

Department of Medical and Clinical Genetics
Institute of Biomedicine
The Sahlgrenska Academy at Göteborg University
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Genetic studies of psoriasis and psoriatic arthritis

Camilla Friberg



GÖTEBORG UNIVERSITY

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camilla.friberg@clingen.gu.se

Department of Medical and Clinical Genetics, Institute of Biomedicine
The Sahlgrenska Academy at Göteborg University

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To my family

“I am among those who think that science has great beauty. A scientist in his laboratory is not only a technician: he is also a child placed before natural phenomena which impress him like a fairy tale. “

Marie Curie (1867-1934)

ABSTRACT

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Psoriasis and psoriatic arthritis are common chronic immune-mediated diseases of the skin and joints. Psoriasis affects approximately 2-3 % of the Caucasian population and about 30 % of all psoriasis patients develop psoriatic arthritis. Both diseases have a strong genetic component but are also affected by environmental factors and are thus regarded as multifactorial. A major genetic factor contributing to susceptibility to both diseases is believed to reside at the HLA locus on chromosome 6, although different alleles within this locus have been found to associate with the respective diseases. While this is the strongest and most replicated locus, other susceptibility loci have also been identified through genome-wide linkage studies and candidate gene approaches.

The studies in this thesis aimed at refining two susceptibility loci for psoriasis identified with linkage analysis, 3q21 and 5q31-32, with a special emphasis on the PSORS5 region on chromosome 3q21. Another purpose was to investigate whether several autoimmune-associated genes and genomic regions are susceptibility factors for psoriasis/psoriatic arthritis.

Association studies on a psoriatic arthritis case-control material revealed an association with a marker in the TNFB locus within the HLA region. Linkage disequilibrium (LD) between TNFB123 and certain HLA-B antigens was also found. Due to the strong LD within this region, it is difficult to identify the disease-causing allele. No association was found with a microsatellite marker within the CTLA-4 gene, previously associated with rheumatoid arthritis (RA), nor with the eight genotyped markers within the PSORS5 region. This region was identified in a data set of southwestern Swedish families with psoriasis and arthritic symptoms. The lack of association is consonant with the hypothesis of a founder mutation in this region.

The 5q31-32 region was refined with 34 markers in multi-affected psoriasis families. We obtained a peak non-parametric linkage value of 3.1 for marker D5S436 in a subgroup of patients with arthritic symptoms. However, no association was found with 3 SNPs reported to associate with RA and Crohn's disease (CD) and to change the functional activity of 2 cation transporters, SLC22A4 and SLC22A5. These results support the existence of a susceptibility region for psoriasis on chromosome 5q32, probably involved in the arthritic phenotype and not caused by the 3 SNPs within SLC22A4 and SLC22A5.

Analysis of two candidate genes, CSTA and ZNF148, within the linkage region of PSORS5 yielded no significant association. It is therefore unlikely that they harbor the genetic cause of psoriasis at this locus. Fine-mapping of the PSORS5 region revealed both point-wise and haplotype associations that might contribute to psoriasis susceptibility. The only gene within this region was also slightly less expressed in skin biopsies from psoriasis plaque than from control individuals. Further genotyping studies are needed to relate the expression data to the associating genotypes, before a disease susceptibility allele can be identified.

Key words: psoriasis, psoriatic arthritis, complex disease, autoimmune disease, linkage analysis, association analysis, PSORS5, 3q21, 5q31-32, SLC12A8, SLC22A4, SLC22A5, CSTA, ZNF148, expression

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numbers:

- I. Alenius GM, Friberg C, Nilsson S, Wahlstrom J, Dahlqvist SR, Samuelsson L. Analysis of 6 genetic loci for disease susceptibility in psoriatic arthritis. *J Rheumatol.* 2004 Nov;31(11):2230-5.
- II. Samuelsson L, Stiller C, Friberg C, Nilsson C, Inerot A, Wahlstrom J. Association analysis of cystatin A and zinc finger protein 148, two genes located at the psoriasis susceptibility locus PSORS5. *J Invest Dermatol.* 2004 Jun;122(6):1399-400.
- III. Friberg C, Bjorck K, Nilsson S, Inerot A, Wahlstrom J, Samuelsson L. Analysis of chromosome 5q31-32 and psoriasis: confirmation of a susceptibility locus but no association with SNPs within SLC22A4 and SLC22A5. *J Invest Dermatol.* 2006 May;126(5):998-1002.
- IV. Friberg C, Inerot A, Zucchelli M, Kere J, Wahlström J, Samuelsson L. Gene expression analysis of, and search for functional variants within the psoriasis candidate gene SLC12A8. *Manuscript.* 2007

CONTRIBUTION TO PAPERS

Contribution of Camilla Friberg to Papers I-IV:

- I. Performed all the genetic analyses, evaluated the results and wrote part of the manuscript. All authors participated in planning the study. Staffan Nilsson was responsible for the statistical calculations and Solbritt Rantapää-Dahlquist and Gerd-Marie Alenius were responsible for collection of blood samples from patients and controls.
- II. Optimized the SNaPshot method used in the genotype analysis and functioned as a lab supervisor for the genetic analyses.
- III. Planned the experimental design of this study together with Lena Samuelsson. Planned and performed all the genetic analyses, evaluated the results and wrote the manuscript. Staffan Nilsson was responsible for the statistical analyses.
- IV. Planned the experimental design of this study together with Lena Samuelsson. Planned, performed and evaluated the results of the sequencing, bioinformatics and gene expression parts of this study. Wrote the manuscript. Marco Zucchelli performed the statistical analyses

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ABBREVIATIONS

APC	antigen-presenting cell
AS	ankylosing spondylitis
ASP	affected sibling pairs
CARD15	caspase recruitment domain 15
CD	Crohn's disease
cDNA	complementary DNA
CDSN	corneodesmosin
CSTA	cystatin A
CTLA-4	cytotoxic T lymphocyte-associated 4
DIP	distal interphalangeal
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide tri phosphate
DZ	dizygotic
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HCR	alpha-helix coiled-coil rod homolog
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hnRNP-A1	heterogeneous nuclear ribonucleoprotein-A1
IBD	identical by descent
ICAM-1	intercellular adhesion molecule-1
IFN- γ	interferon-gamma
IL	Interleukin
IRF2	interferon regulatory factor 2
kb	kilo bases
LD	linkage disequilibrium
LFA-3	lymphocyte function-associated antigen, type 3
LOD	logarithm of the odds
LOR	loricrine
MGST2	microsomal glutathione S-transferase 2
MHC	major histocompatibility complex
mRNA	messenger RNA
MZ	monozygotic
NAT9	N-acetyltransferase 9
NCBI	national center for biotechnology information
NF- κ B	nuclear factor kappa-beta
NPL	non-parametric linkage
PCR	polymerase chain reaction
PDCD1	programmed cell death 1
PPP	palm-plantar pustulosis
PsA	psoriatic arthritis
PSORS	psoriasis susceptibility
PUVA	psoralen plus ultraviolet A
RA	rheumatoid arthritis

