CHAPTER III

LITERATURE REVIEW

Psoriasis

Psoriasis is a chronic inflammatory autoimmune skin disorder that is characterized by sharply demarcated erythematous plaques covered with a silvery scale. Psoriasis is a multiple gene involvement that affected skin, nails, mucous membranes and joints. Clinically, it was considered to be a disease of keratinocyte hyperproliferation and parakeratotic differentiation. The epidermal changes in lesion of psoriatic skin occur in response to cellular immunity. The present day, psoriasis is a T-lymphocyte-mediated dermal immune response with T-type 1 profiles including Interferon-γ (IFN-γ), Tumor necrosis factor-α (TNF-α), Interleukin-2 (IL-2) and Interleukin-12 (IL-12) (Bos and De Rie 1999; Nickoloff and Nestle 2004; Bowcock and Krueger 2005; Luba and Stulberg 2006).

Epidemiology

Psoriasis is a worldwide disease. The prevalence rates of psoriasis is estimated around 2-3% of world's populations (Greaves and Weinstein 1995; Christophers 2001). Ethnic backgrounds and geographical areas also effect to influence the prevalence of psoriasis (Raychaudhuri and Farber 2001; Schon and Boehncke 2005). Generally, psoriasis is more common in colder northern weathers than in tropical regions. The high prevalence rates of psoriasis has been reported 11.8% from Kazach'ye that locate in Arctic region of the former Soviet Union but the lowest prevalence rates has been accounted 0% from Samoa. The prevalence of psoriasis in America was estimated to be around 4.6% while in Canada it was to be 4.7%. In Asia, The population has the largest quantities with abundant races and sub-races. The prevalence rates in India ranges

from 0.5% to 1.5%, 4-5.5% in Malaysia, 0.29-1.18% in Japan and 3.1% in Kuwait, respectively (Christophers 2001; Raychaudhuri and Farber 2001).

Etiology

Psoriasis reveals a complex pattern of inheritance that is consistent with the involvement of multiple susceptibility genes as well as environmental risk factors (Nickoloff and Nestle 2004). Psoriasis is more prevalence among relatives of the affected patients but dose not follows simple Mendelian inheritance patterns. Strong evidence supports the role of genetic component for susceptibility to psoriasis. Initially, in twin who usually shared the same environments, the disease concordance rate is 15-23% for dizygotic twins and 72% for monozygotic twins. There 5-fold difference in the disease concordance rate between identical twins and fraternal twins (Bowcock and Krueger 2005). The degree of familial clustering, measured by comparing the risk of a sibling with the risk in the population as a whole(s), varies between 8-50% (Schon and Boehncke 2005).

Classification of psoriasis

In textbooks, Psoriasis illustrates to be of consequence to classify more precisely the type of psoriasis under study. It is also classified by a major types of lesion into 2 groups including Non-pustular and Pustular psoriasis, as well as is categorized by a lesional morphology (Christophers 2001). (Table 1)

Table 1. Clinical forms of non-pustular and pustular psoriasis (Christophers 2001).

Non-pustular psoriasis	Pustular psoriasis
Type I, early onset	Generalized von Zumbusch type
Type II, late onset	Impetigo herpetiformis
Guttate psoriasis	Localized
	Palmo-plantar pustular psoriasis
Psoriatic erythroderma	Acrodermatitis continua
Drug-induced psoriasis	Annular pustular psoriasis

Chronic plaque psoriasis (Psoriasis Vulgaris)

Chronic plaque psoriasis is the most common form of psoriasis that involves infiltration of leukocytes, altered keratinocytes proliferation and differentiation and increased cytokines, chemokines and inflammatory molecules. Chronic plaque psoriasis is usually identified by erythematous plaques, raised and scaly skin lesions. The differentiation changes in psoriatic epidermis, skin-resident cells proliferate and mature rapidly so that terminal differentiation. Incompletion occurs in squamous corneocytes. Therefore, squamous keratinocytes atypically retain intact nuclei (parakeratosis) (Figure 1). Hence, the characteristic scale or flakes of psoriasis lesion causes poorly adherent of stratum corneum. Infrequently, a few of small pustules can be identified in especially inflamed psoriasis lesions (Lew, Bowcock et al. 2004; Krueger and Bowcock 2005). A schematic of psoriatic epidermis, histopathological features and psoriatic lesions are showed in Figure 1, 2 and 3.

Figure 1. Differentiation of keratinocytes in psoriatic plaques compared with normal skin (or uninvolved skin of patients with psoriasis) (Bowcock and Krueger 2005).

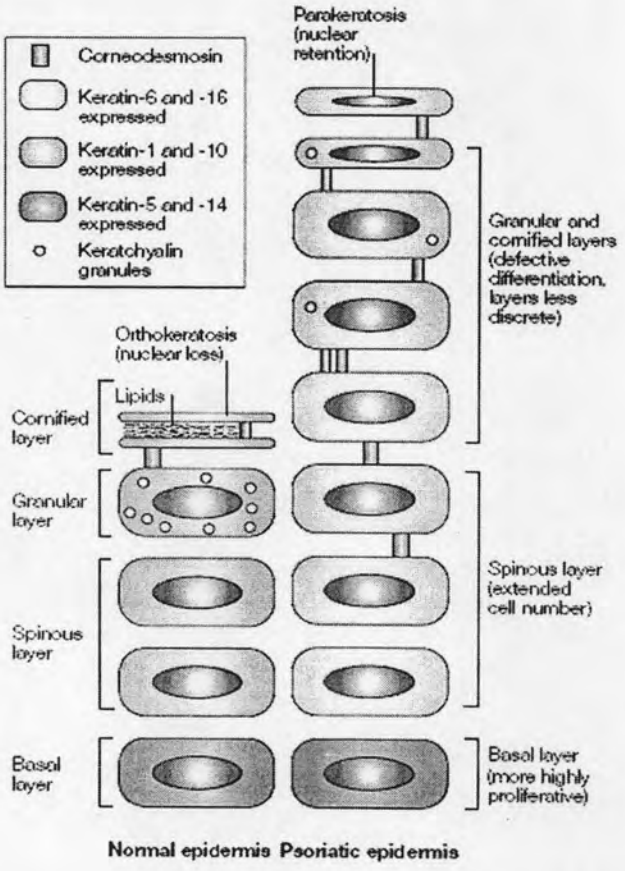


Figure 2. Histopathological features in psoriatic skin. (A) normal skin, (B) is characterized by a thickening of the viable cell layers with elongation of epidermal rete ridges (arrowheads). Moreover, dermal blood vessels in size and number (arrows), (D) psoriatic skin slough off (arrow) and Munro microabscesses (Schon and Boehncke 2005).

