Thesis Title Restriction Fragment Length Polymorphism (RFLP) Analysis of HLA Class II Genes in Thais.

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ABSTRACT

The HLA Taq I DNA-RFLP allogenotyping was employed to study a hundred DNA samples in central Thai population previously characterized by the method of serology (lymphocyte microcytotoxicity test) and PCR-sequence specific oligonucleotides/ sequence specific primers (PCR-SSO/SSP) genotyping. DNA samples were digested with Taq I endonuclease, 0.7% agarose gel electrophoresis, capillary transferred to nylon membrane and subsequently hybridized with ^{32}P-labelled HLA-DRβ, DQβ and DQα probes. A further Msp I restriction digest was performed selectively on samples with DRβ7/9 pattern and hybridized with the DQα probe. The DRβ-RFLP assignment was based on the characteristic RFLP pattern and the known linkage information between HLA-DR/DQ in both Caucasian and Thai populations. Three RFLP variants, the DRβ16b, DRβ12b and DRβ13c were observed. The DRβ16b (defined by the 13.0, 1.7 & 1.65 kb fragments) was different from the DRβ16 (i.e. DRβ16a in this study) by having a smaller upper fragment size of 13.0 instead of 14.8 kb and was found to be the common DRβ16 RFLP in Thais (DRβ16a : DRβ16b = 1:3). The DRβ12b, the rare variant previously

described as DRβx3 in Caucasoid (defined by the 11.1, 4.2 and 4.1 kb fragments) was the most prevalent DRβ12 pattern found in Thais. The newly described RFLP (defined by the 13.0, 6.5 and 4.1 kb fragments) was firstly misassigned as DRβ11 (defined by the 13.0, 6.5 and 4.2 kb fragments), but was found to be DRB1*1303 by the PCR-SSO/SSP typing. Thus, this variant could be a closely related to DRβ13b (defined by the 11.1, 6.5 and 4.1 kb fragments) namely DRβ13c in this study.

Though the majority of RFLP patterns found in Thais were similar to those found in Caucasoid, their DRβ/DQβ/DQα RFLP linkage associations were quite different, particulary in the DRβ15/DRβ16b, DRβ12b, DRβ14b และ DRβ7 RFLP subtypes. The DNA-RFLP typing was highly correlated with serology and PCR-SSO/SSP typing results, only 2 DRβ RFLP discrepancy was noted. In contrast, 5 and 7 discrepancies with 3 and 9 blank assignments were observed with the HLA-DR and -DQ serological typings.

In conclusion, the HLA DNA-RFLP allogenotyping could offer a more accuracy than serology in the HLA-DR/DQ typing in Thais when the known linkage associations were taken into account. However, the PCR-SSO/SSP typing should be used to either verify any typing problem or assign the specific alleles.