RNA	ribonucleic acid
rRNA	ribosomal RNA
RT-PCR	reverse transcription PCR
RUNX1	runt related transcription factor 1
SLC12A8	solute carrier family 12, member 8
SLC22A4	solute carrier family 22, member 4
SLC22A5	solute carrier family 22, member 5
SLC9A3R1	solute carrier family 9, isoform A3, regulatory factor 1
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
STR	short tandem repeats
TCR	T cell receptor
TDT	transmission disequilibrium test
Th1	T helper 1
Th2	T helper 2
TNF- α	tumor necrosis factor alpha
tRNA	transfer RNA
UCSC	university of California, Santa Cruz
UTR	untranslated region
UV	ultraviolet
VCAM-1	vascular cell adhesion molecule-1
ZNF148	zinc finger protein 148

INTRODUCTION

Psoriasis is a skin disease and was first perceived as a distinct disease as early as 1808 (Willan 1808). The name derives from the Greek “psora” meaning “to itch” (Fry 1988). Although psoriasis generally does not affect survival, it can profoundly influence a patient's self-image, self-esteem and sense of well-being. Psoriasis affects all aspects of the quality of life, including physical, psychological, social, sexual and occupational aspects. Psoriasis patients suffer impaired quality of life similar to or worse than patients with other chronic diseases such as ischemic heart disease and diabetes (Finlay et al. 1987). Over the past 20 years, our understanding of the disease mechanisms has advanced significantly and several effective treatments have been developed. However, we still lack a cure for this common and enigmatic disease and many patients experience significant side effects from currently available treatments, including development of skin cancers in non-lesional skin, abnormal renal function and liver abnormalities. It is anticipated that the identification of genes or antigens responsible for the occurrence of psoriasis will increase our understanding of its nature and make it possible to design drugs to increase patient compliance and safety and to reduce medical costs.

GENETICS

DNA

In order to be able to cure human diseases we need knowledge of mechanisms within the human body. The adult human body is made up of between 10 and 100 trillion cells, containing many organelles. One of these is the nucleus, often called the "control center" because it controls cellular activities, including cell reproduction, as well as heredity. This control is mediated through deoxyribonucleic acid (DNA). Every cell in our body has the same DNA, organized into structures called chromosomes. There are 46 chromosomes in every human cell, i.e. 22 autosome pairs and 2 sex chromosomes, XX or XY. The DNA molecule is made up of a double-stranded polymer of four different nucleotides or "bases": adenine (A), guanine (G), cytosine (C) and thymine (T). The double-stranded polynucleotide takes the conformation of a double helix held together by the hydrogen bonding between the bases; A always pairs with T and C always pairs with G. This makes the strands complementary in an antiparallel fashion (Figure 1). One strand can thus serve as a template for the synthesis of the second strand.

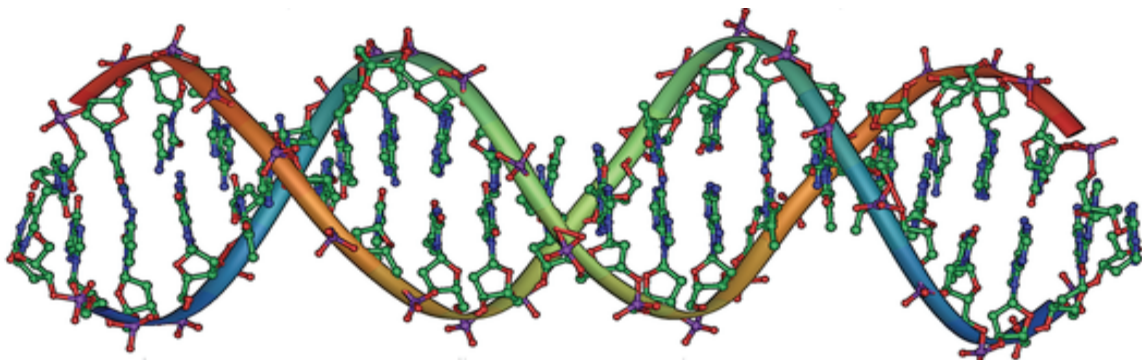


Figure 1. The structure of a DNA double helix

From DNA to RNA to protein

Proteins are the main building blocks and functional molecules of the cell, making up almost 20 % of a eukaryotic cell's weight, the largest contribution after water (70 %). DNA is the template of genetic information for protein synthesis. The flow of genetic information goes from DNA to RNA by a process called transcription and from RNA to protein by a process called translation (Figure 2). In the transcription process, protein-coding genes are transcribed by RNA polymerase II into precursor messenger RNA (pre-mRNA). These pre-mRNAs undergo a number of post-transcriptional modification steps, including capping, polyadenylation and splicing. Splicing is necessary to remove non-coding sequences called introns from the pre-

mRNA. Many pre-mRNAs can generate several types of mature mRNAs via alternative splicing. Mature mRNAs are then translocated from the nucleus to the cytoplasm for translation.

Proteins are formed in the translation process using the genetic code of the RNA, which is based on the order of the nucleotides in the RNA molecule. The nucleotides are grouped into triplets called codons. Each codon codes for a specific amino acid. Since RNA is constructed from four types of nucleotides, there are 64 possible codons ($4 \times 4 \times 4$), three of which specify termination of the polypeptide chain and are thus called "stop codons", leaving 61 codons to specify only 20 different amino acids. Therefore, most of the amino acids are represented by more than one codon; the genetic code is said to be degenerate. The resulting polymer of amino acids will, after extensive modification, fold into an active protein.

The human genome

The genome consists of coding and non-coding DNA. The human genome is estimated to encode between 20,000 and 25,000 protein-coding genes (2004). This is the smallest part and constitutes only 1.2 % of the euchromatic genome (2004). The coding part of a gene is called an exon. A gene often consists of several exons which are separated by non-coding DNA called introns. The intron include a 5' splice site (GT), a 3' splice site (AG) and a branch site which are required for the splicing process. Other parts which are included in a gene are the promoter region, 5' untranslated region (5' UTR), 3' untranslated region (3' UTR) and Poly A signal (Figure 2). The promoter region of a gene is a regulatory sequence, most often located immediately upstream to the gene. It is crucial to controlling gene expression. It is recognized by proteins known as transcription factors which bind to the promoter sequences and recruits RNA polymerase II. Apart from the promoter region, there are also other regulatory sequences, typically short sequences that appear near or within a gene, that are also involved in the controlling of gene expression. Regulatory regions can be identified through comparative genomics studies as they have probably been conserved throughout evolution because of their functional importance.

In addition to protein-coding genes, the human genome contains thousands of RNA genes, including transfer RNA (tRNA), ribosomal RNA (rRNA), microRNA, and other non-coding RNA genes. tRNA transfers the correct amino acid to a growing polypeptide chain during translation, rRNA is the primary constituent of ribosomes and the function of microRNA is mainly to downregulate gene expression.

However, for the vast majority of the human genome the function remains unknown; some of it is comprised of repeat elements, transposons or pseudogenes, but there is also a large amount of sequence not falling under any classification used to be referred to as "junk" DNA. This term may, however, be misleading since there are indications that many sequences may function in ways that are not fully understood. For example, recent studies show that most mRNAs do not encode proteins (Claverie 2005), revealing that a substantial fraction of non-genetic DNA is in fact transcribed

into RNA, with a possibility of an unknown function. Evolutionary conservation studies also indicate a greater degree of conservation across species than can be explained by the protein-coding regions (Waterston et al. 2002).

