CHAPTER I

INTRODUCTION

Psoriasis is a relatively common chronic inflammatory skin disorder affecting approximately 2-4% of the population worldwide, males and females equally, with prevalence varying in accordance with race and geographic distribution (Greaves and Weinstein 1995; Nickoloff and Nestle 2004; Campalani and Barker 2005). Clinically, psoriasis is characterized by sharply demarcated erythematous plaques and covered by a silvery scale. The extensor surfaces, such as knees and elbows, commonly are involved as well as the lower back, scalp and nails (Luba and Stulberg 2006). The pathology of chronic plaque psoriasis is distinguished by altered keratinocyte hyperproliferation and differentiation of the epidermis and inflammation of both the epidermis and dermis (Greaves and Weinstein 1995; Bowcock and Krueger 2005). Patients with early-onset psoriasis are younger; they have strong family histories and more forceful disease. On the contrary, those with late-onset psoriasis are older; they have more stable disease (Greaves and Weinstein 1995). Previous data were suggested that psoriasis was a primary T-lymphocyte-based. The cytokine production by activated T cells is a Type-1 inflammatory profile for instance interferon gamma (IFN-γ), tumor necrosis factor (TNF-α) and interleukin (IL) 12 (Nickoloff 1999; Lew, Bowcock et al. 2004). Activated $CD4^+$ T cells are primarily situated in the lesional dermis and mostly $CD8^+$ T cells exocytose in the psoriatic epidermis (Nickoloff and Nestle 2004). Strongly supported of T-cell immune-mediated pathogenesis by functional study of T cells infiltration isolated and cloned from the psoriatic lesion revealed that they was capable of stimulating keratinocyte proliferation by secreted mediators. In keratinocyte stem cells from psoriasis patients, T-cell-derived mediators could only induced a proliferative response but not happen in those from healthy donors. Furthermore, the apply of human skin grafts transplanted onto severe combine immunodeficiency mice (SCID mice) were injected with activated T cells from psoriasis patients, they develop a typical psoriatic phenotype, enhanced keratinocyte proliferation and granulocyte accumulation. (Prinz

1999). Chronic plaque psoriasis could be transferred by bone marrow transplant from psoriatic donors to recipients without known susceptibility to psoriasis, a result that support the induction of illness through cellular immunity (Bowcock and Krueger 2005). Nowadays, psoriasis is defined a T-cell-mediated autoimmune disease (Bos and De Rie 1999; Luba and Stulberg 2006).

Cumulative studies suggest that interaction of multiple genes and environmental factors are contributed to psoriasis susceptibility (Raychaudhuri and Farber 2001). A strong genetic component associates with chronic plaque psoriasis, although only the susceptibility is inherited and additional exacerbate factors are needed for the disease to appear (Bowcock and Krueger 2005). Especially, twins studies show the concordance rate of psoriasis in monozygotic twins is approximately greater than in dizygotic twins, being around 72% and 23%, respectively. Moreover, The evidence of genetic predisposition to psoriasis in several family studies have demonstrated that as various as half of siblings of persons with psoriasis develops the sickness when both parents are involved, but prevalence cascades to 16% when only one parent has psoriasis and falls to 8% when neither parent is involved (Bowcock and Krueger 2005; Schon and Boehncke 2005). Many genetic studies by linkage analysis and association studies implicated that a variety of genes are related to psoriasis (Bowcock 2005). During the recent years, the search for predisposing to complex disease such as, psoriasis in human can be broadly divided into two strategies: the genome-wide linkage and candidate-gene-association studies (Capon, Dallapiccola et al. 2000). Numerous genome-wide scans and linkage analysis have identified to at least 19 psoriasis-susceptibility loci show significant evidence of linkage to psoriasis. Among the identified linkage is nine "Significant linkage" or "Major loci" to psoriasis. These are *PSORS1* (6p21), *PSORS2* (17q25), *PSORS3* (4q), *PSORS4* (1q21), *PSORS5* (3q21), *PSORS6* (19p13), *PSORS7* (1p), *PSORS9* (4q31-4q34) and *PSORAS1* (16q12) (Bowcock and Krueger 2005; Campalani and Barker 2005). These linkage results are encouraging, considering that confirmation of significant linkage of a locus offers the strongest evidence for existence of putative susceptibility genes. Further efforts in fine mapping to narrow the linked loci for the eventual identification of susceptibility genes are necessary (positional candidate gene). Another strategy is candidate-gene-association studies. In

candidate gene analysis an allele or haplotype, or any DNA polymorphisms, is directly evaluated. A difference in frequency of an allele is demonstrated between affected patients and appropriate controls. Therefore, genetic association with a candidate gene suggests that the polymorphisms being measured is the actual disease-causing allele or one located very closely to the responsible gene. Several candidate genes have been studied and found to be associated with psoriasis such as, MHC class I, *HCR*, *CDSN* (6p21), *SLC9A3R1*, *RAPTOR* and *NAT9* (17q25), *SLC12A8* (3q21) (Bowcock and Krueger 2005).

