CHAPTER VI

DISCUSSION

VEGF is a key cytokine of angiogenic factor in chronic plaque psoriasis. It is produced by both epidermal keratinocytes (KC) and peripheral blood mononuclear cells (PBMCs) (Detmar, Brown et al. 1994; Young, Summers et al. 2006). The epidermal keratinocytes also show to be the main source of VEGF for physiological and pathological cutaneous angiogenesis (Malhotra, Stenn et al. 1989). Some previously reports, a plasma level of VEGF has been shown that are elevated in psoriasis patients with erythroderma (redness) when compared with normal controls (Creamer, Allen et al. 1996). In chronic plaque type, previously reports revealed that a correlation of VEGF levels in lesions of psoriatic skin (Bhushan, McLaughlin et al. 1999). However, a significant increase in new blood vessel formation can also be seen in psoriatic skin lesion. Furthermore, VEGF also can promote chemotaxis of monocytes. Bone marrow-derived cells can be effect by them (Ferrara, Gerber et al. 2003).

Positional genetic study in human also supported the important of chromosome 6, related with psoriasis susceptibility especially on 6p21.3 as the major loci of *PSORS1* region. So far there was only three previous association study in Caucasian using marker at the 5'UTR of VEGF gene with psoriasis susceptibility (Young, Summers et al. 2004; Barile, Medda et al. 2006; Young, Summers et al. 2006). That study gave positive association results including associated with early-onset psoriasis, severity and high or low VEGF producers. In details, Young et al. showed significantly association between +405CC genotype and C allele with early-onset and severe disease. Moreover, this analysis showed significantly association between -460TT genotype and early-onset psoriasis due to high VEGF production. Our result is also correlated with them. Otherwise, Barile, Medda et al. revealed that the -1557AA and -460CC showed to be a significantly associated with developing psoriasis at late-onset. There has not been reported in Asian especially in Thai population.

In this study, we discovered possible associations between the SNPs of the promoter and proximal exon1 region, a regulatory region, of the VEGF gene and Thai chronic plaque psoriasis. We investigated three VEGF gene polymorphisms including -1557C/A, -460C/T and +405C/G). For our result, the frequencies of -460TT or TC compared to -460CC genotype were found to be increased in type-1 or early-onset psoriasis when compared with normal control subjects. Interestingly, our data is correlated with recent report in Caucasian that has shown the -460TT VEGF genotype is associated with genetic susceptibility to develop early-onset chronic plaque psoriasis as well as severe disease (Young, Summers et al. 2006). We suggest that the -460TT or TC genotype may be important to susceptibility of early-onset disease due to high VEGF production by PBMC.

In haplotype analysis, we found that the -1557C/-460T/+405G haplotype significantly associated with both the susceptibility to early-onset and severe psoriasis. These haplotype results revealed that the linkage disequilibrium can cause statistically significant association when compared with each single variation on disease susceptibility. One of the other SNPs, through tight linkage disequilibrium, might be responsible for detected association with chronic plaque psoriasis. There was one functional analysis of the VEGF haplotype by luciferase-reporter assay. The reporter assay showed that one haplotype, carrying -1557A/-460C/+405G polymorphisms was associated with increased production of VEGF *in vitro* both resting and inducing reporter activity. In addition, Haplotype 3 (A) (-1557 or -1540A*/-1451T/-460C*/-160T/-152A/+405G*) showed constantly higher promoter activity when compared with haplotype 4 (B) (-1557A*/-1451T/-460C*/-152A/-116A/+405G*) and haplotype 1 (C) (-1557C*/-1451C/-460T*/-160C/-152G/-116G/+405C*) (Jacobs, Feigelson et al. 2006). The VEGF haplotype of reporter assay were shown in figure 16. That result suggested that the combination of polymorphisms may be responsible for expression level rather than the result from one position.

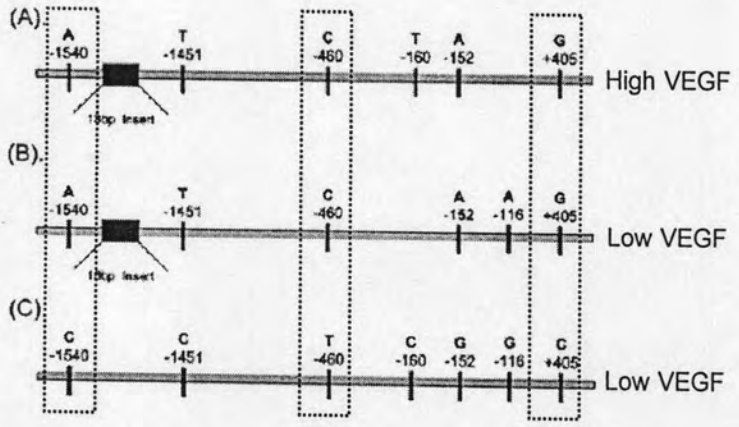
Figure 16. The data of VEGF haplotypes 1(C), 2, 3(A) and 4(C) and haplotype frequency in normal controls (Jacobs, Feigelson et al. 2006; Rogers and D'Amato 2006)

