Molecular Genetic Studies of Psoriasis Susceptibility in 6p21.3

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Molecular Genetic Studies of Psoriasis Susceptibility in 6p21.3
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To my family
Psoriasis is a common and chronic skin disease with multifactorial background. It affects approximately 2% of the Swedish population and is characterized by inflammatory and scaly lesions. The aetiology of psoriasis is not yet known, however, the genetic contribution to the disease is strong. Several loci have been identified and among these, psoriasis susceptibility 1 (PSORS1) in chromosome region 6p21.3 is consistently described in populations of different ethnic background. A major factor involved in disease susceptibility is therefore believed to reside at this locus. Studies in this region are complicated by strong linkage disequilibrium and the previously identified associating allele of human leukocyte antigen C (HLA-C), HLA-Cw*0602, was therefore believed to be a marker of a disease gene located nearby. The purpose of the studies that make up the main body of this thesis work is to characterize alternative candidate genes for association to psoriasis in the Swedish population. Each new candidate was compared to HLA-Cw*0602 and their relationship to this allele was also investigated.

The HCR gene was characterized and found to be a very polymorphic gene and to be strongly associated to psoriasis. However, when compared to the level of association of HLA-Cw*0602, variations in HCR appeared to be in linkage disequilibrium with this allele. Three new genes were identified upon further characterization of the region; psoriasis susceptibility 1 candidate 1-3 (PSORS1C1-C3). Several variants were identified and tested for association, but after comparison, the observed associations appeared to be dependent on the presence of HLA-Cw*0602. HLA-C therefore remains the strongest candidate in the region. The biological function of this molecule is to signal our immune system for self-recognition, and its main interacting partners are specific killer immunoglobulin like receptors (KIR) on natural killer (NK) cells. The presence and absence of these KIR genes and HLA-C alleles were investigated. KIR2DS1 showed association to psoriasis vulgaris. Furthermore, when combining HLA-C and KIR in potential NK cell responses, a clear difference was observed in guttate psoriasis. This group showed an increased potential for inhibition and had less individuals with an undetermined NK cell response. When adding HLA-Cw*0602 to these combinations the effect was even more pronounced. From this analysis HLA-Cw*0602 appears to play an important role in potential thresholds of NK cell responses associated to psoriasis. Continued functional studies on HLA-C and its interaction partners are needed in order to elucidate the involvement of this gene in psoriasis pathogenesis.

Keywords: Complex disease, genetic, Psoriasis Susceptibility region 1 (PSORS1), human leukocyte antigen C (HLA-C), linkage disequilibrium, major histocompatibility complex, single nucleotide polymorphism

I PSORS1 finns de så kallade humana leukocyt antigenen (HLA) vilka fungerar som id-signal för kroppsegna celler. Här har man hittat en stark association mellan en variant av HLA-C, HLA-Cw*0602, och psoriasis, men man vet inte säkert om det här är en sjukdomsorsakande faktor eller en markör för en närliggande sjukdomsarterna. Studier i det här området komplicerar av linkage disequilibrium, ett fenomen där två alleler (varianter av en gen) är ofta oftare tillsammans än vad som förväntas i en frisksjuka populationen. Sökandet efter en genetisk faktor i den här regionen har därför fortsatt. Syftet med den här avhandlingen var att identifiera och karaktärisera alternativa kandidatgener för association till psoriasis. Vi har identifierat tre gener i området Psoriasis susceptibility region 1 candidate 1-3 (PSORS1C1-3) och studerat ytterligare en, HCR. Alla fyra har varianter som associerar till psoriasis när vi jämför frekvensen av dessa varianter mellan patienter och kontroller, men vid jämförelse med HLA-Cw*0602 är denna allel fortfarande den faktor som associerar starkast till psoriasis.

Psoriasis kategoriseras ofta som en autoimmun sjukdom, dvs en sjukdom där immunförsvaret attackerar något kroppseget material. Eftersom HLA-C kommunikerar med immunsystemet utvecklade vi en modell för att testa om vissa kombinationer av HLA-C och de specifika receptorer som känner igen denna molekyl associerar till psoriasis. De här kombinationerna påverkar hur en natural killer (NK) cell, s k mördarcell, reagerar och enligt vår modell har patienter med guttat psoriasis oftare potential för inhibering av dessa celler än aktivering jämfört med den frisksjuka populationen. Detta kan ha en effekt t.ex. vid en infektion med streptokocker. Med dessa resultat har vi visat att HLA-Cw*0602 kan vara en faktor som är involverad i psoriasis patogenes. Fortsatta studier i det här området är nödvändiga för att klargöra vad det är som orsakar sjukdomen. Det kan även finnas bidragande faktorer i andra områden i genomet vilket ytterligare komplicerar sökandet. När de bakomliggande mekanismerna är identifierade kan mer resultatinriktade behandlingar tas fram, men det kan även ge ledtrådar till andra komplexa sjukdomar där orsakerna fortfarande är okända.
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<td>antigen presenting cell</td>
</tr>
<tr>
<td>CDSN</td>
<td>corneodesmosin</td>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>DZ</td>
<td>dizygotic</td>
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<tr>
<td>EA</td>
<td>excess activation</td>
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<td>EGF</td>
<td>epidermal growth factor</td>
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<td>EI</td>
<td>excess inhibition</td>
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<td>EST</td>
<td>expressed sequence tag</td>
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<tr>
<td>HCR</td>
<td>alpha helix coiled coil rod homologue</td>
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<td>HLA</td>
<td>human leukocyte antigen</td>
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<td>IFN-γ</td>
<td>interferon gamma</td>
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<td>kb</td>
<td>kilobase</td>
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<tr>
<td>kDa</td>
<td>kiloDalton</td>
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<td>KIR</td>
<td>killer immunoglobulin like receptor</td>
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<td>Mb</td>
<td>megabase</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<tr>
<td>MIC</td>
<td>HLA class I polypeptide-related molecule</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<td>MZ</td>
<td>monozygotic</td>
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<td>NF-KB</td>
<td>nuclear factor kappa B</td>
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<tr>
<td>NK</td>
<td>natural killer</td>
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<td>OR</td>
<td>odds ratio</td>
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<td>ORF</td>
<td>open reading frame</td>
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<td>POU5F1</td>
<td>POU domain class 5 transcription factor 1</td>
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<td>PPP</td>
<td>palmo plantar pustulosis</td>
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<td>PUVA</td>
<td>psoralen ultraviolet A</td>
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<td>RA</td>
<td>rheumatoid arthritis</td>
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<td>RACE</td>
<td>rapid amplification of cDNA ends</td>
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<td>SCID</td>
<td>severe combined immunodeficiency</td>
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<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<td>TCF19</td>
<td>transcription factor 19</td>
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<tr>
<td>TNF-α</td>
<td>tumour necrosis factor alpha</td>
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<td>TNF-β</td>
<td>tumour necrosis factor beta</td>
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<tr>
<td>UVA</td>
<td>ultraviolet A</td>
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<tr>
<td>UVB</td>
<td>ultraviolet B</td>
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What is it like to have psoriasis?

“At age of fourteen I found out that I had psoriasis. Even though it ran in the family it was quite a shock to be all of a sudden in a relationship with this life-long companion. I was really upset with the disease interfering with my life. Especially after being told to choose a profession that would not worsen my psoriasis. The years that followed were filled with hours of treatment, often at the hospital, precious time for a teenager who would rather spend that time with friends after school. Furthermore, if I became lax with the treatment regime the lesions would spread once again and often become worse.”

“As a psoriatic, you develop a certain talent to hide your ‘spots’. I recognized myself in a story of a young woman who also was a psoriasis sufferer. At one point she had not been wearing any dark clothes (due to skin flaking), shorts or t-shirts in public for 10 years after she had realized that her lesions were actually noticed by others. But for her graduation day she was determined (due to fashion there was no other option) to put on a short skirt. She finally did. After several failures of trying to cover up her lesions with coloured foundation, she got a brilliant idea and spent the very sunny and warm graduation day wearing four pairs of nylon stockings. Today her view of psoriasis is a little different. She states that treating the symptoms is only half the battle, to accept your disease as being a part of you is the crucial step. I couldn’t agree more. Living with psoriasis is so much like a roller coaster, the symptoms rise and fall, the spots come and go. There are good days when you feel strong and secure, and there are bad days when you feel like crying and wonder if maybe you can actually shave your lesions off with a cheese-slicer! The only true power one can have over this disease is knowledge. The more you know, the better you can fight it.”

- A psoriasis sufferer 2005
INTRODUCTION

The first known documented case of psoriasis is probably that of a monk in Peru in 1674 who had fish-like skin covered with scars throughout his life, and fingers which were crooked from joint pains; symptoms that fits the description of psoriasis arthritis (Enlund 2000). As a clinical entity, psoriasis was first described by Robert Willan over 200 years ago (Willan 1801).

Epidemiology

Psoriasis is a common skin disease with a worldwide distribution. The prevalence varies with ethnicity, affecting approximately 2-3% in N. America, N. Europe and Australia, 0.3-3% in Africa (with a higher incidence in east Africa), <1% in Asia and, at <0.01%, almost non-existing in Eskimos and Native Americans (Lomholt 1963; Hellgren 1967; Farber and Nall 1974; Yip 1984; Kavli et al. 1985; Krueger and Duvic 1994; Leder and Farber 1997). The age at disease onset varies widely between patients and psoriasis can develop at anytime throughout life. However, studies have shown that 75-90% will develop psoriasis before the age of 40, with a peak of case onset around puberty, and a smaller peak around 50-60 years of age (Burch and Rowell 1965; 1981; Melski and Stern 1981; Henseler and Christophers 1985; Swanbeck et al. 1994). Both sexes are equally affected, although women tend to develop psoriasis earlier than men (Henseler 1998).