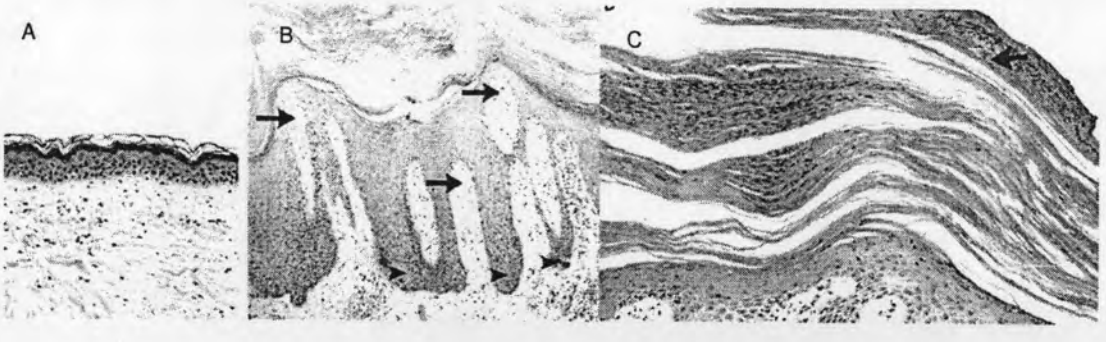
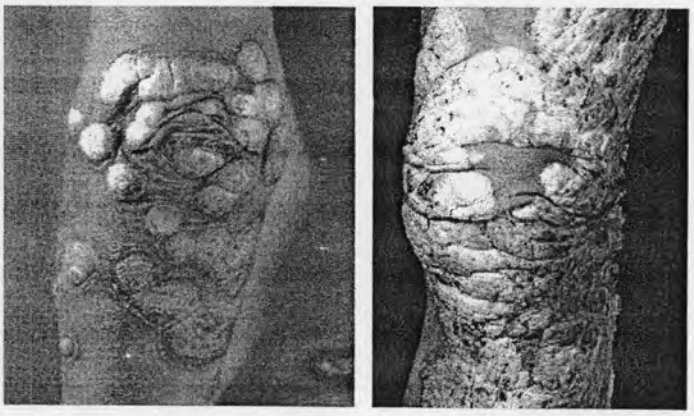


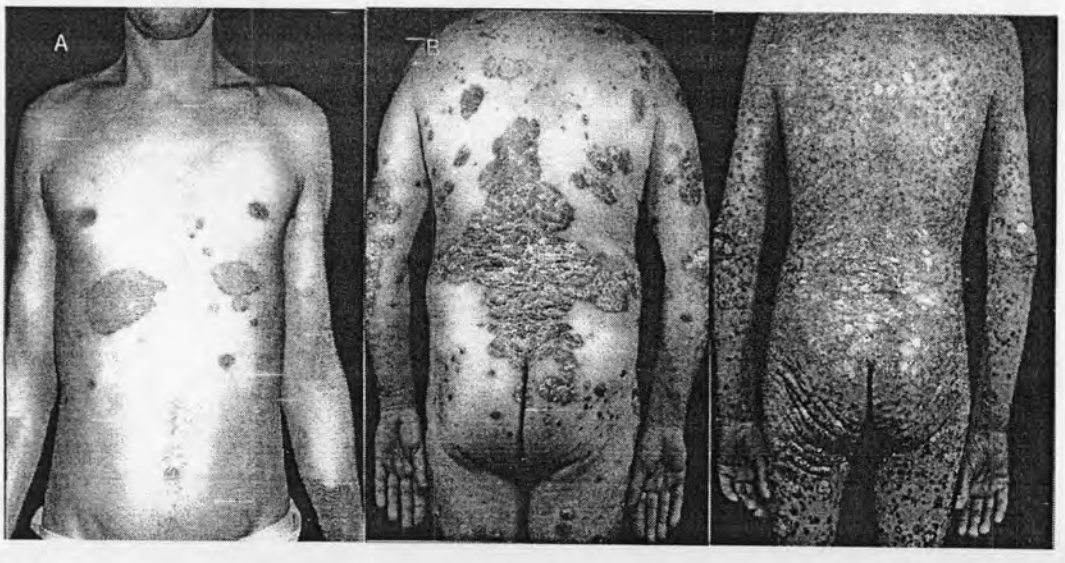
Figure 3. Severe hyperkeratotic plaque psoriasis and confluent lesions covering large body areas (Christophers 2001; Bos, de Rie et al. 2005).



Psoriasis severity

Psoriasis Area and Severity Index is usually identified the severity of involvement, which takes into account the size of the involved area, redness, thickness and scaling (Greaves and Weinstein 1995). The mild, moderate and severe of psoriasis is defined by scores of less than 10, 10 to 15 and greater than 15, respectively. A photograph of psoriasis patients who were defined by severity showed in Figure 4.

Figure 4. Photographs of patients with (A) Mild, (B) Moderate and (C) Severe psoriasis (Greaves and Weinstein 1995).