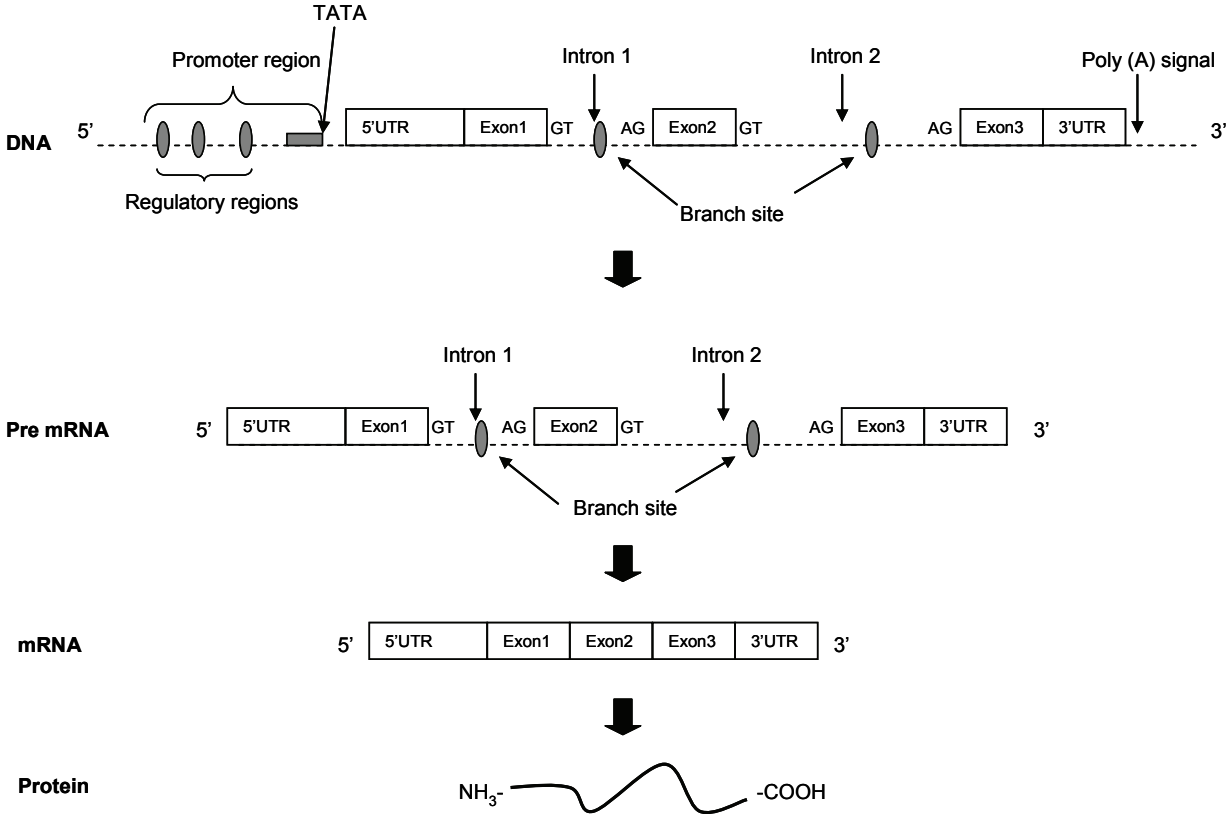


Figure 2. The central dogma of molecular biology. DNA is transcribed into a pre mRNA which is then spliced into an mRNA. The mRNA is then translated into a protein. The DNA picture represents the different parts of a gene.

The most common variations in the human genome are the single nucleotide polymorphisms (SNPs). Most analyses estimate that SNPs occur on average every 1/100 to 1/1,000 base pairs (bp) in the euchromatic human genome, although they do not occur in uniform density. Other variations include repeated DNA sequences, insertions and deletions. Variations can arise as a result of certain drugs and ultraviolet (UV) radiation but the major source comes from spontaneous errors in DNA replication and repair. When a DNA variation is known to cause a pathogenic phenotype it is called a mutation. When a disease is caused by a mutation in only one gene it is said to be transmitted via Mendelian inheritance, whereas if a disease requires the contribution of several genes and possibly also environmental factors, it is called a complex disease with multifactorial inheritance.

Mendelian inheritance

In Mendelian inheritance, a child receives one of two possible alleles for a trait from each parent. A Mendelian trait is controlled by a single locus and exhibits a simple Mendelian inheritance pattern. Mendelian traits may be determined by loci on an autosome or on the X or Y sex chromosomes. Autosomal characteristics in both sexes and X-linked characteristics in females can be dominant or recessive. This results in five basic Mendelian pedigree patterns, i.e. autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive and Y-linked. Over the past decade, about 1,200 genes underlying Mendelian traits have been identified (Botstein et al. 2003). Classic examples of successful positional cloning include hemochromatosis (Feder et al. 1996), nail patella syndrome (Dreyer et al. 1998) and lactose intolerance (Enattah et al. 2002).

Inheritance of a complex disease

Nonmendelian characteristics may be dependent on two, three or many genetic loci, with greater or smaller contributions from environmental factors. Risch's method (Risch 1990) is one way to determine the inheritance of multifactorial diseases and to ascertain whether one or more genes are involved. The risk ratio, λ_R , is defined as the risk of disease in a relative of degree R, in relation to the population prevalence. Risch demonstrated that for a single gene model, $\lambda_R - 1$ decreases by a factor of two with each degree of relationship. In contrast, if there are more genes that must interact to develop a disease, $\lambda_R - 1$ decreases by more than a factor of two with each degree of relationship. Comparing the concordance of the disease in monozygotic (MZ) and dizygotic (DZ) twins is another way of determining genetic inheritance of a complex disease.

Although genetic factors are the cause of many complex diseases, they are much more difficult to identify than the genetic factors in Mendelian diseases, because of the genetic heterogeneity they display. The fact that each contributing locus is neither necessary nor sufficient for a specific phenotype causes a weak relationship between the genotype and the disease phenotype. As a result, the genetic marker used to detect the phenotype will also have a weak relationship with the phenotype and will hence be difficult to detect.

PSORIASIS / PSORIATIC ARTHRITIS

Psoriasis and psoriatic arthritis (PsA) are interrelated disorders, as can be confirmed by several observations. Patients with PsA also have psoriasis. A study performed by Moll and Wright in 1973 shows a 19-fold increase in psoriasis prevalence among first-degree relatives of probands with PsA, compared with the general population. Genome-wide studies have also detected overlapping regions of significance for these two disorders within and outside the major histocompatibility complex (MHC) region.

Epidemiology

Psoriasis is a common skin disease occurring worldwide. Prevalence varies with race and geographical location. The highest prevalence is seen in the northernmost regions of Russia and Norway (5-10 % of the population) (Bhalerao et al. 1998). Scandinavia, Northern Europe, the United States and Australia have prevalences of 2-3 % and the lowest prevalences have been reported in Asia as well as among aboriginal Australians, Native American Indians and West Africans (0-0.3 %) (Lomholt 1963; Hellgren 1967; Farber et al. 1974; Green 1984; Yip 1984; Krueger et al. 1994; Leder et al. 1997; Gladman et al. 2005).