The skin

The skin is the largest organ of the human body. A person with a bodyweight of ~70 kg has a total skin surface of approximately 1.8 m². Acting as a barrier, it helps maintain our internal environment. It is protective against external threats such as microorganisms and mechanical injury and also regulates water balance. The skin is divided into several layers including epidermis and dermis. About 95% of the epidermis is consisted of keratinocytes. It also contains Langerhans’ cells (immune cells) and melanocytes (which are responsible for our skin pigmentation) (Hunter 1989; Bjerke 2002). The keratinocyte produce cytkeratins, polysaccharides, antimicrobial proteins and cytokines and also
plays a role in the immune defense (Barker et al. 1991). Keratinocytes stem from the lower epidermal basement cells and are pushed up towards the skin surface by new keratinocytes growing from underneath, a process that normally takes up to a month. During this procedure the nucleus is lost and the keratinocyte dies. The dermis contains collagen and elastic tissue and thin arterial capillaries that carry nutrition and oxygen to the skin (Hunter 1989).

Figure 1. Histology of normal and psoriasis skin. a) A section of normal skin showing dermis (light grey) and epidermis (dark grey). b) A section of a psoriasis lesion showing the characteristic pattern and thickening of the dermis, and the scaling of the epidermis (at the top). The sections are photographed under different magnification.

The psoriasis skin
The psoriatic lesion is characterized by red inflammatory and silvery scaly plaques. The scaling is caused by impaired differentiation of keratinocytes and proliferation of epidermis (Figure 1). There is a more than an eightfold shortening of the epidermal cell cycle (36 hours versus 311 hours for normal skin). All basal cells of the epidermis appear to enter the growth fraction compared to 60-70 % in normal individuals (Christophers 2003). Inflammatory cells, such as T lymphocytes, macrophages and monocytes, infiltrate the lesions at an early stage and are believed to be involved in both initiation and maintenance of the lesions (Christophers and Mrowietz 1995; Ghoreschi et al. 2003). In addition, the capillary loops in the dermis become dilated, leading to increased blood circulation and hence the redness of lesions (Braverman and Sibley 1982).
Introduction

Clinical features
Psoriasis is a chronic disease where the degree, duration and morphological variants can vary considerable, both between patients and within the same individual. Total remission of symptoms, lasting from a few months up to decades, occurs in about 40-50% of the patients (Lomholt 1963; Farber and Nall 1974; Farber 1991). The most common phenotype is psoriasis vulgaris accounting for 80-90% of cases (figure 2) (Lomholt 1963). The chronic lesions are well defined with nummular and discoid shapes in various sizes generally affecting the scalp and extensor surfaces symmetrically. Guttate psoriasis, affecting 10-20% of psoriasis patients, is a more acute variant where small drop-like lesions appear rapidly often after an upper respiratory streptococcal throat infection (figure 2). The lesions, usually spreading over the trunk and proximal limbs, are not chronic. Instead, the lesions often change to the vulgaris phenotype and in fact patients with vulgaris can also manifest guttate flares (Lomholt 1963; Christophers and Kiene 1995; Naldi et al. 2001).

Other less common variants are; inverse psoriasis, with non-scaling lesions located in groin and axillary regions; erythrodermic psoriasis, covering the entire body surface; and pustular psoriasis, which can be localized such as palmar plantar psoriasis (PPP) or generalized, with sterile pustules in the lesions (Farber and Nall 1992; 1993; 1993). In addition there is a strong association between inflammatory joint disease and psoriasis; psoriasis arthritis (PsA) is a disorder in which most joints may be affected including the soft tissue surrounding them. This phenotype affects at least 10-20% of psoriasis patients and is often associated with psoriatic nail lesions (Espinoza et al. 1992; Hohler and Marker-Hermann 2001; Mallbris et al. 2005).

Triggering factors
Several factors are known to initiate and/or exacerbate psoriasis. The Koebner phenomenon (skin trauma), infections such as throat infection with streptococci, acute viral infections and local skin infections with Staphylococcus aureus or Candida albicans can trigger or worsen psoriasis (Koebner 1872; Lindegard 1986; Telfer et al. 1992; Rosenberg et al. 1994; Naldi, Peli et al. 2001). Also HIV infection has been shown to
Introduction

aggravate psoriasis (Coopman et al. 1993; Mallon et al. 1998). Drugs have been reported to be responsible for the onset or exacerbation of psoriasis, e.g. lithium salts, beta-adrenergic blocking agents, antimalarials, nonsteroidal anti-inflammatory drugs and the withdrawal of steroids (Abel et al. 1986).

Alcohol has been associated with severity of psoriasis and treatment failures, one reason being that heavy drinking might increase the risk of infections and mechanical trauma (Poikolainen et al. 1990; Gupta et al. 1993). A number of diseases linked with smoking have also been connected with psoriasis including hypertension, cardiovascular diseases, respiratory tract neoplasm and kidney cancer, and the risk of psoriasis has been found to increase with rising body mass index (McDonald and Calabresi 1978; Melski et al. 1983; Lindegard 1986; Dunna and Finlay 1989; Lindelof et al. 1990; Olsen et al. 1992; Naldi et al. 1996). It is also known that stressful life events and pregnancy can trigger the disease although contradictory results also have been obtained (Payne et al. 1985; Lindegard 1986; Dunna and Finlay 1989; Gaston et al. 1991; Naldi, Parazzini et al. 1996; Naldi, Peli et al. 2001; Picardi et al. 2003). Exposure to sunlight can aggravate psoriasis in about 10% of patients, but in the majority it has a clear beneficial effect and is often used as a treatment in itself (Stern and Melski 1982; Krueger et al. 1995; Grundmann-Kollmann et al. 2004). Interestingly, it has been suggested that even a patient’s knowledge of, and attitude to, psoriasis influences disease expression (Scharloo et al. 2000). A recent cohort study show that distinct triggering factors are associated with the onset of different psoriasis phenotypes with the event of a life crisis being the predominating factor in psoriasis vulgaris, and streptococcal infection, as previously reported, in guttate psoriasis (Mallbris et al. 2005).
Figure 2. Clinical characteristics of psoriasis vulgaris (top) and guttate psoriasis (bottom).
Established treatments

Topical treatment is the most widely used therapy for psoriasis, the most common being Vitamin D3 analogues and corticosteroids (van de Kerkhof et al. 2000). They act by decreasing the accumulation of inflammatory cells and inhibiting the epidermal proliferation. Lately, studies have shown that a combination of vitamin D3 analogues and corticosteroids improves the efficacy of treatment (Lebwohl et al. 1996; Kragballe et al. 1998; Guenther et al. 2000; van de Kerkhof et al. 2001). Possible side effects of potent corticosteroids are skin with atrophy, striae and teleangiectasias, whereas treatment with vitamin D3 analogs might induce skin irritation. Other common treatments are phototherapy, preferentially narrow-band ultraviolet B (UVB) and ultraviolet A in combination with the photosensitizer psoralen (PUVA). They induce photoproducts that inhibit epidermal proliferation and induce DNA damage. Especially T cells and keratinocytes are susceptible to UVB and PUVA induced apoptosis (Krueger, Wolfe et al. 1995). Acute side effects are erythema and burning, while longterm treatment may enhance photocarcinogenesis (McKenna and Stern 1995; Matsumura and Ananthaswamy 2004).

The efficacy of treatments varies between patients and not all psoriatics will respond to the actions mentioned above. For more severe psoriasis more aggressive treatments are indicated, such as the use of systemic immunosuppressive drugs.

Immunology

The body has a capability to protect itself against invading microorganisms and foreign substances (antigens). The innate immune system is the first line of defence; it is unspecific, has no memory and is present from birth, and is mainly mediated by phagocytes, inorganic molecules and antimicrobial proteins. The acquired immunity is antigen-specific, has memory, is attained through experience, and is mainly mediated by B lymphocytes, T lymphocytes, and antigen presenting cells (APCs) (Delves and Roitt 2000). Immune response to a new antigen requires a proliferation of reactive T cells in lymph nodes, where they become memory T cells, and direction of activated T cells to locations of the triggering antigen (homing). APCs, including macrophages, dendritic
cells and Langerhans’ cells, are key elements in launching an immune response. (Nickoloff et al. 1995).

**T cells**
There are two main types of T cells, T helper cells (CD4+) and cytotoxic T cells (CD8+). CD4+ cells assist in regulating the immune system by activating other immune cells through secretion of cytokines, and CD8+ cells help rid the body of virus-infected cells and tumour cells by mechanisms leading to their destruction by apoptosis (Reiser and Stadecker 1996; Goldsby 2000). In order to become activated, T cells must have antigens presented to them by APCs. Once activated, CD4+ and CD8+ cells secrete two distinct sets of cytokines, type 1; interferon-gamma (IFN-?) , interleukin-2 (IL-2), tumour necrosis factor beta (TNF-ß) and IL-3 which are involved in delayed hypersensitivity reactions and in many chronic inflammatory and autoimmune diseases (psoriasis, diabetes), and type 2; IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 which are involved in the pathophysiology of allergic disorders (asthma, atopic dermatitis) (Reiser and Stadecker 1996; Goldsby 2000; Biedermann et al. 2001). Both sets of cytokines are known to antagonize each others effects (Delves and Roitt 2000).

**Psoriasis immunology**
Psoriasis is considered to be a T cell mediated disease (Ortonne 2004; Prinz 2004). The role of T-cells in psoriasis was initially discovered in psoriasis patients that, undergoing solid organ transplantation, were treated with cyclosporine to prevent graft rejection. This drug was reported having a remarkable effect on the lesions (Mueller and Herrmann 1979). Cyclosporine is an immunosuppressive drug that works primarily through the inhibition of T-cell proliferation and cytokine production.