Trigger factors

Psoriasis has been associated with several exacerbating factors. Cutaneous trauma (such as sunburn, surgery, trauma) can cause the development of psoriatic lesion in about 40% of psoriasis (Koebner's phenomenon)(Raychaudhuri and Gross 2000). Some trigger factors is psychological stress, probably via neuroimmunological mechanisms (Arnetz, Fjellner et al. 1985; Farber, Rein et al. 1991). Some drugs are exacerbated including β-adrenergic-antagonist drugs, angiotensin-converting enzyme inhibitors, lithium and antimalarial drugs as well as bacterial and viral infections (Lazarus and Gilgor 1979; Swerlick, Cunningham et al. 1986; Duvic, Johnson et al. 1987; Gold, Holy et al. 1988; Wolf, Tamir et al. 1990; Greaves and Weinstein 1995).

The onset of psoriasis

Type I psoriasis (Early-onset)

Type I psoriasis, also called early-onset type psoriasis, and begins before the age of 40 years (usually at 16-22 years of age) (Greaves and Weinstein 1995). The majority of patients with positive strong f amilial histories reveal positively to human lymphocyte antigen-Cw6 (HLA-Cw6) (Henseler and Christophers 1985). This is considerable genetic susceptibility and hereditary association in this group of patients. These patients tend to develop more extensive plaques and more severe disease (Greaves and Weinstein 1995).

Type II psoriasis (Late-onset)]

Type II psoriasis, also called late-onset type psoriasis, and occurred after the age of 40 years (usually at 57-60 years). This late-onset type presents with minor hereditary association and no family history. Compared to the early-onset type, type II psoriasis is considered to be mild and localized. This type has more stable disease

(Henseler and Christophers 1985; Greaves and Weinstein 1995; Kormeili, Lowe et al. 2004).

Immunopathogenesis

T-lymphocytes in psoriatic lesions

In psoriatic skin lesions, there is a mixture of innate immune cells (neutrophils, dendritic antigen-presenting (APCs) cells and natural killer T (NKT) cells), adaptive immune cells (T-cells) and inflammatory infiltrates. $CD4^+$ and $CD8^+$ T-lymphocytes are present in psoriatic skin while $CD4^+$ T-lymphocytes are present generally in the dermis. The mature peripheral T-lymphocytes that present in psoriatic skin lesions are skin-homing memory T-cells. The specific antigen of skin T-lymphocytes has not been identified. But, the possibility of antigen recognition consists of self epidermal or even keratinocyte (KC)-derived polypeptides, those derived from microbial antigens or superantigens. Antigen recognition requires T-lymphocytes and mature professional APCs process complex peptides and load them onto major histocompatibility complex class I or class II molecules. The conjugation between the T-cells and the APCs so called immunologic synapse, encompassing the antigen recognition complex, results to complete activation of T-lymphocytes. So, the formations of immunological synapse are targeted to biologic therapies. Thus, the significant efficacy of psoriatic therapies has been applied to interfere with CD4+ and CD8+ T-cell disease-causing effectors activation. In addition, psoriasis is characterized by type 1 cytokines as well as CD4 and CD8 T-cells differentiate into a T-type 1 phenotype. Type 1 cytokines comprise predominately interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (Kormeili, Lowe et al. 2004; Bos, de Rie et al. 2005; Gaspari 2006; Lee and Cooper 2006).

T-lymphocyte have a strongly support by various mouse model including xenotransplantation models, unaffected skin from patients with psoriasis were injected with activated T lymphocytes from the same psoriasis patients onto severe combined immunodeficiency) SCID mice resulted in the development of resembled psoriatic

lesions in this animal models. In this study, using SCID mice engrafted with human skin, they suggested the immunopathogenic immunocyte responsible for causing lesions of psoriasis is a memory $CD4^+$ T-lymphocyte (not naïve $CD4^+$ T-cells) (Nickoloff and Wrone-Smith 1997; Schon, Detmar et al. 1997; Schon and Boehncke 2005). Other finding showed the distinctive observation of transmission of psoriasis via bone marrow transplantation. In addition, chronic plaque psoriasis can be transferred by bone marrow transplantation from psoriatic donors to hosts without known susceptibility to psoriasis that also confirmed the induction through cellular immune response (Bos and De Rie 1999; Bowcock and Krueger 2005).

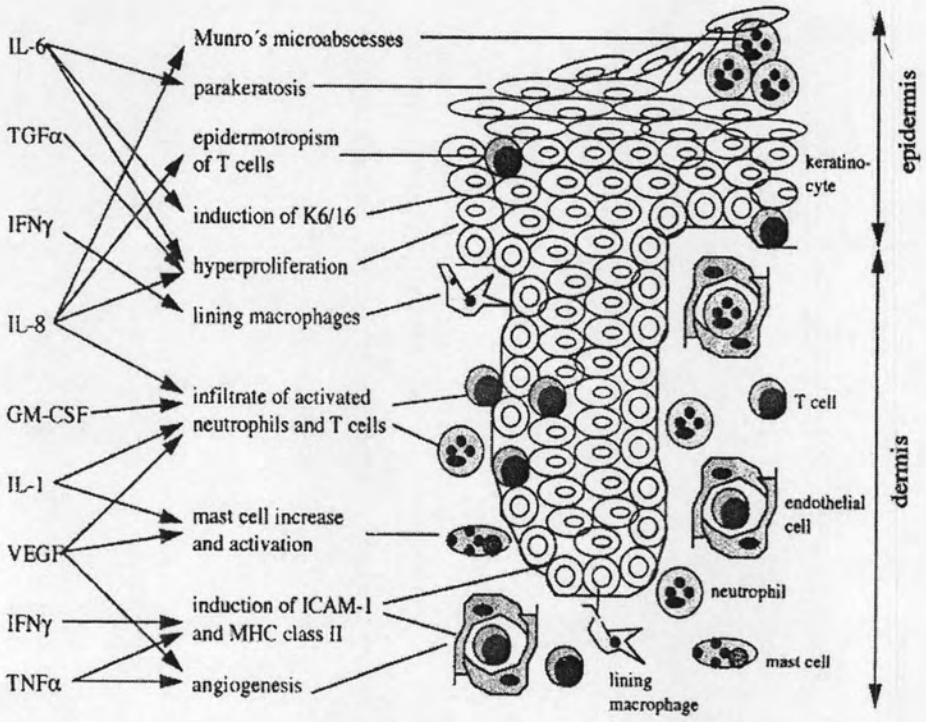
Cytokines, Chemokines and growth factors