The estimated prevalence of PsA has varied widely. Different studies have reported that between 6 and 42 % of psoriasis patients will develop PsA (Gladman 1998). A study from Sweden suggests that PsA occurs in 30 % of patients with psoriasis (Zachariae 2003).

Two types of psoriasis have been described, based on age at onset. The early-onset type (type I) has a peak onset between age 20 and 30. This group has a strong family history of psoriasis, tends to suffer a more severe course and type I is reported to be associated to human leukocyte antigen (HLA). The later-onset type (type II) develops after age 40; peak onset is between age 50 and 60. This type has no HLA association, is more sporadic and usually milder (Henseler et al. 1985). Type I psoriasis is the most common; onset occurs before age 40 in 75 % of all psoriasis patients (Gladman et al. 2005).

All PsA patients must, by definition, also have psoriasis. The most common scenario is onset of psoriasis about 10 years before PsA, but the arthritis may also precede the psoriasis by many years (Gladman et al. 2005). Males and females are equally affected by both psoriasis and PsA (Gladman et al. 2005). Interestingly, a parental gender effect has been demonstrated in both psoriasis and PsA; more probands have an affected father than an affected mother (Burden et al. 1998; Rahman et al. 1999).

Clinical features of psoriasis

Psoriasis vulgaris is usually identified by the clinical appearance of characteristic red, raised, scaly skin lesions which are usually very well circumscribed, forming a psoriatic plaque (Figure 3). The redness is explained by impressive growth and dilation of superficial blood vessels. The epidermis in a psoriatic lesion is thicker than that in normal skin. The epidermal rete is elongated due to abnormal proliferation of epidermal keratinocytes. This pattern is known as psoriasiform hyperplasia. The characteristic scales in psoriatic lesions are formed by the rapid maturation and hyperproliferation of epidermal keratinocytes. The epidermal cell cycle in psoriatic skin is more than ten times shorter than that in normal skin. While a normal keratinocyte usually lives for 4-6 weeks, a psoriatic keratinocyte only lasts a few days (Liu et al. 2007). This short cell cycle results in incomplete differentiation with aberrantly retained intact nuclei (parakeratosis) and the release of fewer of the extracellular lipids that normally cement their adhesion (Bowcock et al. 2005; Krueger et al. 2005). There is also inflammation in the epidermis. Lesions are rich in activated CD4+ and CD8+ T cells that release proinflammatory cytokines and are typically distributed symmetrically on the scalp, elbows, knees and lumbosacral area.



Figure 3. Photograph of an arm covered with plaque psoriasis

Alternatively to this classic presentation, psoriasis can be highly variable in morphology, distribution, severity and cause. It is therefore divided into different types, the most common of which is *plaque psoriasis* or *psoriasis vulgaris*, described above and accounting for 80-90 % of all cases. *Guttate psoriasis*, from the Greek word “gutta” meaning droplet, is characterized by the acute onset of a myriad of small psoriatic lesions, 1-10 mm in diameter. They are usually distributed on the trunk and proximally on the extremities but can also occur on the head. Classically, guttate psoriasis occurs 1-2 weeks after a streptococcal infection of the pharynx or tonsils (Telfer et al. 1992) and predominantly affects children and young adults. It may arise on its own or may complicate existing, often quite limited, chronic plaque psoriasis. If left untreated, guttate psoriasis may clear spontaneously or may develop into chronic plaque psoriasis. *Inverse psoriasis* is morphologically distinct from traditional plaques. It affects the flexures, particularly the armpits, the groin and under the breasts. Flexural lesions are devoid of scales and appear as red, shiny, well demarcated plaques. *Erythroderma* is a scaling,

itching, inflammatory process that involves all or almost all of the body surface. It may either arise from chronic plaque which progresses, becoming confluent and extensive, or it may be a manifestation of unstable psoriasis precipitated by infection, drugs, stress or withdrawal of corticosteroids. Erythrodermic psoriasis can impair

thermoregulation of the skin, leading to hypothermia; it can change metabolism due to loss of keratin, iron and folic acid during the profuse scaling and it can cause edema, especially around the ankles. Some complications can also be life-threatening, e.g. infection, pneumonia and cardiac failure. Patients suffering an erythrodermic psoriasis flare should contact a doctor immediately; severe cases often require hospitalization. *Pustular psoriasis* is characterized by white, sterile pustules surrounded by red skin. It tends to follow a cycle—reddening of the skin followed by formation of pustules and scaling. The pus consists of white blood cells. There are two types of pustular psoriasis, generalised pustular psoriasis (von Zumbusch) and palm-plantar pustulosis (PPP). Generalised pustular psoriasis is rare and represents active, unstable disease. This form is very widespread and the eruptions often occur in repeated waves lasting days or weeks. It appears very rarely in children, although when it does, the prospect of improvement may be much better than for adults. Generalized psoriasis is associated with fever, chills, severe itching, dehydration, a rapid pulse, exhaustion, anemia, weight loss and muscle weakness. Since this form can be life-threatening, immediate medical care is required. PPP causes pustules on the palms and soles; it is uncertain whether it really is a form of psoriasis (Asumalahti et al. 2003). Psoriasis of the nails (also called *psoriatic nail disease*) is seen in 40–45 % of skin psoriasis patients (Gladman et al. 1986). The commonest finding is small pits in the nail plate. The nail may also detach from the bed at its distal or lateral attachments, known as onycholysis. Psoriatic nails also often exhibit yellow-brown discoloration and are deformed and thickened. Psoriatic nail disease is even more often associated with PsA, which will be discussed in the next section.

Clinical features of PsA

PsA is defined as a rheumatoid factor-negative inflammatory arthritis in the presence of psoriasis (Gladman 1998), usually preceded by psoriasis by about 10 years. Moll and Wright characterized five subtypes of psoriasis in their original case series in 1973 (Moll et al. 1973). The mildest form of psoriatic arthritis is called asymmetric psoriatic arthritis. One to three joints in the hip, knee, ankle or wrist are generally involved, often leading to tenderness and redness. When asymmetric arthritis occurs in the hands and feet, swelling and inflammation in the tendons can cause the fingers and toes to resemble small sausages (dactylitis). Symmetric psoriatic arthritis usually affects four or more of the same joints bilaterally. Psoriasis associated with this condition tends to be severe. Distal interphalangeal (DIP) joint PsA affects the small joints closest to the nails (distal joints) in the fingers and toes. Spondylitis can cause inflammation of the spine as well as stiffness and inflammation in the neck, lower back or sacroiliac joints. Inflammation can also occur where ligaments and tendons attach to the spine. As the disease progresses, movement tends to become increasingly painful and difficult. A small percentage of people with psoriatic arthritis have arthritis mutilans — a severe, painful and disabling form of the disease. Over time, arthritis mutilans destroys the small bones in the hands, especially the fingers, leading to permanent deformity and disability.