Psoriasis susceptibility 1 (*PSORS1*), is regarded the most important susceptibility locus, chromosomal location of *PSORS1* is 6p21. *PSORS1* is associated with up to 50% of psoriasis cases (Schon and Boehncke 2005). Many genes encoding proteins with regulatory and adaptive functions or induced vascularization in the immune system have been considered as candidates. One in many that interesting is vascular endothelial growth factor (VEGF) gene. The VEGF gene is categorized in 8 exons divided by 7 introns. The coding region have 14 kb and this gene is located on chromosome 6 at 6p21.3 (Pages and Pouyssegur 2005).

Vascular endothelial growth factor (VEGF or VEGF-A) gene is proposed to play important roles in pathogenesis of various diseases with angiogenesis basis and autoimmune disease including psoriasis. The VEGF gene family consists of VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PLGF). The common isoforms of VEGF has at least 5 different isoforms from 9 subtypes due to the single gene alternative splicing. The more common isoforms consist of $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$, respectively. VEGF-A is generally involved in angiogenesis while VEGF-C and VEGF-D are involved in lymphangiogenesis (Byrne, Bouchier-Hayes et al. 2005; Takahashi and Shibuya 2005). The human keratinocytes revealed mRNA of 3 major splice forms of VEGF such as, $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ (Ballaun, Weninger et al. 1995). VEGF is a more powerful mitogen and permeability factor of vascular endothelial cells, also can promote the growth of new blood vessels (Ferrara, Gerber et al. 2003). Besides, VEGF can promote chemotaxis of monocytes. Bone marrow-derived cells can be effect by them (Ferrara, Gerber et al. 2003).

Evidence indicates that psoriasis is angiogenesis-dependent. Since, psoriasis has many dermal microvascular developments in lesion. Image analysis quantification of immunostained microvessels from paired psoriatic and non-psoriatic skin biopsies in patients with psoriasis have showed a fourfold increase in endothelial of lesional skin (Creamer, Sullivan et al. 2002). Moreover, VEGF expression level were significantly enhanced in lesion of psoriatic skin as compared with healthy control skin (Detmar, Brown et al. 1994; Bhushan, McLaughlin et al. 1999).

Many studies analyzed the association of VEGF gene polymorphism in various positions in Caucasian and non-Caucasian. Most reports in some position that really point to important about SNP with several autoimmune diseases including psoriasis. But nowadays, no any reported SNPs of VEGF gene including function of SNPs with psoriasis in non-Caucasian. We are interested in the role of VEGF gene polymorphisms and genetics susceptibility of psoriasis in Thai population due to both roles in psoriasis pathogenesis and its gene position which lie in these positions. For that reason, we accordingly chose VEGF as the candidate gene in this study.

Thus the aim of this study, VEGF gene was analyzed by candidate gene approach. Population-based case-control studies were used to investigate the polymorphism of VEGF gene in patients with chronic plaque psoriasis compare with control group and determine the association with chronic plaque psoriasis in Thai population. We are interested in the polymorphisms of VEGF gene that might influence disease susceptibility and severity, and operate as marker for the disease. Furthermore, VEGF concentration in plasma of psoriasis patients was determined by Quantitative sandwich enzyme immunoassay technique (Sandwich-ELISA) and the correlation between genetic polymorphisms of VEGF gene and VEGF protein production in plasma of chronic plaque psoriasis patients were investigated.

We select VEGF SNPs in Thai population by searching from SNPper database. We chose 3 SNPs from 136 SNPs in order to represent the SNP within promoter and exon region of VEGF gene by considering the suitable SNP distribution and frequencies (greater than 5%). Additionally, we chose appropriate SNP based on functional and previous studies reports. Moreover, many study report that -1557 C/A polymorphism was associated with VEGF production and it might be also associated with severe

diseases and disease progression (Shahbazi, Fryer et al. 2002; Papazoglou, Galazios et al. 2004; Szeto, Chow et al. 2004; Breunis, Biezeveld et al. 2006). The -460C/T and +405C/G were found to be correlated with VEGF protein production and putative angiogenic basis in many diseases (Young, Summers et al. 2006). Based on previous studies that the +405C allele is associated with elevated serum levels of VEGF in healthy individuals (Watson, Webb et al. 2000). Furthermore, in Caucasian the +405CC genotype and C allele were found significantly more often in early-onset and severe psoriasis (Young, Summers et al. 2004). However, there have not been any studies in Asian population. Interestingly, the reporter assay showed that one haplotype, carrying -2578A/-460C/+405G polymorphisms is also associated with increased production of VEGF *in vitro* both resting and inducing reporter activity (Stevens, Soden et al. 2003).

The genotyping method for VEGF gene polymorphisms were done by PCR-specific sequence priming (PCR-SSP) and PCR-restriction fragment length polymorphism (PCR-RFLP), respectively. After that, genotypes, allele and haplotype frequencies were compared between chronic plaque psoriasis patient and control subjects.

We hypothesized that the specific polymorphism of VEGF gene that determine risk for development and severity of psoriasis in Thai population will be found. This study assist contribute to the identification of psoriasis susceptible gene and might lead to development of new prognostic markers based on these genotypes, with the aim of the better understanding of mechanism of psoriasis and development of new treatment and prevention. In addition, it will provide the frequency of VEGF gene polymorphism in Thai population which it basic knowledge for study these markers in other diseases in the future.