**T cells in psoriasis**
Activated T cells, polarized towards type 1 pathway, are highly prominent in psoriatic epidermis and dermis and have been shown to induce psoriasis in susceptible skin (Bos et al. 1989; Prinz et al. 1994; Gilhar et al. 1997; Nickoloff et al. 2000)). In psoriatic lesional skin, T cells may be continuously stimulated due to persistent presentation by APCs.
(Ozawa and Aiba 2004). Both activated Langerhans cells and dermal dendritic cells are abundant in psoriatic lesions (Cerio et al. 1989; Abrams et al. 2000; Dieu-Nosjean et al. 2000). Another hallmark of psoriasis is the migration of neutrophils into the epidermis and the formation of micro-abscesses (Sticherling et al. 1991; Kulke et al. 1996; Jiang et al. 2001). In addition, keratinocytes can also function like APCs and have been found to function as accessory cells in the stimulation of T cells by bacterial superantigens (Nickoloff et al. 1993; Grousson et al. 1998).

**Streptococcal antigens**

Streptococcal antigens are able to stimulate T cells immediately without involvement of intracellular processing (Tomai et al. 1990; Abe et al. 1991; Tomai et al. 1992; Leung et al. 1993). Streptococcal superantigens can also induce the expression of the skin homing receptor cutaneous lymphocyte-associated antigen (CLA) on T cells, thus directing them towards the skin (Leung et al. 1995). The streptococcal superantigen is homologous to the 50 kDa type I keratin (K14) protein and it has been suggested that T cells may be directed against keratinocytes via a cross-reactive process (McFadden et al. 1991; Valdimarsson et al. 1995; Valdimarsson et al. 1997).

**TNF-a**

TNF-a is a pro-inflammatory cytokine released by keratinocytes, dermal dendrocytes, monocytes, macrophages, mast cells and activated T cells (Nickoloff 1991; Ackermann and Harvima 1998). It increases the synthesis of other pro-inflammatory cytokines (IL-1, IL-6, IL-8) and activates nuclear transcription factors, such as nuclear factor (NF)-kB, promoting keratinocyte proliferation (Yasumoto et al. 1992; Jobin et al. 1999; Kakurai et al. 2001). It also induces vascular endothelial growth factor (VEGF) enhancing vascular permeability and angiogenesis (Sunderkotter et al. 1994).

**IFN-?**

IFN-? is a Th1 cytokine produced in psoriatic lesions and has the ability to inhibit the expression of the anti-inflammatory cytokine IL-10 and provoke new lesions (Fierlbeck et al. 1990; D'Andrea et al. 1993; Austin et al. 1999; Koga et al. 2002). By increasing
factors known to protect keratinocytes from apoptosis, IFN-? and TNF-a may be related to the enhanced survival of keratinocytes and may stimulate the activation and antigen presentation of keratinocytes (Griffiths et al. 1989; Barker, Mitra et al. 1991; Wrone-Smith et al. 1995). Activated T cells can activate endothelial cells by secreting IFN-? and TNF-a, which in turn directly attract Th1 cells into inflamed skin, a process that further upregulates the inflammation (Flier et al. 2001; Rottman et al. 2001).

Keratinocytes
Keratinocytes from psoriatic plaques appears to be resistant to apoptosis compared with normal skin (Wrone-Smith et al. 1997). Supernatant from culture of lesional T cells has been reported to promote proliferation of uninvolved keratinocytes. Only anti-IFN-? antibody was able to neutralize the growth stimulatory effect on psoriatic keratinocyte stem cells. The same growth effect was not promoted on normal keratinocyte stem cells (Bata-Csorgo et al. 1995). These findings suggest that an altered response of psoriatic keratinocytes to the immune system contributes to the pathogenesis.

Autoimmune disease
Psoriasis is considered an autoimmune disease as it appears to be caused by the activation of T cells (Davidson and Diamond 2001). The facts that support this belief are; 1) immunosuppressing drugs such as cyclosporine are effective in psoriasis (Baker et al. 1989; Baker and Fry 1992), 2) during lesion formation, inflammation precedes epidermal hyperproliferation and increased numbers of T cells have been demonstrated in the uninvolved skin of psoriatrics (Baker et al. 1984; Baadsgaard et al. 1990; Baadsgaard et al. 1990), 3) IFN-? is increased in psoriatic epidermis (Barker et al. 1991) 4) T cells isolated from lesions enhance keratinocyte proliferation via secreted products (Prinz, Gross et al. 1994), and 5) appearance or disappearance of psoriasis after bone marrow transplantation (Eedy et al. 1990; Snowden and Heaton 1997).
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**Genetics of psoriasis**

The first observation of the inheritance of psoriasis was reported by Willan, 1801 (Willan 1801). Later two classical epidemiological studies in the Faroe Islands and in Sweden showed that the prevalence of psoriasis was higher among relatives of cases than unaffected controls. The conclusion of Lomholt’s was that “.. psoriasis is genetically determined.. beyond doubt..” (Lomholt 1963; Hellgren 1967). In a re-evaluation of this data, high risk ratios ($\mathcal{R} = 8$ and 4) for first-degree relatives of psoriatics were obtained, and in a more recent study a risk ratio of 10 was observed for first-degree relatives of patients with juvenile onset (Henseler and Christophers 1985; Elder *et al.* 1994). These are indications that make a search for the genes feasible by genetic linkage techniques (Risch 1990).

Also twin studies have indicated a strong genetic predisposition to psoriasis. Monozygotic (MZ) twins have identical genomes while dizygotic (DZ) twins only share half their genes in common. Even if a disorder is caused by several genes, disease should more often be concordant in MZ twins than in DZ, which is the case in psoriasis; a concordance in 35-72% of MZ twins and in 14-23% of DZ twins have been noted. Also ages at onset and disease manifestations were very similar in concordant MZ twins, which were not particularly observed in DZ twins (Farber *et al.* 1974; Brandrup *et al.* 1982; Duffy *et al.* 1993).

The heritability for psoriasis, $h^2$ (proportion of variability of the trait that is due to genetic factors), has been calculated to between 60-90% based on twin and family studies which is among the highest of all the multifactorial genetic disorders (Elder *et al.* 2001). These observations clearly demonstrate that a genetic predisposition contributes to the development of psoriasis, however environmental factors are likely to play some role in triggering psoriasis since concordance rate for MZ twins does not reach 100%.
**Introduction**

**Complex genetics**

With the finding that psoriasis segregates in families, researchers have been trying to fit psoriasis into a mode of inheritance using Mendelian models e.g. autosomal dominant with incomplete penetrance and recessive mode of inheritance (Elder, Nair et al. 1994). Other suggestions are; genetic heterogeneity, where one or more different genetic factors cause the same phenotype, and multifactorial inheritance, where a combination of genetic factors and external triggers influence the onset and development of the disease (Lomholt 1963; Burch and Rowell 1965; Farber 1991; Elder, Nair et al. 1994). Revealing the mode of inheritance of a common and complex disease could be problematic due to several reasons; 1) when a disease is common, sporadic cases can be mistaken for familial segregations, 2) existence of susceptible individuals with incomplete penetrance of symptoms, 3) phenotypic expression may vary depending on age, gender, environmental factors and genes that contribute to severity, and 4) existence of genetic heterogeneity (Bhalerao and Bowcock 1998). Today the common belief is that psoriasis follows a multifactorial mode of inheritance with variable distribution and expression. To map genes in complex multifactorial diseases like psoriasis, a combination of several approaches may be necessary including: linkage analysis, allele sharing methods, association studies and animal models (Lander and Schork 1994; Risch and Zhang 1996).

**Studying genetic disease**

Linkage is a method that can be used to map disease genes by following the segregation of highly polymorphic markers through a disease in many generations. The efficacy of the test is dependent on prior assumptions of inheritance patterns and it has therefore been used to study Mendelian diseases, i.e. when a genotype at a single locus causes the expression of the character. In complex diseases the inheritance patterns do not fit any simple genetic explanation and instead of large families, sib-pairs or affected-relative-pairs can be used. This tests whether affected pairs share parental alleles at the locus of interest either more or less often than predicted by Mendelian expectations. The calculated lod score is the logarithm of odds of the likelihood that a genetic marker is located close to the gene that causes disease (linked), relative to that marker located far away on the same chromosome or on a different chromosome (unlinked) (Ott 1991).
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Statistical significance for linkage is reached with a lod score of 3, i.e. a 1000-fold higher probability of the observed outcome if the gene and the marker are linked.

When genes are located close to each other their alleles can be inherited together through generations more often than expected. They are then said to be in linkage disequilibrium. This is a phenomenon that can complicate genetic studies as it becomes difficult to separate markers from the actual disease-causing factor without using functional studies. It can, however, also be used to help narrow down regions and identifying ancestral haplotypes for the disease gene in relation to several marker loci (Haines 1998).

Once a critical region has been identified, it should be examined for any known genes that might have a biological function related to the trait. Any genes of interest should subsequently be tested for association to the disease by comparing frequencies of alleles or polymorphisms in patients and the general population. If the variant(s) occur more often, or less, than expected by chance in individuals with the disease, it is suggestive of either direct action of the polymorphism or linkage disequilibrium to such a functional difference.

Identified psoriasis susceptibility locus (PSORS)

A number of linkage analyses for psoriasis have been performed, identifying at least 19 potential susceptibility loci on 15 different chromosomes. Of these regions, nine have been confirmed in other populations or have yielded strong enough results to be designated as psoriasis susceptibility locus (PSORS1-9) (figure 3). Additional loci on 2p, 4q13, 4q21, 6q, 7, 8q24, 11p13, 14q31-32, 15q, 18p11 and 20p have also been indicated (Nair et al. 1997; Trembath et al. 1997; Bhalerao and Bowcock 1998; Samuelsson et al. 1999; Veal et al. 2001; Asumalahti et al. 2003; Karason et al. 2003).