Triggering factors

Psoriasis or psoriatic flares are known to be triggered by several environmental factors. The Koebner reaction, i.e. the appearance of a psoriasis lesion resulting from and located at the site of injury to the epidermis, was first described in 1872 (Koebner 1872). This reaction can, for example, be triggered by tape stripping. Bacterial infections can also induce psoriasis (Leung et al. 1995; Baker et al. 1997; Skov et al. 2000). Streptococcal throat infections frequently precede outbreaks of guttate psoriasis which can in turn lead to chronic plaque psoriasis. Psoriasis can also be triggered by drugs such as lithium and beta-blockers or by rapid withdrawal of immunosuppressive drugs such as corticosteroids (Barisic-Drusko et al. 2004; Dika et al. 2007). Stress, smoking and alcohol are common environmental factors inducing psoriasis (Ockenfels 2003). HIV infections and UV light have been shown to exacerbate psoriasis (Ros et al. 1987; Obuch et al. 1992; Mallon et al. 1998), although UV light has a clear beneficial effect in the majority of cases and is often used as a treatment (see the Treatment section).

Treatment

There is currently no cure for psoriasis or PsA. Currently available treatments suppress rather than modify the disease. A realistic goal of psoriasis treatment is reduction of the disease to a manageable level, with minimal toxicity from treatment, rather than complete remission.

Three therapeutic modalities can be used, alone or in combination: topical agents, appropriate wavelengths of UV radiation and systemic medications. Choice of treatment depends on a number of factors, including the nature and extent of the disease as well as quality of life, anatomical location, coexistent PsA, triggering factors and the patient's commitment to therapy. If less than 5 % of the body's surface area is involved, the psoriasis is defined as mild (Menter et al. 2007); this form affects some 75-80 % of individuals. Moderate psoriasis affects 5-10 %, and severe psoriasis affects more than 10 %, of the body's surface (Menter et al. 2007). About 20-25 % of patients have moderate to severe psoriasis.

Topical therapy

Topical treatments are usually the first line in psoriasis treatment, especially in cases with limited disease. Although effective for individual plaques, it is time-consuming and compliance is a substantial issue.

Corticosteroid therapy is the most frequently used treatment for psoriasis, entailing rapid effects as well as drawbacks such as loss of efficacy, with recurrence of disease and skin atrophy. Extensive treatment with potent corticosteroids can also cause systemic effects such as iatrogenic Cushing's syndrome and hypothalamic-pituitary-adrenal axis suppression (Bruner et al. 2003).

Vitamin D₃ derivatives have become the first-line therapy for plaque psoriasis. They lack the risks associated with corticosteroids but have slow onset of action and cause skin irritation in about 20-25 % of users. Other therapeutically active topical agents licensed for psoriasis include coal tar, dithranol and tazarotene (a retinoid).

Phototherapy

For moderate psoriasis or when topical therapy is inadequate, treatment is usually combined with phototherapy. About 80 % of the patients respond positively to light therapy, 15 % do not respond and 5 % react with exacerbation. The most common forms of phototherapy are ultraviolet B (UVB) and Psoralen plus ultraviolet A (PUVA). The presumed mechanism of action of both UVB and PUVA is modulation of the expression of cellular adhesion molecules and induction of T cell apoptosis (Krutmann 1998). Both treatments have proven extremely effective for psoriasis but premature ageing of the skin and increased risk of skin cancers are side effects.

Systemic treatments

Systemic treatment is administered to patients with moderate to severe psoriasis as well as to those unresponsive to topical agents or phototherapy and those with associated psoriatic arthritis or significant quality of life issues. The most common systemic drugs are methotrexate, acitretin and ciclosporin. Methotrexate is a folic acid antagonist that interferes with purine synthesis and thus inhibits DNA synthesis and cell replication. It has also a specific T cell-suppressive activity. It is, however, teratogenic and can cause severe side effects such as bone marrow suppression and liver fibrosis. Synthetic hormones such as acitretin act by normalizing keratinocyte proliferation and differentiation by binding to retinoid receptors, thereby altering gene transcription. They too are teratogenic and can cause mucocutaneous side effects resembling hypervitaminosis A, hyperlipidemia, osteoporosis and skeletal abnormalities. Ciclosporin blocks the intracellular components of T cell activation by binding to a cytosolic immunophilin and affects epidermal keratinocytes (Santini et al. 2001). Treatment with ciclosporin requires careful monitoring for nephrotoxicity and hypertension (Lowe et al. 1996; Zachariae et al. 1997).

Biological agents

For severe psoriasis, we now have biological therapies which have been approved as recently as during the past 3 years. Unlike earlier treatments for psoriasis, biological agents are proteins or antibodies that target specific molecules thought to be essential in psoriasis pathogenesis. These drugs cannot be administered orally and must be injected. There are two groups of biological agents, T-cell agents, including Alefacept and Efalizumab, and TNF α inhibitors, including Etanercept, Infliximab and Adalimumab., They have been well tolerated in clinical practice but the main concern is long-term chronic immunosuppression which may facilitate infection and increase the risk of cancer. The high cost of these treatments, compared with traditional systemic therapy, is another issue.

Psoriasis as an autoimmune disease

Autoimmune diseases are characterized by the presence of autoantibodies and/or autoreactive T cells to a specific antigen within a target organ (Eisenberg 2003). Psoriasis is considered to be a T lymphocyte-mediated autoimmune disease, although no epidermal autoantigens have been identified (Horrocks et al. 1997). The classification stems most directly from the positive effects of immune antagonists in clinical studies (Bowcock et al. 2005). However, studies of human skin xenografts in mice (Nickoloff et al. 2000) and the identification of clonal populations of T cells within intact skin lesions (Prinz et al. 1999; Vollmer et al. 2001) strongly support the hypothesis of immune-mediated pathogenesis.

