptide antigen and the amino acid side chain of the HLA peptide binding groove may clarify the pathogenesis of Graves' disease in our population.