PSORS9, 4q31

A genome wide scan in the Chinese Han population revealed a locus on 4q31, a region also observed previously (Nair, Henseler et al. 1997; Zhang et al. 2002). A meta-analysis
Figure 3. Potential psoriasis susceptibility regions (PSORS) 1-9. This is a figure indicating the location of the identified susceptibility regions for psoriasis in our genome. The chromosomes are numbered in order of size and the names of the loci are indicated in the text. In the locus name, short arm locations are labelled p (petit) and long arms q (queue).
using six genome wide scans was recently carried out revealing linkage to PSORS1 and PSORS9 (Sagoo et al. 2004).

**PSORS8, 16q**
In a genome-wide scan a region on chromosome 16q was detected by non-parametric analysis (Nair, Henseler et al. 1997). This region has later been identified when searching for a genetic locus for psoriasis arthritis and indications of paternal transmission from this region has been shown (Karason, Gudjonsson et al. 2003).

**PSORS7, 1p35-p34**
A locus on 1p was identified by sib pair analysis in all UK families studied. The EPS15 gene, encoding an intracellular substrate for the EGF receptor, is located within the critical region defined, and is known to be overexpressed in psoriatic epidermis (Veal, Clough et al. 2001).

**PSORS6, 19p13**
Assuming a recessive model a new susceptibility locus was identified on 19p13 in a German study (Lee et al. 2000). This result was recently confirmed by the same group using a linkage disequilibrium approach (Hensen et al. 2003). Further refinement of the region is needed to identify putative candidates.

**PSORS5, 3q21**
A region on 3q21 was identified in a Swedish study using pair-wise linkage analysis (Enlund et al. 1999; Enlund et al. 1999). Further refinement of the region led to the identification of SLC12A8A, a member of the solute carrier 12 family. A five marker haplotype of this gene showed significant association to psoriasis in Swedish psoriasis patients, however no association was detected in a study of US families (Hewett et al. 2002; Bowcock and Barker 2003).

**PSORS4, 1cen-q21**
This region maps within a cluster of related genes involved in epithelial differentiation, the epidermal differentiation complex (Bhalerao and Bowcock 1998; Capon et al. 1999).
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Existence of epistasis between PSORS1 & PSORS4 regions has also been detected (Capon et al. 1999).

**PSORS3, 4q**
This region was detected through a genome wide scan of large families from Ireland and England assuming a dominant mode of inheritance and a penetrance of 70% (Matthews et al. 1996). Initially, the family material used in the study was tested for linkage to 17q. This lead to the exclusion of one family, as it was the only one linked to this locus, supporting the theory of genetic heterogeneity.

**PSORS2, 17q25**
In 1994, Thomforde et al. reported the finding of a psoriasis susceptibility locus on 17q. Eight families from USA were used in the study and half of them showed linkage to 17q, a finding that suggested genetic heterogeneity (Tomfohrde et al. 1994). This linkage has been confirmed by additional studies in different populations (Matthews, Fry et al. 1996; Nair, Henseler et al. 1997; Enlund, Samuelsson et al. 1999). In a more recent study this locus was re-confirmed and fine-mapped to 17q23-q25 with a peak close to a cluster of genes coding for a cluster of genes that belong to the immunoglobulin superfamily (Speckman et al. 2003). The identified region contains several genes and after additional refinement two peaks were located over the genes SLC9A3R1 (solute carrier 9, isoform 3 regulatory factor 1), NAT9 (new member of the N-acetyltransferase superfamily) and RAPTOR. The RAPTOR gene still awaits investigation but an allele located between SLC9A3R1 and NAT9 was found to eliminate a putative binding site for the runt related transcription factor (RUNX) 1. RUNX1 sites have been associated with diverse autoimmune diseases suggesting an important role for RUNX1 in tolerance (Prokunina et al. 2002; Nielsen et al. 2003; Tokuhiro et al. 2003). What is intriguing is that SLC9A3R1 is involved in immune functions like HLA-C, such as antigen recognition and immune synapse formation (Speckman, Wright Daw et al. 2003).

**PSORS1, 6p21.3**
Using both parametric and non-parametric models this locus has consistently been linked to psoriasis in independent studies of populations of different ethnicity and also further
refined (Nair, Henseler et al. 1997; Trembath, Clough et al. 1997; Jenisch et al. 1998; Leder et al. 1998; Balendran et al. 1999; Capon, Novelli et al. 1999; Enlund, Samuelsson et al. 1999; Oka et al. 1999; Samuelsson, Enlund et al. 1999; Lee, Ruschendorf et al. 2000; Nair et al. 2000; Zhang, He et al. 2002). It has been estimated that the contribution of this region to psoriasis predisposition is approximately 30-50% (Trembath, Clough et al. 1997) and it is therefore believed to contain a major factor(s) involved in susceptibility to psoriasis. It is this locus that is the main focus of the studies that form this thesis.

**Genetics of PSORS1**

PSORS1 is located in the major histocompatibility complex (MHC), a region that harbours the human leukocyte antigen (HLA) genes. Intriguingly the first identified associations to psoriasis were those of HLA antigens obtained from serologic typing during the 1970s before any genome wide linkage studies were performed (Tervaert and Esseveld 1970; Russell et al. 1972; White et al. 1972; Tiilikainen et al. 1980). Associations to both HLA class I and class II antigens have been identified in psoriasis, e.g. HLA-DR7, -B13 and -B57 but the most consistently and highest significance has been obtained with an allele of \( HLA-C \), HLA-Cw*0602. The strong association of HLA-Cw*0602 to psoriasis is especially prominent in patients with young age of onset. This led to the categorization of psoriasis patients in two groups; type I, with age of onset <40 years of age, increased frequency of HLA-Cw*0602 and an established family history and type II with age of onset >40 years of age, lower frequency of HLA-Cw*0602 and lower degree of family history (Henseler and Christophers 1985).

A complicating factor for association studies is the high linkage disequilibrium along the PSORS1 region. It appears that most of the modern HLA haplotypes are derived from a limited group of ancestral haplotypes (Degli-Esposti et al. 1992). Knowing this and with experience from other diseases, where associated HLA molecules have eventually turned out to be markers for the true disease genes located nearby, the role of \( HLA-C \) in psoriasis susceptibility has been questioned. Thus major effort has been put into the identification and characterization of other potential candidate genes located in the vicinity of \( HLA-C \). In 1999 the complete structure and gene map of
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the continuous genomic sequence of the entire 3.6Mb MHC region was published, facilitating these type of studies [, 1999 #472;TheMHCsequencingconsortium, 1999 #17]. Since then several attempts have been made to narrow down the PSORS1 region leading to variously defined high risk regions (Balendran, Clough et al. 1999; Oka, Tamiya et al. 1999; Nair, Stuart et al. 2000; Veal et al. 2002). However, the region most likely stretches around 200 kb from HLA-C to corneodesmosin (CDSN). This area encodes for several genes and pseudogenes, some more interesting as candidates than others. POU5F1 is a transcription factor involved in the regulation of pluripotency in embryo and TCF19 is believed to play a role in transcription of genes. Identified polymorphisms in these candidates do not show strong association to psoriasis (Gonzalez et al. 2000; Teraoka et al. 2000). The CDSN gene, previously known as the S gene, encodes for a protein that is expressed in the late stages of keratinocyte differentiation and is considered to be essential for normal desquamation (Zhou and Chaplin 1993; Haftek et al. 1997; Guerrin et al. 1998; Guerrin et al. 2001). Involvement of CDSN in psoriasis has been suggested on the basis of association analysis, especially with the CDSN*5 allele, and its expression in psoriatic lesions. However contradictory results have also been obtained (Jenisch et al. 1999; Enerback et al. 2000; Enerback et al. 2000; Schmitt-Egenolf et al. 2001; Orru et al. 2002; Asumalahti et al. 2003; Romphruk et al. 2003; Capon et al. 2004).

The HLA class I genes (HLA-A, -B, -C) are expressed on somatic cells as an identification of self/non-self. The main interaction partners to the HLA class I antigens are the killer immunoglobulin receptors (KIRs). KIRs are presented on the surface of natural killer (NK) cells and a subpopulation of T-cells, NK-T cells, continuously screening our body for recognition of self. The are both activating (S) or inhibitory (L) receptors and they are sensitive to both the type and level of HLA class I antigens expressed. Any alterations from the normal state will therefore lead to killing of the target through KIR(S), while normal HLA class I expressing cells avoid attack through KIR(L) (Colonna et al. 1993; Mandelboim et al. 1996; Winter et al. 1998). In psoriasis, the number of circulating NK cells appears to be reduced, which is also the case in other autoimmune diseases, and NK-T cells carrying KIRs, are able to induce psoriatic plaques
in severe combined immunodeficient (SCID) mice (Nickoloff and Wrone-Smith 1999; Nickoloff et al. 1999; Nickoloff, Bonish et al. 2000; Cameron et al. 2002; Gilhar et al. 2002). In addition, studies have shown association of KIR2DS1 and KIR2DS2 to psoriasis and psoriasis arthritis, especially in the absence of their allotype specific HLA-C ligand (Griffiths et al. 1986; Martin et al. 2002; Luszczek et al. 2004; Suzuki et al. 2004). This suggests that specific communication patterns between HLA-C and KIRs may have a role in altered immune reactions, which is a major feature in psoriasis pathogenesis.
AIMS OF THE STUDY

The most consistently identified psoriasis susceptibility locus in genome wide scans is PSORS1 on chromosome 6p21.3. This locus is therefore believed to harbour a major genetic factor(s) involved in the predisposition to psoriasis. This study was designed to:

- characterize newly identified gene sequences in the region for association to psoriasis

- identify new genes in the region and characterize them with regard to psoriasis susceptibility using expression studies and sequencing methods

- investigate putative interactions between genes located in PSORS1 and related biological partners
MATERIALS AND METHODS

Study populations
We have been working in close collaboration with the Swedish Psoriasis Association in collecting psoriasis patients. All studies have been approved by the Regional Committees of Ethics in Stockholm and Gothenburg and the samples have been used with informed consent. The patients enrolled, have individually been examined by a dermatologist to confirm the diagnosis of psoriasis.