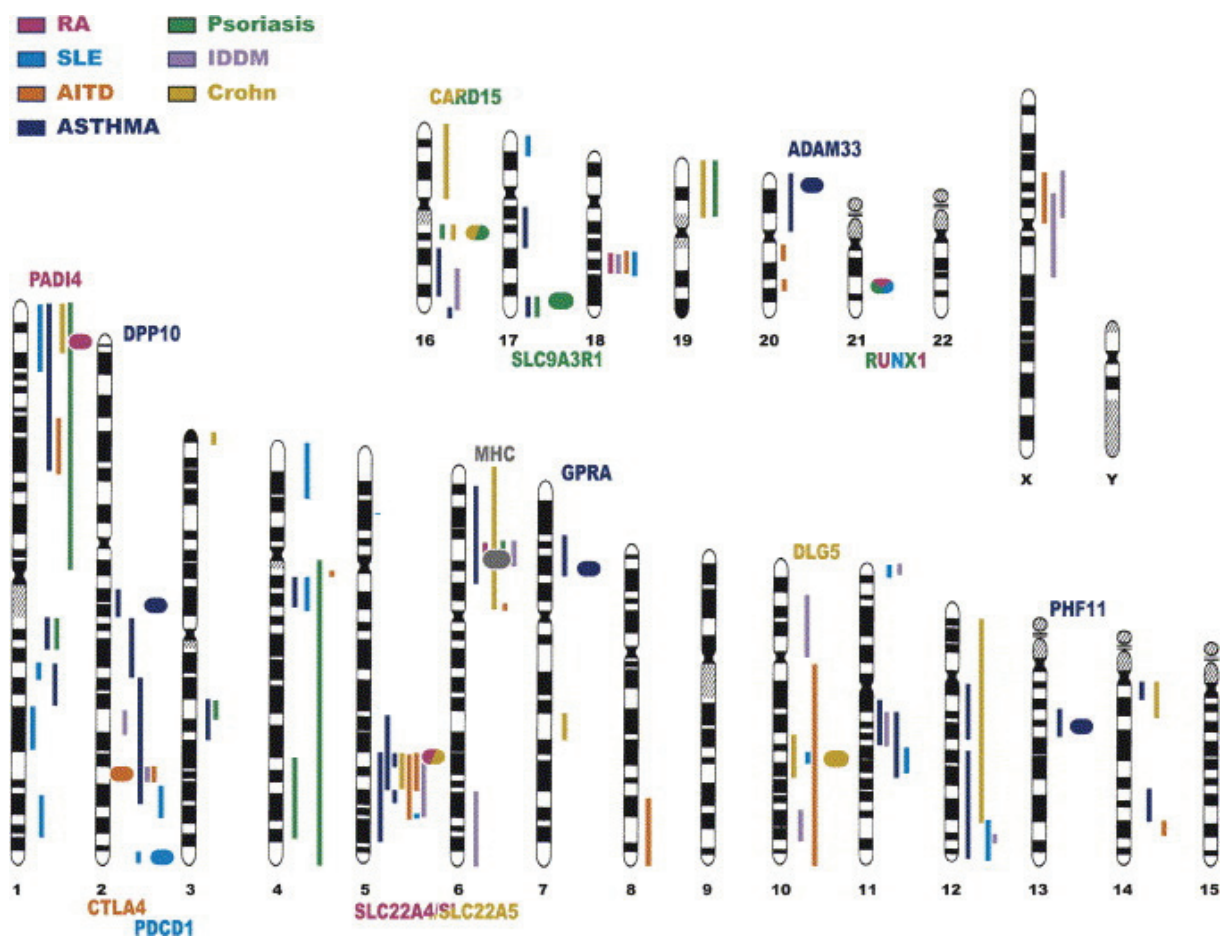


Figure 4. Whole genome map of inflammatory disease-related loci and genes. Lines parallel to chromosomal karyotypes represent linked loci. Eclipses are disease-associated genes. Insulin-dependent diabetes mellitus (IDDM), autoimmune thyroid diseases (AITD) (Yamada et al. 2005).

The MHC is a large genomic region or gene family that has a significant function in the immune system and autoimmunity. The best-known genes in the MHC region are the subset encoding cell surface antigen-presenting proteins, referred to as human leukocyte antigen (HLA) genes in humans. Their important role in autoimmune diseases has been repeatedly confirmed by the localization of

autoimmune loci to the MHC region and by the genetic risk conferred by the different HLA class I and class II alleles. In the case of psoriasis, many researchers have shown that a major locus lies within the HLA class I region. Although MHC is the most consistently overlapping locus in autoimmune disease, other loci also show a substantial degree of overlap. Figure 4 shows the disease-related loci and genes for 7 inflammatory diseases. Five genes (HLA, RUNX1, CARD15, SLC22A4/5) have been associated with four autoimmune diseases (psoriasis, (SLE), rheumatoid arthritis (RA), Crohn's disease (CD)) in a pattern displayed in Figure 5. RUNX proteins are transcription factors or repressors for various target genes. RUNX1 is expressed in all hematopoietic lineages and is known to regulate the expression of genes specific for hematopoiesis and myeloid differentiation. SLE, psoriasis and RA have reported associated SNPs that disrupt the RUNX1 binding sites in PDCD1 (Prokunina et al. 2002), the intergenic region between SLC9A3R1 and NAT9 (Helms et al. 2003), and SLC22A4 (Tokuhiko et al. 2003), respectively. SLC22A4 and SLC22A5 are organic cation transporters that have been associated with both RA (Tokuhiko et al. 2003) and CD (Peltekova et al. 2004). CARD15 is suggested to act as an intracellular receptor for bacterial products in monocytes and to transmit signals via activation of NF- κ B. It was initially associated with CD (Hugot et al. 2001; Ogura et al. 2001) but was later also shown to be associated with PsA (Rahman et al. 2003). Another connection between psoriasis and CD is the increased prevalence of psoriasis among CD patients (Yates et al. 1982; Lee et al. 1990). All these results suggest that there are pleiotropic autoimmune and/or inflammatory genes that confer susceptibility to more than one autoimmune/inflammatory disease.

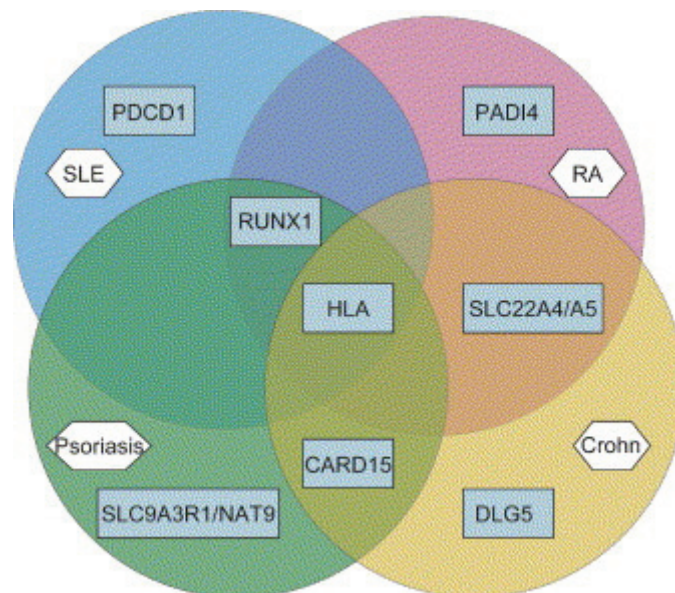


Figure 5. Four autoimmune diseases are mutually connected by five genes: HLA, RUNX1, SLC22A4/A5 and CARD15 (Yamada et al. 2005).

Immunology of psoriasis

Like many other autoimmune/inflammatory diseases, psoriasis is driven and maintained by multiple components of the immune system, which consists of an innate and an adaptive part. It is the innate system that responds first to an infection. Macrophages, neutrophils, dendritic cells, natural killer cells, mast cells, eosinophils and basophils are among the cells of the innate system. They recognize and respond to pathogens in a non-specific manner. This system is present from birth but has no memory and hence does not confer long-lasting or protective immunity to the host. Adaptive immunity is, in contrast, a highly specialized system, the major functions of which are to recognize non-self antigens, eliminate specific pathogens or pathogen-infected cells and develop an immunologic memory. It is highly diverse, takes longer to develop and is mainly mediated by B and T lymphocytes. In fully developed psoriatic skin lesions, there is an admixture of innate immune cells, T cells and pro-inflammatory cytokines and chemokines. B lymphocytes are the major cells involved in the creation of antibodies. There are two main types of T cells, helper T cells (CD4+) and cytotoxic T cells (CD8+)(Gaspari 2006).

