

## CHAPTER 4

### DISCUSSION

Early studies of the association of HLA genes with Graves' disease had been conducted mostly in Caucasian populations (Farid et al., 1979, Dahlbreg et al., 1981, Yanagawa T, 1993). These studies almost invariably revealed strong association of the HLA-DR3 which is in strong linkage disequilibrium with the HLA-DQB1\*0201 and HLADQA1\*0501 alleles (Yanagawa T, et al,1993) as the positive marker of Graves' disease. Recently, a few studies were conducted in several Oriental populations with GD but no association with the HLA-DR3 allele was observed. (Azuma Y, et al, 1982, Yeo PP, et al, 1989, Dong R, et al, 1992, Huang SM, et al, 2003) Similar to other studies, our study in the Southern Thai population is also shown no association of HLA-DR3 allele with Graves' disease. The former study, conducted in Thai GD patients of King Chulalongkorn Memorial Hospital , Bangkok, Thailand (T. Wongsurawat. 2006) had demonstrated the significant association of the DRB1\*1602-DQA1\*0102-DQB1\*0502 haplotype in GD patients (P = 0.0209, OR = 2.55). DRB1\*07-DQA1\*0201-DQB1\*0201 haplotype (P = 0.039, OR = 0.32) and HLA-DRB1\*12-DQA1\*0601- DQB1\*0301 haplotype (P = 0.0025, OR = 0.28). The significant association of DRB1\*1602-DQA1\*0102-DQB1\*0502 and HLA-DRB1\*12-DQA1\*0601- DQB1\*0301 alleles and haplotypes with GD was also reported in Korean population. (Park HP, et al, 2005). Thus HLA-DRB1\*1602-DQA1\*0102-DQB1\*0502, might serve as a marker for genetic susceptibility to GD in Asian population. (T. Wongsurawat. 2006) Similarly, the data have shown a positive association between HLA-DQB1\*05 and GD, and also supported evidence of HLA-DRB1\*0701 as a protective effect against development of the disease in Thai population. In at least two studies, the association appears to involve only the HLA-DQB locus. For examples, Graves' disease has been shown to associate with the HLA-DQB1\*0303 allele in Hong Kong Chinese (R.R. = 3.2; n = 90) (Cavan et al., 1994). In another study, HLA association with GD involves serologic determinant governed by the HLA-DQB allele, such as the HLA-DQw4 allele in the Japanese (RR = 3.2; n=88) (Inoue et al., 1992). On the other hand, a study of Singaporean Chinese revealed no positive association of any HLA class II alleles (HLA-DQA1, -DQB, -DRB1,-DRB3, -DRB4, -DRB5 and -DPB1 loci were considered) with Graves' disease (n = 33; HLA-DR3 = 11.4% antigen frequency in normal control) (Chan et

al., 1993). The discrepancy could be due to the low number of cases studied in this Singaporean report. The advances in molecular technology have altered the HLA typing methodology. The increasing number of polymorphic alleles identified by DNA typing could lead to the loss of significant association after the correction of p-value with the number of polymorphic alleles. The lack of association of HLA-DR3 with Graves' disease in the Asians correlates with the low frequencies of this allele in most Oriental populations (~5% vs ~14%) (Cerna et al., 1993, Rønningen et al., 1990, Gao et al., 1991, 1993, Doherty et al., 1992). In addition, The primary etiological variants in the HLA region, however, remain unknown, largely because of the strong linkage disequilibrium (LD) between the class II alleles and other variants in the region. The study of MHC susceptibility/protective alleles in populations of distinct ethnic background or the realization of transracial analyses to look for common alleles in different homogeneous populations, may help to discriminate alleles which are relevant immunogenetic markers for a specific disease, supporting or ruling out previous MHC and disease associations. (Maciel L, et al, 2001)

Class II molecules are attractive primary candidates for etiological determinants of GD because of their involvement in both antigen presentation in the periphery and in thymic deletion of potentially auto reactive T cells and positive selection of a T-cell repertoire, some of which may be capable of recognizing self-epitopes and causing autoimmune damage in genetically susceptible individuals (Weetman 1994). Ban et al., (2004) recently sub typed HLA-DR3, by direct sequencing, HLA-DR3b chain in a population of DR3- positive GD patients and controls and identified critical amino acids for the susceptibility to GD. The allelic frequency of DRB1\*0311 (a protective allele in Caucasians) was significantly lower in patients than in controls. The specific amino acids occupying the peptide-binding pockets of DRB1\*0311 showed that the lack of arginine at position 74 of the DRb chain(DRb74Arg) was significantly more frequent in the DR3-positive controls than in the DR3-positive patients (Ban et al., 2004). These results suggested that GD is associated with specific sequences of the HLA-DR3 allele. Another recent study by Simmonds et al. (2005) also confirms a strong association of arginine at position 74 with GD. Interestingly, the lack of arginine at position 74 of HLA-DRB\*0311 is also found on the DRB1\*07 which is the protective allele in Thai population and several other studies (33 Lavard L, et al, 1997, 34 Schmidt D, et al, 1997). The arginine 74 is replaced with a glutamine,

which is not only a polar residue, but also has a shorter side chain than arginine. Glutamine is substituted in this position and may have a role in the protection of GD, possible that DRB1 position b74 is a primary determinant of susceptibility. However, it is also possible that DQA1 and DQB1 alleles as well as other genes in linkage disequilibrium to this haplotype might contribute to the protective effect in GD.

So, the substitution of the amino acid residue which change the structure or property of the binding groove is now known to change its binding affinity to antigenic peptides (Brown et al., 1988). One possible explanation for the inconsistency of HLA-disease association with regards to population is the difference in HLA allele frequency in various populations, especially when small sample size was used for the study of this common disease. The chance of detecting the statistically significant association of a particular allele of HLA molecules would depend on the prevalence of that allele in each population. Another possible explanation may reflect the presence of non-HLA susceptibility factors. In an effort to explain the lack of a common HLA susceptibility marker with Graves' disease when comparing patients from different races, Caven et al. (1994) suggested that the difference was due to the influence of non-HLA factors. the HLA class II alleles that are decreased association with of Graves' disease are also varied in various populations. One possible explanation is the lack of immunogenicity of bound peptide or lack of peptide binding in certain HLA alleles. Our findings support the idea that patients with Graves' disease have multiple distinct HLA susceptibilities with regard to HLA genotypes. Complete sequence determination in very large sample sizes and additional functional studies will be required to further distinguish between the various explanations for the association of the HLA region with GD and other HLA associated disorders.