Paper I
42 patients with psoriasis vulgaris were recruited from the Swedish Psoriasis Association in the Southwest of Sweden. They show a mean, median and range of age of onset of 19, 16, and 6-51 years respectively and the distribution of males and females were equal. The control group consisted of 38 population matched controls.

Paper II
To investigate PSORS1C1 and PSORS1C2 (SEEK1 and SPR1) a total of 87 psoriasis patients with psoriasis vulgaris were used. Of these 63 were recruited from the Stockholm area and 24 from Southwest of Sweden. The age of onset shows a mean, median and range of 27, 22, and 1-72 years. As a control group 50 population matched controls were recruited with no known personal or family history of psoriasis.

Paper III
In the PSORS1C3 study we extended the material used in paper II with 131 patients with psoriasis vulgaris and 77 controls from the Stockholm region. In total 218 patients, mainly with young age of onset, and 127 controls were investigated. The all showed an equal sex distribution and the patients had a mean, median and range of age of onset of 20, 18, and 1-72 years respectively.

Paper IV
In total 396 psoriasis patients and 372 controls from the Stockholm area were investigated for HLA-C/KIR interactions. All patients were identified within their first year of disease onset. Eighty patients were diagnosed with guttate psoriasis, 75 had
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psoriasis arthritis diagnosed by a rheumatologist and 241 had psoriasis vulgaris. The range of age of onset varied between 12 and 84 years with a mean of 41 years.

Identification of new candidate genes
Expressed sequence tag characterization
In the search for new genes we screened the PSORS1 region for unmapped expressed sequence tags (ESTs). Identifying a match would provide indications of exons and putative genes. By comparing the genomic sequence of the region to databases of cDNA libraries we identified several candidate sequences. The ESTs of interest were obtained and sequenced using vector-specific primers. If publicly available chromatograms were available they were also imported by file transfer protocol from www.wustl.genome.edu and assembled using the Pregap4 and Gap4 programs of the Staden package (Bonfield et al. 1998).

Characterization of gene sequences with bioinformatics
The exon-intron structures of the identified sequences were obtained by comparing their genomic sequences to the corresponding mRNA. Using the Dotter program (Sonnhammer and Durbin 1995) dot matrix plots were produced, where matching strings of nucleotides or amino acids of the two sequences compared are marked on a chart. The sequences were also compared using the Pregap4 and Gap4 programs. For the investigation of putative functions we used the blast family (www.ncbi.nlm.nih.gov) for homology searches; Scanprosite (www.expasy.ch), Pfam (pfam.wustl.edu), Blocks (www.blocks.fhcrc.org) and Smart (smart.embl-heidelberg.de) to search for known protein families and domains; and Psort (psort.nibb.ac.jp) in the prediction of protein sorting signals and localization sites. Also the programs of Coils (http://www.ch.embnet.org), Paircoil (nightingale.lcs.mit.edu/cgi-bin/score) and Multicoil (nightingale.lcs.mit.edu/cgi-bin/score) were used to investigate coiled-coiled domains.

To further study the sequences, gene specific primers were designed for amplification and sequencing after blocking any repetitive sequences using the
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RepeatMasker program (repeatmasker.genome.washington.edu). It is critically important to have perfect matching at the 3’ end of the primer to the target sequence in order to avoid spurious products. For the HLA-C and KIR amplifications we used primers designed by others and since these genes are known to be very polymorphic wanted to ensure their proper specificity. All known allele sequences were imported from the IMGT/HLA sequence database (www.ebi.ac.uk/imgt/hla) and the IPD-KIR sequence database at EMBL-EBI (www.ebi.ac.uk/ipd/kir) and compared to each other using the Pregap4 and Gap4 programs. All primer sequences were compared to the human genome using BLAST. In addition, primers designed for the pyrosequencing of HLA-C were compared with SNP and STS databases to search for unknown SNPs that could affect primer binding and amplification in this very polymorphic region.

Amplification of DNA
Polymerase chain reaction
Polymerase chain reaction (PCR) is an in vitro method used for copying small segments of DNA in order to have enough amounts for subsequent analysis. To amplify a target sequence, the sample is first heated to separate the DNA into two pieces of single-stranded DNA. Next, temperature is lowered for primers to anneal, indicating where to begin the synthesis of the copy. The temperature is then raised to the optimum for the enzyme, Taq polymerase, to create two new strands of DNA using the original strands as templates. This process results in a duplication of the original DNA. Each of these strands can then be used to create two new copies, and so on, and so on, and repeated up to 30 or 40 times, more than one billion copies of the original DNA segment are created in a few hours.

Phototyping of allele specific sequences
Alleles of a gene can vary at only a single nucleotide. Using primers where the last nucleotide at its 3’ end (i.e. the starting point of the synthesis) is located at the particular variation, distinct alleles of a gene can be amplified (figure 4). To further reduce the risk of unspecific amplification, the annealing temperature can be lowered stepwise during the PCR cycling in order to keep the stringency of hybridization high and favour
Figure 4. Allele specific PCR. The allele-specific primers (ASP) are designed to exactly match the region previous to the variant and to terminate in the variant nucleotide. The ASP1 will bind perfectly to the target sequence and amplification will continue while ASP2 mismatches with the variant making amplification impossible.
amplification of the specific fragment, i.e. touch-down PCR. When these conditions are optimized, an amplicon is produced only if the specific allele is present, hence prevalence can be determined by visualizing the result on agarose gels, with ethidium bromide and UV-light, and record presence or absence. This method was used to determine the status of HLA-Cw*0602 and KIR genes. In addition, primers for DRBI were included as a control for the reaction.

**Competitive and nested PCR**

When a gene is highly homologous to other sequences in the genome it is essential to reduce the possibilities of amplifying the wrong sequence. One option is to perform a nested PCR; a first amplicon is produced, which is used as template for a second PCR amplification with a different set of primers, corresponding to sequences located internal to those used in the first reaction. For HLA-C the first amplification was carried out in the presence of competitor primers in order to block any unwanted sequences and favour amplification of HLA-C. The competitor primers were degenerated and dideoxy capped, thus unable to elongate any sequence.

**Expression analyses**

**Reverse transcriptase PCR**

Reverse transcriptase PCR (RT-PCR) is used for converting mRNA into cDNA (complementary DNA) in order to study expression of genes in tissue. Its advantage is that low levels of a transcript can be detected with high accuracy, but it is at best only a rough method for quantification and therefore the strength of the signal should be interpreted with caution. First strand cDNA synthesis is carried out using Reverse Transcriptase and random hexamers. Then, using pairs of exon-specific primers, cDNA fragments can be PCR amplified under standard conditions if the sequence is expressed in that tissue. For this purpose total RNA was extracted from HaCaT cells, psoriasis skin biopsies of untreated lesions, and tonsil from a psoriasis patient with recurrent streptococcal infections. Total RNA from normal skin was obtained commercially.
Rapid Amplification of cDNA Ends

Rapid amplification of cDNA ends (RACE) is an anchor PCR modification of RT-PCR (Frohman 1993). Using this method it is possible to investigate whether the 5’ and/or 3’ ends of a transcript can be extended, i.e. when there is suspicion of a longer fragment than obtained by prior knowledge. The aim is to amplify sequences between a previously known region in the mRNA (cDNA) and an anchor sequence that is coupled to the 5’ or 3’ end of the target. In 3’ RACE a primer with an anchor sequence in the 5’ end is incorporated into the cDNA transcript at the reverse transcription step. Using an internal primer a second strand is then generated ending in a sequence complementary to the original anchor sequence. Then PCR is initiated using the same two primers.

For 5’ RACE an internal primer is first used for synthesis from a partial cDNA strand. A poly(dA) is added to the 3’ end of the cDNA using terminal transferase. Second strand synthesis is performed using a primer complementary to the poly(Ad) extended with a specific anchor sequence. This strand is then used as a template for further synthesis steps using the internal primer in order to produce a complementary copy of the anchor sequence. PCR can then be accomplished using internal and anchor sequence primers. RACE was performed trying to extend the transcripts found for PSORS1C1-C3 (paper II and III). The resulting products were sequenced as described below using the same primers as in the amplification step (figure 5).

Northern blot analysis

Northern blot is used to identify tissue specific expression of the mRNA of interest. The advantage of this analysis is that differential splicing can be detected and mRNA levels can be quantified. Briefly RNA (preferably poly(A)+) is size separated on an acrylamide gel, transferred onto a membrane and thereafter hybridized with a labelled sequence. The blot is washed under controlled temperature and salt concentrations to remove unspecifically bound probe. If the transcript of interest is present the probe will bind to it and the size and abundance of the complex can be determined using photographic film or phosphorimager.
Materials and methods

Figure 5. Rapid amplification of cDNA ends. In 3’ RACE a starting primer with an 5’ anchor sequence gets incorporated into the cDNA. An internal primer is then used to generate a short strand complementary to the strand ending with a complementary anchor sequence. Thereafter, PCR is initiated using the internal primer and an anchor sequence primer. In 5’ RACE an internal primer is used for amplifying the mRNA template and then a poly(dA) is added to the 3’ end of the cDNA. The second strand is amplified using a primer with a specific anchor and this resulting strand is then used as a template for further amplification using the internal primer. PCR can then be accomplished using the internal and anchor sequence primers.
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To explore the expression patterns of the identified genes, probes specific for coding regions in PSORS1C1-C3 were amplified. The products were size-separated and excised from low melting point agarose and labelled radioactively with $^{32}$P using random-priming as described (Sambrook et al. 1989). The probes were then tested in multiple human tissues. As control for tissue expression, the membranes were also hybridized with a probe for human $\beta$-actin. Signals were then visualized using phosphorimager after overnight exposure.