The predominant type of Th1 cytokines present in psoriatic plaques such as IFN-γ, IL-2 and TNF-α but not Th2 cytokines such as IL-4, IL-5 or IL-10. Various antigen presenting cells infiltrate into lesions that contribute to the localized inflammatory infiltrated skin lesions, which contains IL-18 and TNF-α. IFN-γ production by T-type 1 lymphocytes is induced by both IL-18 and IL-23 (Gaspari 2006). In general, the concentration of TNF-α also increase in psoriatic lesions, as a key primary cytokine involved in the induction and maintenance of psoriatic plaques (Nickoloff and Nestle 2004). The important role of inflammation related to a biological activity of TNF-α in psoriasis. This cytokine has seen to be extremely expressed in the endothelium, dermal dendrocytes, epithelium and fibroblasts of lesional skin. Thus, TNF-α is a critical cytokine in immunopathology of psoriasis (Gaspari 2006). APCs derived cytokine production includes TNF-α and IL-23. T-cells are the probable source for IFN-γ, IL-15 and IL-17, while keratinocytes can generate IL-1, IL-6 and IL-8, as well as IL-18 and IL-20 (Nickoloff and Nestle 2004). Variety of other cytokines are increased in psoriatic skin that supply to the pathophysiology, as well as IL-15 which can promote $CD8^+$ T-lymphocyte expansion, but also promotes keratinocyte (KC) hyperplasia and conflict to apoptosis (Gaspari 2006) or IL-8 is the proinflammatory cytokine to induce the traffic lymphocytes as well as a neutrophils into psoriatic lesions (Kormeili, Lowe et al. 2004).

Numerous chemokines and chemokine receptors were found in immunopathogenesis of psoriatic plaques for instance thymus and activation-regulated chemokine (TARC or CCL17), monokine induced by interferon γ (MIG or CXCL19), interferon-inducible protein-10 (IP10 or CXCL10), macrophage-derived chemokine (MDC or CCL22) and regulated on activation, T-cell expressed and secreted (RANTES or CCL5), as well as chemokine-related receptor2 (CXCR2), CXCR3, chemokine receptor4 (CCR4), secondary lymphoid tissue chemokine27-CCR10 (CCL27-CCR10), macrophage inflammatory protein3α (MIP3α or CCL20), MIP3β (CCL19), and CCR6. In addition, in psoriatic lesions they can promote the migration of bone marrow-derived cells (Nickoloff and Nestle 2004; Gaspari 2006).

Several of growth factors are also found in psoriatic plaques, vascular endothelial growth factor (VEGF), transforming growth factor-α (TGF-α), insulin-like growth factor-1 (IGF-1), KC growth factor (KGF), nerve growth facor (NGF), as well as amphiregulin and IL-20. The accomplishment of a growth factors are contributing epidermal hyperplasia, apoptotic resistant, neo-angiogenesis and stimulation of T-cells proliferation (Gaspari 2006). A schematic of pathology of psoriasis are outlined in Figure 5.

Figure 5. This is a schematic representation of pathogenic events in human psoriasis. On the right, a cross section of psoriatic skin showed the tissue alteration. On the left, showed several function of major cytokines, chemokines and growth factors (Schon 1999).



Study Approaches

Study approach for susceptibility loci can be classified into two main approaches: family-based studies and population-based studies.

Family-based studies

This approach is a tool for searching susceptibility loci that co-segregate with disease in families with Mendelian pattern of inheritance. The principle of family-based is based on the fact that two genes or markers which are closely on a chromosome will be co-segregate together with disease in families due to a recombination between genes is low. Linkage analysis has been performed at various regions or one regions of interest. Strategies to identified linkage analysis can use genome-wide scanning, microsatellites, SNPs or candidate genes. The approach of family-based studies is a power tool for detecting major genes in complex disease. However, this method has limited power to detect less influential genes and it is difficult to collect samples from families that include both parents particularly in late-onset diseases.

Population-based studies

Population-based studies are used to investigate whether a marker allele is associated with susceptibility to the disease by comparing the frequency of the allele in a disease population with unrelated control. Similar to the strategies to identified linkage analysis, this approach can use genome-wide scaning, microsatellites, SNPs or candidate genes. The association between genetic marker and susceptibility to the disease may be due to that marker itself or due to another gene in linkage disequilibrium in a neighboring gene. Moreover, false positive arising from population stratification or small data sets can be found (Gough, Saker et al. 1995). However, this method has a lot of advantage since the identification and collection of samples from subjects is easier and more efficient than the collection of family samples. Besides, population-based

studies are sensitive method that can be detected less influential genes in complex diseases and late-onset diseases.

Genetic markers

The difference in the genome from one individual is about 0.1%. Moreover, this difference has the potential to effect the function of the gene. Most commonly used genetic markers are microsatellites and SNPs because of their advantageous over first generation DNA markers (restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (RAPD) etc.).

SNPs occur more than 1% in human population as the coding fraction of the DNA in humans is about 5%. They are distributed randomly over the genome and passed from generation to generation. In complex disease might result from a combination of inherited and obtained mutations in more than one gene, besides expose to environmental factors that might modulate the susceptibility, severity and clinical outcomes of the disease. Various diseases associated mutations are supposed to be SNPs. Some of SNPs will be located in the coding regions and can cause adjustments in the function of the protein since a result of substitution of one amino acid by a new ones or created stop codons. Other SNPs that association with complex trait diseases might be situated in non-coding regions, might adapt gene function or also affected on the level of gene expression. Furthermore, some SNPs can altered binding sites for transcription factors that also effect on expression of targeted genes of this transcription factors (Shastry 2003; Prokunina and Alarcon-Riquelme 2004).