The immunopathogenesis of psoriasis is initiated when an antigen-presenting cell (APC) from the epidermis or dermis captures an antigen and becomes activated. As described above, the antigen specificity for psoriasis has not yet been identified. However, different antigens have been proposed, including self polypeptides such as keratin 13 (K13) (Sigmundsdottir et al. 1997) and heterogeneous nuclear ribonucleoprotein-A1 (hnRNP-A1) (Jones et al. 2004), and microbial agents, such as streptococcal M protein (Sigmundsdottir et al. 1997), retrovirus (HIV) (Mahoney et al. 1991), human papillomavirus (Favre et al. 1998) and microbial superantigens (a group of bacterial and viral proteins characterized by their capacity to stimulate a large number of T cells simultaneously) (Olsen et al. 1999)) (Baker et al. 1993; Rosenberg et al. 1994; Valdimarsson et al. 1995; Nickoloff et al. 1998).

A molecular mimicry hypothesis exists in which bacterial antigens and skin determinants have similar epitopes, causing the immune system to cross-react and induce an immune reaction to the self peptide, creating an autoantigen (Christen et al. 2004).

The activated APCs travel to the peripheral lymph nodes where they activate naïve CD4 or CD8 T cells. Activation includes the presentation of the antigen to the T cell receptor (TCR) on the T cell. (Intracellular antigens are presented by a MHC I molecule and extracellular antigens are presented by a MHC II molecule on the APC.) The second step in the activation process is a variety of non-antigen-specific costimulatory signals mediated, for example, by CD86, CD80, CD40, LFA-3 and CD54 on the APC. As T cell activation occurs, two distinct lines of differentiation are possible. T helper 1 (Th1) cells develop under the influence of IL-12 and IFN- γ , participate in cell-mediated immunity and are responsible for controlling intracellular pathogens such as viruses. They secrete type 1 cytokines, including IL-2, TNF- α and IFN- γ (Austin et al. 1999; Bonifati et al. 1999; Nishibu et al. 1999). T helper 2 (Th2) cells develop under the influence of IL-4, IL-6 and IL-10. They help B cells and are thus important for antibody-mediated immunity, required to control extracellular

pathogens such as bacteria. Th2 cells secrete type 2 cytokines, including IL-4, IL-6, IL-10, and IL-11.

Psoriasis is considered to be a type 1 disease, characterized by type 1 cytokines and a predominance of CD4 T cells in the dermis and CD8 cells in the epidermis (Uyemura et al. 1993; Schlaak et al. 1994). These are released at the site of inflammation in the skin, aided by the activated T cell expressing a new surface protein known as CLA. CLA helps the T cell to tether to the endothelium in the cutaneous postcapillary venules by interacting with E-selectin and P-selectin which are overexpressed on cutaneous microvessels during inflammation (Picker et al. 1991; Fuhlbrigge et al. 1997). The T cells can finally enter the skin after binding to the ICAM-1 and VCAM-1 on the blood vessels with LFA-1 and VLA-4 integrins (Lee et al. 2006). The released cytokines from the migrating epidermal lymphocytes disrupt the basement membrane and desmosome connections between adjacent keratinocytes. The net result is increased proliferation of keratinocytes manifested by the elongation of rete ridges, loss of granular layer, parakeratosis and endothelial hyperproliferation (Krueger 2002; Lebwohl 2003).

Genetics of psoriasis

It has been known for a long time that there is familial occurrence of psoriasis. In his classic epidemiological study of psoriasis among 10 000 inhabitants of the Faroe Islands, Lomholt observed that the incidence of psoriasis was much greater among first- and second-degree relatives of sufferers than among unaffected control subjects (Lomholt 1963). Comparing disease concordance in MZ twins (which have identical genomes) and DZ twins (which only share half of their genomes) is another method of investigating the heredity and penetrance of a disease. Based on twin and family studies (Elder et al. 1994), the heritability for psoriasis, h^2 (the proportion of phenotypic variation of a trait attributable to genetic variability), has been estimated at between 60 % and 90 %, among the highest for all multifactorial genetic disorders (Elder et al. 2001). The concordance of psoriasis in MZ twins is much higher than in DZ twins, indicating a strong genetic component to the disease. Danish twin registry studies reveal that MZ twins have a concordance of 72 %, compared with 15 % in DZ twins (Brandrup et al. 1978). Similarly, Faber et al. observed concordance rates of 70 % in MZ twins and 23 % in DZ twins in the USA (Farber et al. 1974). However, in an Australian study, the concordance rate was only 36 % in MZ twins and 12 % in DZ twins (Duffy et al. 1993). Concordant MZ twins are also very similar regarding age at onset, anatomical distribution, severity and course (Farber et al. 1974). However, concordance in MZ twins never reaches 100 %, which implies that environmental factors participate in the triggering of psoriasis. Over the years, several models for the inheritance of psoriasis have been proposed. Some of the first studies suggested a dominant mode of inheritance with incomplete penetrance (Abele et al. 1963). Today, common belief is that psoriasis follows a multifactorial mode of inheritance. The genetic contribution to psoriasis has been extensively described and at least 20 different psoriasis susceptibility loci have been identified (Bowcock et al. 2004). Nine of these regions have been confirmed in other populations or have yielded strong enough results to be designated as psoriasis susceptibility loci (PSORS1-9).

PSORS1

The psoriasis susceptibility 1 (*PSORS1*) locus on chromosome 6p21 is the most consistently replicated of all loci. It is estimated to account for 30-50 % of the genetic contribution to psoriasis (Trembath et al. 1997) and is located within the MHC. The best-known genes in the MHC region are the subset that encodes cell-surface antigen-presenting proteins, HLA. Strong association of HLA-Cw6 with psoriasis was reported as early as over 26 years ago (Tiilikainen et al. 1980). This association is particularly strong in patients with a young age at onset (Henseler et al. 1985). However, proof of this association is still lacking, due to the extensive linkage disequilibrium (LD) across the class I region, resulting in the existence of particularly strong extended haplotypes (Walsh et al. 2003). It is thus unclear whether this allele is the predisposing psoriasis gene or simply a marker in strong LD with the true disease gene (Jenisch et al. 1999; Nair et al. 2000; Veal et al. 2002; Gudjonsson et al. 2003). The search for an alternative to HLA-Cw6 in the *PSORS1* region has led to the characterization of the corneodesmosin (*CDSN*) and α -helical coiled-coil rod homolog (*HCR*) genes. They are located about 160 kilobases telomeric to HLA-C and both have alleles which are associated with psoriasis (*CDSN**TTC and *HCR**WWCC) (Allen et al. 1999; Tazi Ahnini et al. 1999; Asumalahti et al. 2000; Asumalahti et al. 2002; Veal et al. 2002). They have also been shown to be differently expressed in lesional psoriatic skin than in normal skin (Allen et al. 2001; Suomela et al. 2003). The predicted structure of the risk allele of *HCR* protein differs from the wild-type allele in that it has a shorter first alpha-helical domain, which can affect the antigenicity of the protein (Asumalahti et al. 2002). The *CDSN* gene product is expressed in terminally differentiated keratinocytes and localizes to the modified desmosomes that ensure intercellular cohesion of keratinocytes. The serine- and glycine-rich terminal domains of *CDSN*, essential for cell adhesion, are sequentially cleaved during skin desquamation (Simon et al. 2001; Jonca et al. 2002). Strong LD between these three genes has made it difficult to distinguish their individual genetic effects. To overcome this challenge, sufficient numbers of subjects are analyzed to find individuals carrying only portions of the ancestral *PSORS1* risk haplotype. Results of these studies indicate that the location of *PSORS1* is closer to the HLA-C/HLA-B region, excluding *CDSN* and *HCR*. (Helms et al. 2005; Nair et al. 2006).