Polymorphism analyses

Detection of polymorphisms

For the detection and analysis of polymorphisms (paper I-III), each of the exons of HCR and PSORS1C1-3 were PCR amplified from genomic DNA, extracted from blood samples. Amplified products were purified and sequenced (figure 6) and the resulting sequences from the separation of fragments on polyacrylamide gels were compared against the corresponding genomic sequences using the Pregap4 and Gap4 programs of the Staden package (Bonfield, Rada et al. 1998). Polymorphisms were detected using the TRACE_DIFF program of the same package and any novel variants or ambiguous sequences were re-sequenced and re-analyzed.

Pyrosequencing (paper III and IV)

With pyrosequencing, only one of the DNA strands is sequenced. Briefly, short PCR a fragment of 200-300 bp in size of the target sequence is amplified with one of the primers biotinylated at the 5’-end. The two strands are then separated using streptavidin-coated magnetic beads and the sequencing primer is annealed in close proximity to the variation investigated. Nucleotides are released one by one in a predetermined order into the reaction mix also containing enzymes and substrate. If the added nucleotide is complementary to the next base at the template strand, a luminous signal is released with the intensity corresponding to the number of nucleotides inserted (figure 7). Excess nucleotides are degraded before the next is added. The result is recorded as a chromatogram and evaluated using appropriate software. This method was used to genotype known nucleotide polymorphisms (SNPs).
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**Figure 6. Sanger sequencing.** Sequencing is performed by random incorporation of labelled dideoxynucleotides (ddNTPs) at the 3’ terminus in a PCR reaction creating DNA fragments of various lengths. After size separation and visualization on polyacrylamide gels (A), the sequence can be determined by reading the incorporated ddNTPs in order of appearance. Today this method is automated and each ddNTP (ddATP, ddCTP, ddGTP and ddTTP) is labelled with a specific dye. The emission spectra of the DNA fragments are recorded and visualized as chromatograms (B).

**Figure 7. The chemistry of pyrosequencing.** If the nucleotide added is complementary to the sequenced strand, a lightsignal is emitted after an enzyme reaction with sulfurylase and luciferase. The signal is recorded with a peak level corresponding to the number of nucleotides incorporated. Any excess nucleotides are removed by apyrase before the next nucleotide is added.
Materials and methods

Classification of HLA-C/KIR combinations
The amino acid at position 80 of the HLA-C protein determines which KIR can elicit a NK cell response; KIR2DL1 and KIR2DS1 recognize Lysine (K80) and KIR2DL2 and KIR2DS2 recognize Asparagine (N80). Since an individual can carry either both ligand and receptor, only ligand or only receptor, we predicted the following possible biological outcomes of NK cell response; balanced (B), if both inhibitory and activating HLA/KIR combination for either or both ligand group(s) are present simultaneously; excess inhibition (EI), if one or two inhibitory HLA/KIR combinations are present and activating HLA/KIR combination is in minority or missing; excess activation (EA), if one or two activating HLA/KIR combinations are present and inhibitory HLA/KIR combination is in minority or missing; undetermined (U) when no matching HLA/KIR combination is available (table 1).

Table 1. Classification scheme over HLA-C/KIR combinations and their potential NK cell response. The combinations are based on the amino acid present at position 80 (N or K) of the HLA-C protein and presence or absence of the corresponding KIR receptors (L1, S1, L2, S2).

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Statistical analysis
The frequencies of alleles in a population are expected to follow a normal distribution under the assumption of Hardy-Weinberg equilibrium, i.e. random distribution of alleles. If this assumption is violated, e.g. through assortative mating or non-random selection of individuals, skewed results can be obtained leading to wrong conclusions of an
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association. When investigating association of an allele to a disease this bias is expected to occur in patients but not controls. Testing for Hardy-Weinberg equilibrium was performed using the GenePop website (http://wbiomed.curtin.edu.au/genepop/) and the R language and program for statistical computing (http://www.r-project.org/index.html). The significance of the distribution of alleles and genotype frequencies between patients and controls was tested by the chi-square ($\chi^2$) method. Odds rations (OR) and 95% confidence intervals (CI) were calculated and Fisher’s exact test used to obtain exact P values. These were corrected for multiple testing by Bonferroni method (Elston R et al. 2002). In order to test for confounding factors the data was stratified according to presence or absence of HLA-Cw*0602 as well as for each of the associating SNPs and then tested for homogeneity of the odds ratios (Breslow NE and NE 1980). Estimation of haplotypes analysis was done with HPlus (http://qge.fhcrc.org/software.php) (Li et al. 2003).
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Characterization of the HCR gene in Swedish psoriasis patients (paper I)

The HCR gene was first reported as Pg8, a novel gene located approximately 110 kb telomeric to HLA-C. Initially it was only represented in GenBank by genomic and mRNA sequences. By comparing the available sequences to each other, and to orthologs, it was determined from the longest cDNA (NM_019052) that HCR consists of 16 exons stretching 14.7 kb. The produced transcript of HCR was identified as a 2.4 kb long sequence encoding 782 amino acids. Using various prediction programs the putative function of this protein was investigated. HCR contains nuclear localization signals and leucine zipper motifs suggesting that the protein interacts with DNA in the nucleus. This was further supported by the presence of multiple coiled-coil domains.

Polymorphism analysis and association to psoriasis

In order to characterize this gene for association to psoriasis we performed polymorphism analysis of the coding sequence on an ongoing basis. The exons were sequenced in all individuals in the study and we were able to identify 25 single nucleotide polymorphisms (SNPs) spread throughout the gene. Of these 13 encoded amino acid changes and the remaining 12 were silent, i.e. did not produce any changes to the protein sequence. All SNPs were confirmed by sequencing of both strands, no other polymorphisms were detected. When comparing the frequencies of the SNPs between patients and controls significant association was found to several of the variations. The highest association was found with SNPs +1328 and +1364, both located in exon 8. The SNP +1364 does not produce any amino acid change and was therefore regarded as having very little effect, if any, on the HCR protein. The SNP +1328 appeared to co-segregate with SNP +386 and +404 as an allele in 69% of the patients and 38% of the controls. With HLA-Cw*0602 being the strongest associated allele in the region so far, we wanted to compare its frequency to the identified HCR SNP combination. For the patients carrying the three SNPs, 97% were also positive for HLA-Cw*0602 whereas only 36% of the controls. This is an inferred allele based on information from patients and controls homozygous for these variations, however SNP +386 and +404 in combination with HLA-Cw*0602 were
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also identified in families in a Finnish population (+251 and +269 according to their coordinates) and were reported to associate strongly to psoriasis (Asumalahti et al. 2000). In the same study no association to psoriasis was found with SNP +1328 (+1193).

The association of HLA-Cw*0602 was highly significant in our study population. To continue the investigation of the relationship between HLA-Cw*0602 and HCR, the various SNPs were evaluated by stratifying the data according to presence or absence of HLA-Cw*0602. By doing so, only SNP +1364 remained associated. When investigating the association of HLA-Cw*0602 while stratifying for all the SNPs there was still highly significant association of this allele to psoriasis. Knowing that most HLA-haplotypes appears to derive from a restricted number of ancestral haplotypes it is likely that a non-disease causing association would occur by linkage disequilibrium. This could be the case with the HCR SNPs as they appear to be associated mainly due to the enrichment of HLA-Cw*0602 positive haplotypes containing these SNPs. In addition, the SNP +1364 was not associated in the Finnish psoriasis population and is therefore believed to have little to do with disease pathogenesis. Based on these data we concluded that the HCR gene in itself is unlikely to be the major factor in psoriasis development assuming a one locus – one gene hypothesis.

HCR has been further analyzed and two additional exons have been identified adding 135 bp to the 5’ end of the gene and 26 amino acids to the protein (Asumalahti et al. 2002). No additional polymorphisms were detected. This did however change the numbering of the SNPs as they are determined from the first methionine of the sequence. Our numbering of the SNPs is given in brackets. Additional psoriasis populations have been tested for HCR association in comparison to the Finnish results (Asumalahti, Veal et al. 2002). A new susceptibility allele named HCR*WWCC (HCR-307 (+386), HCR-325 (+404), HCR-1723 (+1802) and HCR-2327 (+2406)) was identified being present in 35% of the patient and 18% of control chromosomes. Also SNPs for testing the CDSN*5 allele (CDSN-619 and -1243) were included in the study however, the strongest association was found with HLA-Cw*0602. Albeit a large study
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population in total they were not able to separate the effects of HCR*WWCC and HLA-Cw*0602 due to strong linkage disequilibrium.

In a third study by the same group, the difference in association between HLA-Cw*0602 and HCR SNPs were even more pronounced when investigating patients with guttate psoriasis, strongly signifying an involvement of HLA-Cw*0602 (Asumalahti, Ameen et al. 2003). Individuals in this phenotype group are known to show association to this allele, often in correlation to young age of onset, which are the criteria for type I psoriasis. Interestingly, type I patients appear to have more severe disease indicating a major effect of the HLA-C allele (Mallon et al. 2000; Gudjonsson et al. 2002). Looking for a major factor one cannot disregard the constantly stronger association of HLA-Cw*0602. The argument used that not all patients are positive for this allele, and the presence of HLA-Cw*0602 negative HCR haplotypes, may be explained by involvement of other HLA-C susceptibility alleles. Another option is heterogeneity that likely exists in psoriasis (Elder, Nair et al. 1994; Bowcock and Barker 2003). There are families that do not link to PSORS1 but instead appear to have a main psoriasis factor located elsewhere, e.g. 17q25 or 18p (Asumalahti, Laitinen et al. 2003; Speckman, Wright Daw et al. 2003). Nevertheless, it is possible that more than one gene in this region contributes to the disease, and HCR can therefore not be excluded only based on genetic association. But assuming a synergistic effect of more than one gene in PSORS1, then all the genes previously investigated (and excluded) would have to be re-assessed in a different hypothesis model.