VEGF gene promoter haplotypes

From ATG	-2578	-2549	-2489	-2447	-1498	-1198	-1190	-1154	-634	-7
From TSP	-1540	-1511	-1451	-1409	-460	-160	-152	-116	+405	+1032
Haplotype 1 (C)	C	---	C	G	T	C	G	G	C	C
Haplotype 2	C	---	C	G	T	C	G	G	G	C
Haplotype 3 (A)	A	18bp	T	-	C	T	A	G	G	T
Haplotype 4 (B)	A	18bp	T	-	C	C	A	A	G	C

Haplotype frequency in normal controls (%)							
Haplotype	Japanese	UK	Poland, Germany	Korea	China	India	Thai (This study)
Haplotype 1 (C)	34.3	25.5	29	42.8	38.5	25.0	36.32
Haplotype 2	31.8	19.6	22	32.3	35.1	28.3	33.33
Haplotype 3 (A)	17.8	21.2	*48	12.5	*24.2	*44.3	*27.35
Haplotype 4 (B)	12.7	26.5		12.5			

*Fused boxes indicate that haplotype 3 and 4 cannot be distinguished using the markers typed in those studies.



Unfortunately, there was no reporter activities with haplotype containing -1557C/-460T/+405G (haplotype 2) which is significantly associated with susceptibility and severity of psoriasis in Thai population. The frequencies of this haplotype in Asian populations (32-35%) are higher than in Caucasians (19-22%) (Fig 16).

Interestingly, some previous studies showed the higher levels of VEGF production were significantly observed in stimulated PBMCs which have the -1557C and +405G alleles (Watson, Webb et al. 2000; Shahbazi, Fryer et al. 2002). We can hypothesize that haplotype containing -1557C/-460T/+405G is associated with high VEGF production. In this study, we attempt to determine a relationship between the -1557C/-460T/+405G haplotype and plasma level of VEGF in psoriasis patients. Plasma from psoriasis patients containing different haplotype but with the same condition including early-onset, mild, stopped systemic treatments were assayed for VEGF protein expression by ELISA. We could not observe any significant relationship. However, our sample size is very limited and various factors can pressure circulating VEGF in the plasma such as other environmental factors. We suggest that the *in vitro* reporter assay with -1557C/-460T/+405G haplotype should be performed in future study.

The VEGF gene locates on chromosome 6p21.3 in *PSORS1* as well as the MHC gene. Therefore, the associations may due to linkage disequilibrium (LD) between the MHC especially HLA-Cw6 and VEGF polymorphisms. This question was investigated by Young et al. that there was no linkage disequilibrium examined between either of the VEGF and HLA-Cw6 polymorphisms in UK population. Thus, our positive association of VEGF gene is not shared likely due to LD with MHC gene.(Young, Summers et al. 2004).

We have also summarized previous studies of VEGF polymorphism in many diseases that show similar trend to our result (table 4 and 5).

At position -1557C/A, Seven of ten studies revealed that the -1557CC genotype or -1557C allele were found to be associated with many diseases due to higher VEGF production on PBMC.

At position -460C/T, five of seven studies revealed that the -460 TT genotype or -460T allele were found to be associated with many diseases.

At position +405C/G, nine of fourteen studies revealed that the +405 CC genotype or +405C allele were found to be associated with many diseases due to strong VEGF expression in lung tumor. Five studies revealed that +405GG genotype or +405G allele were found to be associated with various diseases due to highest VEGF expression on PBMC.

In conclusion, our data suggests that the VEGF polymorphisms in this study confer an increased risk in chronic plaque psoriasis, especially in early-onset psoriasis. Therefore, -1557C/-460T/+405G VEGF haplotype may be used as a genetic marker for early-onset psoriasis in Thai population. However, it is well known that the genetic association studies are commonly prone to result heterogeneous varying by ethnics and geographical areas. This polymorphism may be representing associated haplotype in Thai early-onset psoriasis but may not represent in other ethnic backgrounds. Nevertheless, Psoriasis is a complex disease. The single gene study is not enough. For that reason, the association study of other genes or markers should be performed in further study. Interestingly, the frequencies of -1557C/-460T/+405G haplotype was increased in Thai psoriasis patients. We suggest that this haplotype is associated with high VEGF protein production. One of our future study is *in vitro* reporter assay with the haplotype containing -1557C/-460T/+405G. Furthermore, epigenetic mechanisms that can pressure a regulation of gene including methylation will be included in future investigation because epigenetic regulation may influence with susceptibility to psoriasis as well.