Genetic association studies in psoriasis

Plentiful evidence that psoriasis has an extraordinarily strong genetic trait comes from different population, family and twin studies. The mode of inheritance of the disease has been discovered in various pedigree analyses of psoriasis familial aggregation studies. This information after studying large North Carolina kindred of British descent suggested a simple autosomal dominance pattern with reduced

penetrance. Otherwise, some studies proposed a recessive mode of inheritance. The mainly evidence sustaining a genetic predisposition to psoriasis is supplied by a higher concordance rates in monozygotic twins than dizygotic twins. Few studies of 61 twin pairs with at least one twin member affected, these studies establish a concordance rate of monozygotic twins 75% higher than that in 25% of dizygotic twins, 5-10 fold increased risks (Balendran, Clough et al. 1999; Barker 2001; Campalani and Barker 2005). Several experiments have calculated the risk of sibling to develop psoriasis based on their family history. The risk is 14% if one parent was affected, 41% if both parent are affected, 6% if one sibling affected (but not parents), compared to 2% when no parent or sibling is affected. In a great Swedish family data, the approximate risk of psoriasis was 28% if one parent was affected, 65% if both parents were affected and 24% if an affected sibling was present. Very interestingly, the offspring have a higher risk of increasing the disease and also might do at earlier age (genetic anticipation), when the father undergo from psoriasis (Campalani and Barker 2005).

Numerous genome-wide linkage analysis have identified putative susceptibility loci at least nine total, 9 susceptible loci which reveal significant linkage to psoriasis (showed in table 2) (Campalani and Barker 2005).

Table 2. Overviews of genetic susceptibility loci of psoriasis (Bos, de Rie et al. 2005).

Susceptibility locus	Chromosome	Candidate gene/marker	Possible gene function
PSORS1	6p21.3	HLA-Cw*0602	MHC I-dependent antigen presentation
PSORS2	17q25	SLC9A3R1/NAT9	Dysregulation haematopoietic and polarized epithelial cells
PSORS3[65]	4q32–35	D4S1535	Interferon regulatory factor 2 (IRF2) (transcription factor)
PSORS4	1cen–q21	Epidermal differentiation cluster (EDC)	Barrier function, induction of immune cells, chemotaxis
PSORS5	3q21	SLC12A8	Potassium/chloride transporter
PSORS6	19p13–q13	D19S425	Unknown
PSORS7	1p35–34		Unknown
PSORS8	16q12–13		Unknown
PSORS9[66]	4q31–34	D4S1597	Unknown
PSORAS1[67]	16q12	NOD2 protein	(?)Intracellular receptor for bacterial products in monocytes

PSORS, Psoriasis susceptibility locus; PSORAS, psoriasis arthritis susceptibility locus.

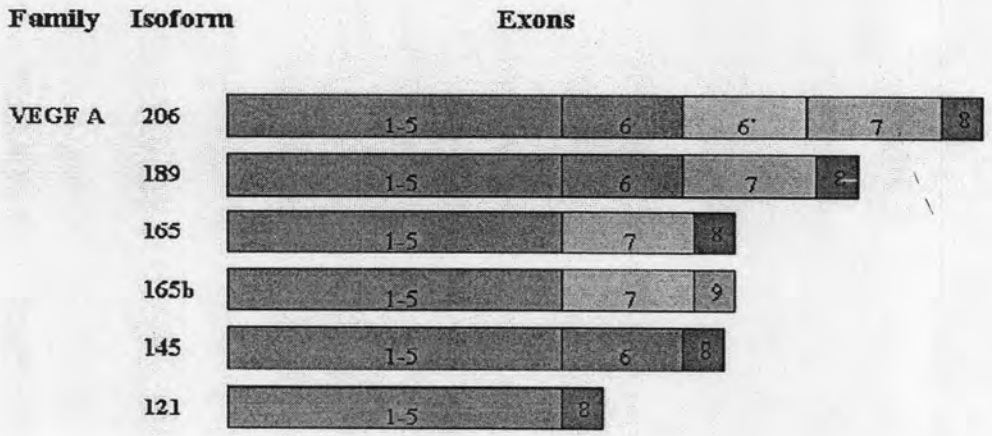
The mainly reported association has also been explained on the basis of genome-wide linkage studies. The mostly reported associations occupy a region in the major histocompatibility complex (MHC or HLA) region on the short arm (p) of chromosome 6p21.3 (*PSORS1*) (Barker 2001). Many studies narrowed down in the area of MHC about 200 kb segment containing eight well-known genes, HLA-C, TCF19, OTF, HCR, CDSN, SEEK 1, SPR 1 and STG. The Three essentially candidate genes are HLA-Cw*0602 and the *corneodesmosin* (*CDSN*) gene as well as *coiled-coilα-helical rod protein1* (*HCR*) (Nickoloff and Nestle 2004; Bowcock and Krueger 2005). As well HCR, this might negatively regulate the differentiation or proliferation of keratinocytes. Other genes included CDSN, are important for cell adhesion that are consecutively cleaved during skin desquamation (Bowcock and Krueger 2005).

Recently, linkage studies of two genome wide and region specific analysis have mapped a major susceptibility locus, namely *PSORS1* to a 12–cM region at chromosome 6p21.3 (Balendran, Clough et al. 1999; Barker 2001). These studies supporting case-control studies reveal the association of MHC class I and II alleles (such as HLA-B13, HLA-B57, HLA-Cw6, HLA-DR7) with psoriasis. *PSORS1* is estimated to report for 30-50% of familial psoriasis. Nevertheless, these physically powerful genetic associations seem to be impounded to type I (early-onset) chronic plaque psoriasis. Additionally, numerous epidemiology studies that showed the more severe and generalized psoriasis would tend to an early–onset patients. HLA tissue typing studies of 112 patients who randomly selected showed that HLA-Cw6 positive patients about 85% potent to the early-onset psoriasis. Positive family histories of psoriasis were found that more than one half of patients with early-onset psoriasis while was not present in the late-onset groups (Trembath, Clough et al. 1997; Capon, Dallapiccola et al. 2000; Campalani and Barker 2005).