The structure of the HCR*WWCC sequence appears to decrease the probability of coil dramatically, in comparison to the wild type protein. This structural change could affect the interaction potential of the protein. Looking into the expression pattern in skin, HCR mRNA is highly upregulated in keratinocytes of psoriasis whereas normal and non-lesional skin appeared almost negative (Asumalahti, Laitinen et al. 2000). In lesional skin, the expression pattern is reversed compared to Ki67, a marker of proliferation, and HCR has therefore been suggested to have a role in regulating keratinocyte differentiation and/or proliferation. Altered expression was not seen in other
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inflammatory diseases (Asumalahti, Veal et al. 2002). The effect of the altered protein structure has been investigated in transgenic mice carrying either the wild-type allele or the risk allele (Elomaa et al. 2004). However, no phenotypic or histological changes were detected. First when comparing expression of genes, similarities to previous findings in psoriatic lesions was observed, such as upregulation of cytokeratins 6, 16 and 17. Also differences in expression of genes involved in terminal differentiation and formation of the cornified cell envelope was observed and the authors concluded that HCR might contribute to psoriasis development (Elomaa, Majuri et al. 2004). Whether this upregulation is a contributing factor or not remains to be investigated.

Identification and characterization of novel genes (paper II & III)

PSORS1C1-3 structures

The MHC is one of the most gene dense regions in our genome {TheMHCsequencingconsortium, 1999 #17}. When comparing the areas surrounding HLA-C to the rest of MHC, they appeared to have a low gene number. Suspecting that unknown candidates could be located herein we started a screening of the regions, trying to identify unknown open reading frames (ORFs) to be characterized regarding psoriasis. The genomic sequence between HLA-C and POU5F1, and between HCR and CDSN were compared to ESTs, proteins and protein domains and this search resulted in the identification of three potential gene sequences; Psoriasis Susceptibility 1 Candidate 1-3 (PSORS1C1-3). During the progress of our studies two gene sequences that were shorter but largely overlapping with two of our sequences were released in public databases; SEEK1 and SPR1, however the names accepted by the nomenclature committee are PSORS1C1 and PSORS1C2 (www.gene.ucl.ac.uk/nomenclature).

PSORS1C1 stretches a region over 25 kb and contains six exons. It is located in a telomeric to centromeric orientation on the opposite strand to the introns of CDSN and PSORS1C2 (figure 8). From the screening we identified at least two splice-isoforms, one with exon 1 spliced to exon 6 and one containing all six exons. From this full-length isoform the most likely ORF starts in exon 3 and encodes for a 152 amino acids protein
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Figure 8. Organisation of exons of PSORS1C1-3. The PSORS1C1 is indicated by grey shaded boxes, PSORS1C2 by striped and PSORS1C3 by dotted. The figure is not to scale.
sequence. When investigating the putative function of the product, weak similarity to Cu-Zn superoxide dismutase proteins was identified. The shorter splice-isoform has a dissimilar N-terminus encoded by exon 1.

*PSORS1C2* has a centromeric to telomeric direction and consists of two exons stretching 1.8 kb (figure 8). A transcript of 1185 bp was obtained for this sequence and it produces a putative protein of 134 amino acids. This protein sequence has two regions of low complexity and is rich in proline. This is a common feature of an activation domain in transcription factors. By comparison to other proteins and known domains there were indications of a N-terminus signal peptide located in the first 22 amino acids, a signal necessary for sorting of proteins, and supports a functional role for this sequence. We also cloned the murine and porcine orthologs, which show that PSORS1C2 is a highly conserved protein.

*PSORS1C3* consists of three exons with a centromeric to telomeric orientation (figure 8). *PSORS1C3* produces a transcript of 599 bp with the longest identified ORF being 58 amino acids. This putative protein does not show any similarity to known proteins or domains but the sequence is conserved between human, chimpanzee (Pan Troglodytes) and pig (Sus scrofa).

**Expression patterns of PSORS1C1-3**

Using RT-PCR on total RNA, expression was detected for all three genes in HaCaT cells, tonsil (only tested for *PSORS1C3*) and both normal and psoriasis skin. Next we investigated the expression by Northern blotting. For *PSORS1C2*, a strong band was detected in heart and skeletal muscle. For *PSORS1C1*, two different probes were used in order to separate the two isoforms identified. They did however generate the same pattern with weak expression consistently detected at 1.8 and 6 kb in heart, placenta, liver, skeletal muscle and pancreas. The larger identified band could indicate the presence of more exons, although no additional sequence was detected using RACE. During the characterization of ESTs for *PSORS1C1* we found an alternative terminal exon spliced to
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exon 1-3, however this exon did not reveal any signal neither by RT-PCR nor by Northern blot.

Different sizes for PSORS1C3 were also obtained. A 1 kb band in testis and pancreas and a 2 kb band in skeletal muscle were observed. This difference in size could result from alternative splicing. It may also indicate a larger gene than reported here, though no additional sequence was detected and no splice-variants were seen in the ESTs/RT-PCR sequencing results. It is quite common to find larger transcripts than expected on Northern blots. In addition, it is known that isoform expression can be organ and/or time-specific in up to 70% of genes (Lander et al. 2001) therefore one cannot rule out that other splice variants exist. In fact, regulation of mRNA stability has emerged as a major control point in gene expression and different splice variants appear to confer different stability on the mRNA level (Guhaniyogi and Brewer 2001; Kozak 2004).

With these analyses we were quite confident that all three candidates are active transcriptional units. They have the expected structure of a gene and are conserved between species, and transcripts have been identified in various tissues with RT-PCR and Northern blots. Although PSORS1C3 did not show any similarities to known proteins and functional domains, it may still be active on the RNA level. The experience in the field of RNA is evolving and functions at this level may be more important and developed than previously thought (Eddy 2001; Hirotsune et al. 2003; Mattick 2003).

Polymorphism detection and association with psoriasis

To investigate the presence of polymorphisms in PSORS1C1 and PSORS1C2 all exons were completely sequenced from both directions in all individuals. For PSORS1C3 polymorphism detection was performed in 87 patients and 50 controls and the additional genotyping of 131 patients and 77 controls was completed using pyrosequencing.

A total of nine SNPs were detected in PSORS1C1, of which five associated to psoriasis. Of these a silent SNP, +39604 located in exon 2, showed the highest association and of the additional four only one changed an amino acid, SNP +26680. In
PSORS1C2 we identified four SNPs. Two of these encoded amino acid changes but did not associate to the disease. Only SNP +26276 showed significant difference between the study groups. In PSORS1C3, the polymorphism analysis revealed a total of 11 SNPs. When comparing the frequencies in patients and controls three SNPs were not analyzed further, as they were rare variants. After pyrosequencing a fourth SNP was excluded from the study due to deviation from Hardy Weinberg equilibrium. Of the remaining seven SNPs, three located in exon 1 (+12019, +12072 and +12343) demonstrated significant association to the disease.

Association of HLA-Cw*0602 (paper II & III)
The strongest association detected was with HLA-Cw*0602. In the PSORS1C1/C2 study (paper II) HLA-Cw*0602 was present in 48% of the patients and 10% of the controls and in the PSORS1C3 study (paper III) it was even higher, 59% compared to 12%. In comparison to PSORS1C1/C2 we also investigated the association of the inferred HCR*WW allele detected in HCR (paper I) and the frequency of this allele differed significantly between patients and controls, however not as strong as with HLA-Cw*0602. When combining the two alleles HCR*WW and HLA-Cw*0602 the association appeared even stronger indicating the strong linkage disequilibrium between these variants. This could however, be due to observation bias. It has been demonstrated that haplotypes shared between populations represent only a fraction of the total number of haplotypes observed (Crawford et al. 2004).

Testing for confounding factors
Knowing that HLA-Cw*0602 is a possible confounding factor due to the high linkage disequilibrium in the region, we stratified the data to see the effect of this allele on the association. After stratification for all SNPs HLA-Cw*0602 still showed significant association, however when stratifying for HLA-Cw*0602 status only two SNPs from PSORS1C1 showed independent association to psoriasis (+39610 and +39604) both located in exon 2. The remaining SNPs were concluded to be associated to psoriasis due to linkage disequilibrium with HLA-Cw*0602. In a similar manner, we tested the effect
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of the inferred haplotype HLA-Cw*0602-HCR*WW on the association of \textit{PSORS1C1} and \textit{PSORS1C2} and the same two SNPs in \textit{PSORS1C1} remained associated.

Although more information is necessary, the stratification shows that association of variations in these three genes are most likely due to their presence on the same haplotype as HLA-Cw*0602. In addition the associations detected are not near the levels obtained with HLA-Cw*0602, but for one SNP in \textit{PSORS1C1} (+39604), which also displayed independent association. Despite this, \textit{PSORS1C1} does not emerge as a strong candidate. The two HLA-Cw*0602-independent SNPs do not code for any amino acid changes and the exon in which they are located, does not appear to be translated. However, if more splice variants exist, also indicated by the large fragment detected by Northern blot, there might be isoforms where exon 2 is part of an ORF. Another possibility is that this exon is involved in the regulation of the gene hence variations in it may have a potential effect on the function.

Continued studies of the putative function of all three genes are required in order to evaluate them as psoriasis genes and especially the functional role of \textit{PSORS1C3}. Today the understanding of the relationship between genetic information and biological function is rooted in the one gene - one protein hypothesis (Mattick and Gagen 2001). With the emerging number of reports of genes presenting alternative splicing, and of non-coding RNAs performing a variety of tasks (gene silencing, catalysis, regulation of development), we may have to re-consider this hypothesis and not as easily discard sequences not behaving according to the existing dogma (Eddy 2001).

