DHAPTER VI

DISCUSSION

In the present study, we focus on the TGFß2 gene (located on chromosome 1q41) within TGFß family as SLE susceptibility gene. Emerging data suggests that this family is an important cytokine in immunoregulation. TGFß is an important factor in the generation of regulatory T cells that down-regulate B cells function. It is thought that SLE is a T cell dependent autoimmune disorder with T cell dysfunction; thus the mechanisms that maintain self tolerance and down-regulate B cell function were broken down. In addition, many studies in glomerulonephritis disorder point that TGFß has important role in renal fibrosis and chronic kidney disease. Positional genetic study in both murine and human also supported the important of chromosome1, related with SLE susceptibility especially on 1q41 as the major loci. So far there was only one previous association study in Causasian using one marker at the 5'UTR of TGFß2 gene with SLE susceptibility (Alansari, Hajeer et al. 2002). That study gave negative association result. Since TGFß2 gene have gave span approximately 100 kb and consisted of 2 haplotype blocks with in one gene, the negative association using 1 marker doesn't rule out the importance of this gene. In this study, polymorphism within all exons and nearly introns within TGFß2 gene were analyzed and reported by THAISNP project by sequencing 32 Thai healthy controls. Out of the seven polymorphisms that were detected by THAISNP, we selected 4 markers as haplotype tagging SNP to represent all common haplotypes. The two markers (+71_72insACAA and +720(T/G) are located in 5'UTR and proximal intron1 region are located within one haplotype block. The other 2 markers (+89835(A/G) and +94400_94401insA) located in intron 5 and intron 6 which are located within another block. However, each of the 2 markers belong in the same hap-block were not tight linkage disequilibrium with each other.

Briefly, we genotyped 153 Thai patients with SLE and 133 normal controls and found that the wild type allele _ at position +71_72insACAA, allele T at position +720(T/G) and wild type allele _ at position +94400_94401insA were associated with the increased risk of SLE disease (p = 0.02, OR = 1.83, 95%CI = 1.09-3.08, p = 0.01, OR = 2.37, 95%CI = 1.19-4.77 and p = 0.00008, OR = 2.91, 95%CI = 1.66-5.15,respectively). Furthermore, haplotype analysis at 4 positions reveal a strongest association in _/T /A/_(common) haplotype with SLE susceptibility in Thai population (p = 0.0001, OR = 2.64, 95%CI = 1.58-4.42).

I will particularly of on the forth position due to the highly significance (p = 0.0001, OR = 2.64, 95%CI = 1.58-4.42) at this position. There are at least three possible hypothesis for the existence of a strong association between an allele or genotype and the disease susceptibility at this position. Firstly, TGFß2 at position

+94400_94401insA is on the causative SLE. However, the allele that associated with disease risk is a wild type allele (_ in normal control). So this hypothesis is unlikely. In addition, since this position is located within the intron, it should be hypothesized that the polymorphism in this region might affect RNA splicing. RNA splicing is a series of processing reactions where by the intronic RNA segments are snipped out and discarded and the exonic RNA segments are jointed end-to-end to give a shorter RNA product. RNA splicing requires the nucleotide sequences at the exon/intron boundaries (splice junctions) to be recognized. In the vast majority of case intronics start with GT and end with AG, it compound splice donor site, branch site and splice acceptor site. Intronic sequence that is known to be functionally important in splicing is the so-called branch site which is usually located very close to the end of the intron, at most 40 nucleotides before the terminal AG dinucleotide but mutation from position +94400_94401insA has 300 nucleotides far from the end of AG dinucleotide in the intron6. Therefore, this hypothesis might not true. Secondly, the risk in this position has linkage disequilibrium with the causative polymorphism or mutation. This is the most likely possible hypothesis. Therefore, further study using other polymorphism nearby or within the same block is required to discover the putative causative position. We hypothesize that the causative polymorphism or mutation may occurred in the exon region in SLE patients might be performed. Since TGFß2 consisted of various splicing form, it is also possible that the causative position will affect the splicing position which subsequently cause the dysregulation of the immune system. The analysis of splicing forms of TGFß2 in SLE patients might help elucidate this hypothesis. Thirdly, it might be a false positive association finding for this position +94400_94401insA. Although this is unlikely due to quite a strong association, independent study inorder to confirm the result is preferred. As for the association with clinical regression, SLE patients were divided to subgroup with and without specific organ involvement (lupus nephritis, skin manifestation arthritis efc.). However, no significant association was observed in this study.