Vascular endothelial growth factor (VEGF)

The interaction between vascular endothelium and signaling molecules results in the development of new blood and lymphatic vessels. These signal molecules include vascular endothelial growth factors (VEGF) that are dimeric endothelial cell mitogens. VEGF can produce by endothelial cells, macrophages, activated T-cells and other cell types including predominately by keratinocytes. The VEGF gene family consists of VEGF (also known as VEGF-A), VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PlGF) (McColl, Stacker et al. 2004; Young, Summers et al. 2004; Byrne, Bouchier-Hayes et al. 2005; Takahashi and Shibuya 2005; Ng, Krilleke et al. 2006). The human VEGF-A gene is mostly involved in angiogenesis while VEGF-C and VEGF-D are mainly involved in lymphangiogenesis. It signals via tyrosine kinase receptors which are high affinity. VEGF proteins have various isoforms. The more common isoforms consist of $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$, respectively. Other isoforms have been established consisting of $VEGF_{148}$, $VEGF_{162}$, $VEGF_{183}$, correspondingly. $VEGF_{165}$ is common predominant isoform which is the most potentially for stimulating angiogenesis whereas $VEGF_{165}b$ is an inhibitor of VEGF (Byrne, Bouchier-Hayes et al. 2005). In previous studies showed human keratinocytes expressing the three major splice forms of VEGF such as $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$, correspondingly (Ballaun, Weninger et al. 1995). Interestingly, psoriatic lesions have also found predominantly $VEGF_{121}$ isoform. This finding was suggested a mojor role of $VEGF_{121}$ isoform to be altered structure of blood vessels in psoriatic plaques (Zhang, Matsuo et al. 2005). VEGF isoforms are outlined in Figure 6. Besides, VEGF receptors were identified on endothelial cells. VEGF can binds to three receptor tyrosine kinases, including flt-1 (fms-like tyrosine kinase, VEGFR-1), Flk-1/KDR (fetal liver kinase 1-murine homologue/Kinase insert Domain containing Receptor-human homologue, VEGFR-2) and flt-4 (VEGFR-3) (Ferrara, Gerber et al. 2003; Ferrara 2004; Byrne, Bouchier-Hayes et al. 2005).

Figure 6. The schematic of VEGF isoforms. There are at least 6 different isoforms (Byrne, Bouchier-Hayes et al. 2005).



VEGFR-1 and VEGFR-2 are common found on the vascular endothelium while VEGF-3 is abundantly found on the lymphatic endothelium. VEGF-A, PLGF and VEGF-B can bind VEGFR-1. Additionally, VEGFR-1 is also expressed by monocytes, osteoblasts, macrophages and pericytes (Byrne, Bouchier-Hayes et al. 2005). However, VEGF is also mainly a survival factor for endothelial cells, both *in vitro* and *in vivo*. Furthermore, VEGF can induce the expression of the anti-apoptotic protein Bcl-2 and A1 in endothelial cells. VEGF also effects on bone marrow-derived cells and induce hematopoietic stem cells mobilization. It can sustain monocyte chemotaxis and also induces colony formation by granulocyte-macrophage progenitor mature subsets as well as induces vascular leakage that enhance permeability activity underlies significant roles of these molecules in inflammation pathology (Ferrara, Gerber et al. 2003; Tammela, Enholm et al. 2005).

Role of VEGF in psoriasis pathogenesis

Psoriasis is a chronic autoimmune skin disorder caused by inflammation, immune infiltration and altered proliferation of blood vessels. Psoriasis is an angiogenesis-dependent disease. The psoriatic features that consist of highly abnormal dermal blood vessels whereas are extremely permeable and edema. The keratinocytes

also increased expression of VEGF and its receptors that lead to neovascularisation and microvascular permeability. Over expression of VEGF in psoriatic skin can stimulate a vascular inflammatory response. Some evidence showed that psoriatic epidermal alterations precede capillary leakiness and vascular abnormalities. Previous data by light microscopy revealed that non-skin lesions to show short lengths of microvessels in the superficial and papillary dermis while psoriatic lesions represent dilated and elongated superficial capillaries. Additional, the immunostaining of microvessels that compared normal skin and psoriatic skin biopsies in psoriatic patients have displayed a four-fold increase of microvascular density in lesional skin (Detmar, Brown et al. 1994; Creamer, Sullivan et al. 2002; Byrne, Bouchier-Hayes et al. 2005).

Some experimental evidences, VEGF was significant enhanced in psoriasis compared with normal skin ($p < 0.0001$). Furthermore, VEGF level in psoriatic plaques associated closely with the clinical severity (Young, Summers et al. 2004).

The transgenic delivery of VEGF to mouse skin results in an inflammatory condition resembling human psoriasis phenotype (Xia, Li et al. 2003). Chronic VEGF expression in *in vivo*, the murine $VEGF_{164}$ gene was expressed in basal epidermal keratinocytes in transgenic mice. The results showed an enhanced density of cutaneous blood capillaries and increased leukocyte rolling (Detmar, Brown et al. 1998).

Genetic studies of VEGF polymorphism in psoriasis and various diseases

The VEGF gene is identified in 8 exons separated by 7 introns. The coding regions comprise approximately 14 kb. This gene is founded at short arms on chromosome 6p21.3 which also located in *PSORS1* susceptibility locus. From SNPper database has shown numerous SNPs in VEGF gene at chromosome 6 which consists of 136 SNPs (http://snpper.chip.org.). Individual of VEGF expression levels are also underneath genetic power. Preceding studies demonstrate that the single nucleotide polymorphisms (SNPs) of VEGF gene are associated with increased or decreased levels of circulating VEGF in healthy individuals. Moreover, investigated data was shown in type-1 chronic plaque psoriasis genotype and allele of VEGF polymorphisms at position +405C/C or +405C associated with severe psoriasis patients and psoriasis at early-

onset (Detmar 2004; Young, Summers et al. 2004). Some reports have also revealed that the +405C allele is correlated with increasing of VEGF serum levels in healthy donors (Watson, Webb et al. 2000). Addition, recent data have shown that the -1540AA (-1557), -1512InsIns, -1451TT, -460CC and -152AA homozygous VEGF genotypes compared to controls have a statistically significant two-fold increased risk in developing late-onset psoriasis. The results of each haplotype showed that only the -1540, -1512ins18, -1451, -460 and -152: C+CTA haplotype has significantly increased as compared with control groups (Barile, Medda et al. 2006). Besides, the -460TT VEGF and the +405CC VEGF genotypes are associated with highly VEGF production by PBMCs and also associated with a genetic susceptibility to develop early-onset and severe psoriasis (Young, Summers et al. 2004).

In haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter, 4 different common haplotypes were identified in the promoter region alone (Rogers and D'Amato 2006). Haplotype A has been showed consistently higher promoter activity compared with haplotype B and C. Haplotype C has also been found to be a wild-type sequence. (Stevens, Soden et al. 2003). The VEGF gene promoter haplotypes were shown in table 3.

Table 3. VEGF gene promoter haplotypes (Rogers and D'Amato 2006).

VEGF gene promoter haplotypes

From ATG	-2578	-2549	-2489	-2447	-1498	-1198	-1190	-1154	-634	-7	Haplotype frequency in normal controls					
From TSP	-1540	-1511	-1451	-1409	-460	-160	-152	-116	+405	+1032	[12]	[14]	[84]	[15]	[16]	[18]
Haplotype 1 (C)	C	---	C	G	T	C	G	G	C	C	34.3	25.5	29	42.8	38.5	25.0
Haplotype 2	C	---	C	G	T	C	G	G	G	C	31.8	19.6	22	32.3	35.1	28.3
Haplotype 3 (A)	A	18bp	T	-	C	T	A	G	G	T	17.8	21.2	48	12.5	24.2	44.3
Haplotype 4 (B)	A	18bp	T	-	C	C	A	A	G	C	12.7	26.5		12.5		

Locations of the polymorphism in relationship to the translation start point (ATG) and transcription start point (TSP) are indicated. The polymorphisms and its distribution in haplotypes as compiled from multiple sources are indicated light shading indicating the "wild type" genotype and darker shading indicating the polymorphic genotype. Boxed numbers indicate that haplotypes 3 and 4 cannot be distinguished using the markers typed in those studies.

[12] = Japanese, [14] = UK, [84] = Poland, Germany, [15] = Korea, [16] = China, [18] = India

Various studies of VEGF polymorphism in many diseases as well as chronic plaque psoriasis were showed in Table 4 and 5.

Table 4. Genetic studies of the effect of VEGF polymorphisms in many diseases (Rogers and D'Amato 2006).

Polymorphism position	Biological effect of allele, genotype or haplotype	Condition associated with allele, genotype or haplotype
-1540 (- 1557 or - 2578) C/A	Higher peritoneal VEGF production(Szeto, Chow et al. 2004), VEGF plasma levels were significantly higher in patients with the early phase of kawasaki disease (KD) and there was a trend toward higher VEGF plasma levels in KD with the -2578CC (Breunis, Biezeveld et al. 2006) The -2578C/A alleles were linked with high lung tumor VEGF expression and microvascular density (MVD) (Koukourakis, Papazoglou et al. 2004). Higher resting and phorbol myristate acetate (PMA) inducing reporter activity (Hap 3 vs. 1 and 4) (Stevens, Soden et al. 2003). -2578 C allele was associated with higher VEGF production and lower PMA/LPS/PDGF stimulated PBMC VEGF production (Shahbazi, Fryer et al. 2002).	Peritoneal VEGF level was higher in patients with CC genotype than those with CA/AA genotype, excess mortality in peritoneal dialysis(Szeto, Chow et al. 2004), The haplotype -2578C, +405G, -73C, +936C was significantly associated with the development of KD (Breunis, Biezeveld et al. 2006). VEGF -2578 CC and CA genotypes were associated with increased rejection risk (Shahbazi, Fryer et al. 2002). -2578CC has been shown to be associated with higher VEGF expression than AA, consistent with a protective effect for VEGF in atherosclerosis development (Howell, Ali et al. 2005). -2578C, -1154G, -634G and -2578C, -1154G, -634C were associated with a lower mean number of diseased vessels (atherosclerosis) (Howell, Ali et al. 2005). -2578A allele was associated in worse graft survival (Lemos, Mol et al. 2005). -2578AA genotype was associated with an increased risk for Alzheimer's disease (AD) (Del Bo, Scarlato et al. 2005). The correlation was observed between the -2578CC genotype and high-grade cancers (Kim, Jeong et al. 2005).

		-2578C,-1154G, -634C, +936T and -2578A,-1154A, -634G, +936T were significantly increased in rheumatoid arthritis (Hap 1 and 4) (Han, Kim et al. 2004). -2578AA genotype and the -2578A/-634G haplotype with low grade breast cancer (Hap 3 and 4) (Jin, Hemminki et al. 2005). -2578C,-1154A,+405C haplotype was significantly associated with less advanced melanoma (Howell, Bateman et al. 2002).
-1512 (-2549) 18 bp Del./Ins.	Higher resting and PMA inducing reporter activity (Hap 3 vs. 1 and 4) (Stevens, Soden et al. 2003). 18 bp deletion had a 1.95-fold increase in transcriptional activity (Yang, Cross et al. 2003). Higher LPS-stimulated PBMC VEGF production (Salvarani, Boiardi et al. 2004).	Insertion alleles were associated with behcet's disease (Salvarani, Boiardi et al. 2004). Insertion alleles are associated with susceptibility to developing giant cell arteritis (Boiardi, Casali et al. 2003). Decreased diabetic nephropathy (Yang, Cross et al. 2003)
-460(-1498) T/C	Higher resting and PMA inducing reporter activity (Hap 3 vs. 1 and 4) (Stevens, Soden et al. 2003).	The -460C allele was associated with decreased breast cancer survival (Lu, Shu et al. 2005). TT increased oral cancer risk (Ku, Wan et al. 2005) T/T homozygotes and the T allele of the VEGF-460 gene are associated with a higher risk of endometriosis. Moreover, Heterozygotes and C allele are related to the lower risk of endometriosis formation (Hsieh, Chang et al. 2004). -460C allele was associated with proliferative diabetic retinopathy (Ray, Mishra et al. 2004). TT homozygous genotype indicated a significant risk factor for prostate cancer (Lin, Wu et al. 2003). The T allele was associated with a relative high risk of kidney stones (Chen, Chen et al. 2003).
-160 (-1198) C/T	Higher resting and PMA inducing reporter activity	-1198C/T genotypes were significantly greater in