Searching for a candidate in the PSORS1 region has led to the identification and characterization of several genes, including the four discussed above. The majority of these are significantly associated to psoriasis, but the strongest association that consistently is observed across populations is that of HLA-Cw*0602. The debate regarding its role in psoriasis has been ongoing and several arguments are used against the involvement of this gene in disease pathogenesis; low frequency of psoriasis in HLA-Cw*0602 positive individuals, more than 30% of psoriasis patients are negative for HLA-
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Cw*0602, other candidate variants reach the same level of association as HLA-Cw*0602 and haplotypes carrying HLA-Cw*0602 associate more strongly to psoriasis than the allele itself (Jenisch et al. 1999; Asumalahti, Laitinen et al. 2000; Barker 2001; Elder, Nair et al. 2001; Veal, Capon et al. 2002).

Psoriasis is a multifactorial trait where several factors are likely to interact in the development of disease. Therefore it does not follow Mendelian inheritance patterns, a circumstance further complicated by heterogeneity. The high level of association seen with HLA-Cw*0602 is practically invariable and presence of this allele has been associated to severity of disease (Gudjonsson, Karason et al. 2002). Additionally, homozygotes for HLA-Cw*0602 has been shown to have a higher risk of developing psoriasis (Gudjonsson et al. 2003). Considering that HLA-C plays an essential role in T cell biology, and T cells are involved in both the formation and maintenance of a psoriatic plaque, this molecule is very likely part of psoriasis aetiology and deserves thorough investigations.

*HLA-C and KIR interactions (paper IV)*

The main function of HLA-C is signaling to NK cells and NKT cells through recognition by killer immunoglobulin like receptors (KIRs). Any alterations in the expression of HLA-C are likely to initiate immune responses through NK cells, where KIR(L) regulate NK cell cytotoxicity by antagonizing the activating signals from KIR(S) activating receptors. Since the MHC and KIR loci are located on different chromosomes (6p21 and 19q13.4) and the number of genes at the KIR locus naturally varies between 7-14 genes, individuals can inherit both ligand and receptor, only ligand or only receptor. As a result, different HLA/KIR combinations could lead to variations in thresholds for NK cell activation. Assuming all alleles involved are expressed, four classes were defined as representing the likely functional outcome of the possible HLA-C/KIR combinations identified (table 1).
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As KIRs can discriminate HLA class I alleles by their amino acid at position 80 of the α1 helix, a key factor for the division of individuals in different classes is zygosity information for HLA-C. Alleles carrying K80 at this position are HLA-Cw2, 4, 5, 6 and 15, and alleles carrying N80 are HLA-Cw1, 3, 7, and 8 (Mandelboim, Reyburn et al. 1996; Winter and Long 1997; Boyington et al. 2001). We therefore genotyped all individuals for the sequence at this amino acid position in HLA-C. For comparison we genotyped for HLA-Cw*0602 and all individuals carrying this allele had a corresponding Lysine at position 80. As expected, the frequency of HLA-Cw*0602 was high. The association was even stronger when dividing the patients according to their phenotype, with 73% positive for this allele in the guttate group. Patients carrying K80 were more frequent in the guttate and vulgaris groups than among patients with arthritis. Additionally the guttate patients correlated with an early onset of psoriasis in agreement with previous findings and the observed association of position 80 (Enerback et al. 1997; Mallon, Bunce et al. 2000; Gudjonsson, Karason et al. 2002). Of the KIR genes the frequency of the activating KIR2DS1 was significantly different among patients with psoriasis vulgaris and a similar trend was observed in the arthritis group. This has also been observed in other studies where an inability to control activating responses has been suggested to contribute to disease (Martin, Nelson et al. 2002; Luszczek, Manczak et al. 2004; Suzuki, Hamamoto et al. 2004).

With this information all individuals were classified according to their HLA-C/KIR combinations (table 1). With this model, a trend for increased potential for activation (EA) was found among psoriasis arthritis patients. This is consistent with the findings reported for KIR2DS1, but overall there was a low frequency of individuals in this class. Looking at previous indications such that inhibitory receptors have higher affinity for their ligand than activating receptors, and inhibition of NK cell activation is crucial during pregnancy, the number of individuals with potential for NK-cell activation would be kept at low frequencies in the population (Winter, Gumperz et al. 1998; Hiby et al. 2004).
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The most striking difference was observed with guttate patients showing a distinctive profile compared to the other phenotypes. Significantly less guttate psoriasis individuals were classified as having undetermined (U) HLA-C/KIR responses and more guttate individuals were classified as having an increased potential for NK cell inhibition (EI). When dividing the data according to HLA-Cw*0602 status, individuals were more likely classified as EI, and among the U class most individuals were lacking this allele. This is in line with previous indications of guttate psoriasis emerging as a distinct phenotype of psoriasis (Gudjonsson, Karason et al. 2002; Carlen et al. 2005; Mallbris 2005).

The frequency of EI individuals among the guttate patients is differing from the assumption that a lower threshold for activation contributes to disease (Martin, Nelson et al. 2002). One explanation could be that a lack of NK cell response in relation to an infection would favour the microbe agent, with consequences that may contribute to immune responses leading to development of disease. Indeed, the divergence towards Th1 response in psoriasis may be mediated by cytotoxic T cells secreting Th1 cytokines. Of the guttate patients with predicted balanced responses one third had a concomitant streptococcal throat infection, which could be the agent that perhaps disturbs their stability. In other patients it might add to the already imbalanced HLA-C/KIR interactions. In U class individuals it is not clear what causes the NK cell imbalance. It is likely that other receptors and their ligands need to be included into the model suggested here before we have a more complete picture of the process leading to NK cell activation in psoriasis. For example, in patients with HIV, the disease progression is delayed for people carrying a certain HLA-B/KIR combination and in type I diabetes and rheumatoid arthritis patients HLA-C/KIR2DS2 appears to be involved. Other immune related molecules, such as HLA-B and MIC, have been associated to psoriasis and it would be interesting to see the effect of these molecules on the predicted NK cell responses. In addition, this model is based on the assumption that all genes involved are expressed and it is therefore necessary to investigate the allelic expression. In order to get a more detailed view of the mechanisms behind initiation of a HLA/KIR response according to
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these combinations, also the level of expression of both receptors and ligands need to be considered as they may have an effect on the threshold.

What is shown here is an association between $HLA-C/KIR$ combinations in psoriasis indicating an altered communication between $HLA-C$ and $KIR$, in which $HLA-Cw^*0602$ contributes significantly. This is especially prominent in guttate patients and likely in conjunction with a streptococcal infection, supporting an immunity-based theory of psoriasis. Since the NK cell response profiles in this study were based on zygosity at position 80, not only $HLA-Cw^*0602$ is expected to contribute to the outcome but also other $HLA-C$ variants. Hence it might be advantageous to incorporate $KIR$ into studies where weak association to $HLA-Cw^*0602$ is observed, thus accounting for the effect of other $HLA-C$ alleles.
Psoriasis is a common disease with a complex inheritance pattern. Researchers worldwide make major efforts in the search for an explanation to the origin of psoriasis. It is well established that psoriasis is a genetically inherited disease and the PSORS1 region on chromosome 6p21.3 constantly appear as the main source for such a factor in various populations.

The studies included in this thesis have contributed to the description of the PSORS1 locus. Four genes, \( HCR \) and \( PSORS1C1-C3 \), have been characterized with regard to psoriasis susceptibility in the Swedish population. Of these genes, three were identified by us and they all carry variations associating to psoriasis in case-control studies. However, analyses in the PSORS1 region are complicated by strong linkage disequilibrium. It is therefore necessary to compare the observed variations to the strongest factor previously identified in order to test whether they show similar or even stronger association. The allele HLA-Cw*0602 has consistently shown high association to psoriasis and in comparison the characterized genes from this study appear less significant.

Studying a multifactorial disease there are several complicating factors. The identification of different psoriasis susceptibility loci in diverse populations may indicate that psoriasis is a genetically heterogeneous disease. If a major factor is involved it will therefore not be present in all individuals studied and it will be common in the general population. In addition, the option remains that more than one factor in PSORS1 is involved, however all genes previously rejected will then have to be re-investigated using a different approach. A continued thorough characterization of the areas surrounding \( HLA-C \) is needed in order to understand the underlying mechanisms for psoriasis association at this locus. Considering the location of \( PSORS1C1 \) in relation to \( CDSN \) and \( PSORS1C2 \), and the increasing awareness of a complex RNA world there may still be undetected genes in this region.
Concluding remarks and future studies

The understanding of psoriasis has been swinging back and forth from being a keratinocyte mediated disease to today’s belief of an autoimmune T cell mediated disease. The involvement of T cells in initiation and maintenance of psoriatic skin lesions is quite clear and the development of new treatments for psoriasis is currently directed towards T cell biology. Additionally, recent data shows that activated T cells are required for development of psoriatic lesions in K5.Stat3C mice (Sano et al. 2004). With the high association between \( HLA-C \) and psoriasis, and the biological role of this molecule in our immune system, it is essential that this gene be further characterized. No other inflammatory disease manifests such a strong \( HLA-C \) association and to date this remains the strongest candidate in this region. Therefore we continued to investigate this gene and in the fourth study we propose a model to study the potential biological effect of \( HLA-C \) when considered together with its natural interacting partners. Using this approach HLA-Cw*0602 appears to contribute significantly to altered immune responses in psoriasis. This suggested model also allows for the addition of other factors, such as members of the PSORS1 region, or other proteins known to be active in the immune system and should therefore be developed further.

Several diseases have been associated with psoriasis. The best established is arthritis, but also inflammatory bowel disorders, diabetes and non-melanoma skin cancer has been associated (Moll and Wright 1973; Yates et al. 1982; Frentz and Olsen 1999; Hannuksela-Svahn et al. 2000; Shbeeb et al. 2000; Thumboo et al. 2002). This may reflect involvement of common etiologic factors and revealing the source behind traits such as psoriasis would very likely give clues for other complex diseases that await deciphering of the underlying disease causing patterns. A variant interfering with a binding site for RUNX1 has been identified in the PSORS2 region, a phenomenon also associated with other autoimmune diseases. Whether such sharing of common molecular background also exist in the PSORS1 region remains to be investigated.
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