	(Hap 3 vs. 1 and 4) (Stevens, Soden et al. 2003).	AD (Del Bo, Scarlato et al. 2005).
-116 (-1154) G/A	The -1154A/A alleles were linked with low lung tumor VEGF expression and MVD (Koukourakis, Papazoglou et al. 2004). lower PMA/LPS/PDGF stimulated PBMC VEGF production (Shahbazi, Fryer et al. 2002).	-1154AA genotype was associated with thinner melanoma (Howell, Bateman et al. 2002). -1154AA genotype, low producer, was significantly decreased in prostate cancer when compared with controls (McCarron, Edwards et al. 2002). -2578C,-1154G, -634C, +936T and -2578A,-1154A, -634G, +936T were significantly increased in rheumatoid arthritis (Hap 1 and 4) (Han, Kim et al. 2004). -2578C,-1154A,+405C haplotype was significantly associated with less advanced melanoma (Howell, Bateman et al. 2002).
+405 (-634) G/C	The -634C/C alleles were linked with high lung tumor VEGF expression and MVD (Koukourakis, Papazoglou et al. 2004). Higher serum VEGF levels (Awata, Inoue et al. 2002). Highest VEGF production was observed for the +405GG genotype (Watson, Webb et al. 2000). Lower resting reporter activity (vs. Hap 3) and lower PMA induction of reporter activity (vs. hap 3) (Stevens, Soden et al. 2003).	Significantly association between -634CC genotype and -2578/-634CC haplotype and high tumor aggressiveness (p=0.01) (Jin, Hemminki et al. 2005). -634C were significantly more frequent in behcet's disease (Salvarani, Boiardi et al. 2004). The haplotype -2578C, +405G, -73C, +936C was significantly associated with the development of KD (Breunis, Biezeveld et al. 2006). -2578C,-1154G, -634C, +936T and -2578A,-1154A, -634G, +936T were significantly increased in rheumatoid arthritis (Hap 1 and 4) (Han, Kim et al. 2004). -634C alleles are associated with susceptibility to developing giant cell arteritis (Boiardi, Casali et al. 2003). The +405G allele was associated with decreased breast cancer survival (Lu, Shu et al. 2005). -2578C,-1154A,+405C haplotype was significantly associated with less advanced melanoma (Howell,

		Bateman et al. 2002). -634C allele was a risk factor for diabetic retinopathy and diabetic macular thickness (Awata, Kurihara et al. 2005). Significantly association between +405CC genotype (p=0.04 and p=0.02) and C allele (p=0.03 and p=0.02) with severe disease and early-onset of psoriasis (Young, Summers et al. 2004). +405CC genotype was associated with poor prognosis in chronic heart failure(adverse clinical outcome) (van der Meer, De Boer et al. 2005). +405GG genotype was found more often in endometriosis (Bhanoori, Arvind Babu et al. 2005). Endometriosis patients showed a higher incidence of +405CC genotype compared with controls (Kim, Choi et al. 2005). -634G allele was twice as likely to develop threshold retinopathy of prematurity (Cooke, Drury et al. 2004)
+1032 (-7) C/T	Higher resting and PMA inducing reporter activity (Hap 3 vs. 1 and 4) (Stevens, Soden et al. 2003).	-
+1974 (+936) C/T	70% circulating VEGF were significantly lower in carriers of the +936T allele (Renner, Kotschan et al. 2000).	-2578C,-1154G, -634C, +936T and -2578A,-1154A, -634G, +936T were significantly increased in rheumatoid arthritis (Hap 1 and 4) (Han, Kim et al. 2004). +936T allele was significantly associated with severe pre-eclampsia (Papazoglou, Galazios et al. 2004). T allele may decrease susceptibility to sarcoidosis (Morohashi, Takada et al. 2003). Individuals with +936TT or +936CT genotype were significantly association with spontaneous preterm delivery (Papazoglou, Galazios et al. 2004).

		The CT and TT genotype were increased in acute respiratory distress syndrome (Medford, Keen et al. 2005). +936T allele was significantly associated with decreased risk for breast cancer (Krippl, Langsenlehner et al. 2003).

Table 5. Association studies of VEGF polymorphisms in psoriasis.

Country/Ethnic	Cases/Controls	Specificity	OR/ RR	References
UK/Caucasian	137/102	Significantly association between +405CC genotype (p=0.04 and p=0.02) and C allele (p=0.03 and p=0.02) with severe disease and early-onset of psoriasis.	>1	(Young, Summers et al. 2004)
UK/Caucasian	333/101	Genotype-dependent significant association between -460TT genotype and early-onset psoriasis (p=0.03). -460TT or +405CC genotypes has significantly association between genotype and high VEGF producers (p<0.001)	>1	(Young, Summers et al. 2006)
Italy/Caucasian	117/215	Significant risk association between homozygous -1540AA, -1512InsIns, -1451TT, -460CC and -152AA and developing psoriasis. Significantly two-fold increase risk association between -1540AA, -1512InsIns, -1451TT, -460CC and -152AA homozygous genotypes and developing psoriasis at late-onset (p=0.02, 0.02, 0.02, 0.04 and 0.02, respectively.	>1	(Barile, Medda et al. 2006)