However, since the penetrance of the strongest associating allele at this locus, HLA-Cw*0602 (Nair et al. 2006), is only about 10 % it is evident that other genetic variants or environmental effects are necessary (Elder et al. 2001; Bowcock et al. 2004).

PSORS2

This locus, on chromosome 17q25, was the first non-MHC locus found to confer susceptibility to psoriasis (Tomfohrde et al. 1994). This linkage has also been confirmed by several other groups (Matthews et al. 1996; Nair et al. 1997; Enlund et al. 1999). There are two distinct loci within *PSORS2*.

One locus includes the solute-carrier family 9 isoform 3 regulator 1 (SLC9A3R1) and *N*-acetyltransferase 9 (NAT9) genes, both of which encode proteins that play a role in negatively regulating immune cell activation. A common haplotype that carries SLC9A3R1 and NAT9 is associated with susceptibility to psoriasis (Helms et al. 2003). One psoriasis-associated allele from this five-marker haplotype leads to loss of a putative site for the RUNX family of transcription factors. This is interesting as RUNX1 and RUNX3 play a major role in hematopoietic development and thymic selection.

The second, more distal, locus within PSORS2 encodes regulatory associated protein of mammalian target of rapamycin (RAPTOR). RAPTOR is a serine/threonine protein kinase that regulates cell growth and proliferation in response to environmental stimuli such as growth factors, mitogens or cytokines. It is a target for immunosuppressive drugs. Intronic SNPs have been shown to be associated with psoriasis and are therefore likely to be regulatory (Helms et al. 2003; Capon et al. 2004).

PSORS3

Using parametric linkage analyses, Matthews et al found evidence of linkage to 4q in a single large multiplex psoriasis family. The maximum total pairwise LOD score obtained with the microsatellite marker D4S1535 at $\theta = 0.08$ was 3.03 (Matthews et al. 1996). The human interferon regulatory factor 2 (IRF2) gene is located within this locus. Association of IRF2 with type 1 psoriasis was detected for two markers in the IRF2 gene (Foerster et al. 2004). Hypersensitivity to type 1 interferon signaling causes a psoriasis-like skin disease in IRF2-deficient mice.

PSORS4

A linkage study of Italian families detected a putative linkage to chromosome 1cen-q21. The highest two-point LOD score was obtained with D1S305 marker (3.75 at $\theta = 0.05$) (Capon et al. 1999). This region is of interest because it contains the so-called epidermal differentiation complex, a cluster of at least 20 genes expressed during epithelial differentiation. Fine mapping of this region localized the susceptibility gene to the genomic interval spanned by D1S2346 and 140J1D (Capon et al. 2001). Further refinement of this locus revealed a 100 kb region containing only the loricrine (LOR) gene. However, as no association could be detected, the gene was ruled out as a candidate gene for the PSORS4 locus (Giardina et al. 2004). Later studies have also reported involvement of other genes within this region, e.g. S100A8, S100A9, PGLYRP3 and PGLYRP4 (Zenz et al. 2005; Sun et al. 2006).

PSORS5

This region on 3q21 was identified in a linkage study of Swedish families. The strongest linkage results were seen when only families originating from the southwestern part of Sweden were included in the study. The localization was confirmed by an association study using an independent replication cohort of single affected families in which at least one parent originated from southwestern Sweden (Enlund et al. 1999). A genome-wide scan yielded a non-parametric linkage (NPL)

value of 2.64 for marker D3S1551 when the families were stratified with regard to arthritic symptoms (Samuelsson et al. 1999). When this region was fine-mapped using the transmission disequilibrium test (TDT), three SNPs showed significant association with disease. The SLC12A8 gene was found within this region and an association was found with a five-marker haplotype spanning the 3' half of this gene (Hewett et al. 2002). Association with SLC12A8 has also been confirmed in a German study (Huffmeier et al. 2005).

PSORS6

A locus on chromosome 19p13 was identified by a linkage analysis of 32 German extended families (Lee et al. 2000). An association scan of this region revealed both a protective and a susceptibility locus (Hensen et al. 2003). JunB, a gene localized within PSORS6, has been reported to exhibit decreased expression in epidermal keratinocytes in psoriatic lesions (Zenz et al. 2005). It has also been shown that 100 % of JunB/c-Jun double-mutant mice have a psoriasis-like phenotype (Zenz et al. 2005).

PSORS7

A novel locus at 1p35-p34 was identified in a genome-wide linkage analysis by Veal et al (2001). The EPS15 gene was pointed out by the markers that contributed to the NPL score. This gene was previously known to be overexpressed in psoriatic epidermis (Veal et al. 2001).

PSORS8

In 1997, Nair et al found suggestive linkage to a region on chromosome 16p (Nair et al. 1997). This linkage was also indicated in a subsequent analysis of affected sibling pairs (ASPs) by the International Psoriasis Genetics Consortium, especially when analyzing only those families carrying either of two psoriasis-associated MHC haplotypes (2003). This locus has also been implicated in the paternal transmission of PsA (Karason et al. 2003).

PSORS9

This locus is situated proximal to the PSORS3 locus on distal chromosome 4q. It was suggested as a psoriasis locus by a genome-wide scan in the Chinese Han population (Zhang et al. 2002; Yan et al. 2007). This region was also recorded in a meta-analysis combining the results of six genome-wide studies (Sagoo et al. 2004). Within this region, IL-15 has long been recognized as a strong candidate gene for psoriasis. Highly significant evidence of association was identified at the 3'-untranslated region (UTR) of the IL-15 gene. It was also demonstrated that the identified risk haplotype is associated with increased IL-15 activity (Zhang et al. 2007). Recently, a psoriasis patient with a balanced translocation disrupting the microsomal glutathione S-transferase 2 (MGST2) within this region was reported (Tzschach et al. 2006). Interestingly, a novel non-synonymous mutation in MGST2 has been seen in one Chinese family with psoriasis (Yan et al. 2006).

Genetics of psoriatic arthritis

Evidence for a strong genetic contribution in PsA comes from family studies (Moll et al. 1973; Gladman et al. 2003). Just as in psoriasis, association in PsA has also been found with the HLA loci on chromosome 6. The strongest associated allele in psoriasis, Cw6, is reported to be more strongly associated with psoriasis than with PsA. The situation in PsA is considerably more complex and genetic studies have shown associations with several HLA antigens including HLA-B13, B17, -b27, B38, B39, Cw6, DR4, DR7 and DQ3 (Murray et al. 1980; Gladman et al. 1986; McHugh et al. 1987; Salvarani et al. 1989; Torre Alonso et al. 1991). Association with PsA has also been found with the MICA-A9 triplet repeat polymorphism and with polymorphisms in the TNF- α region (Gonzalez et al. 2001; Hohler et al. 2002).

To the best of our knowledge only one genome-wide scan has been completed in PsA (Karason et al. 2003). This study was performed on 39 Icelandic families using 1000 microsatellite markers. A LOD score of 2.17 was reported on chromosome 16q and when conditioning the analysis on paternal transmission, the LOD score increased to 4.19. Addition of markers to this region further increased the LOD score to 5.69 also when analysis was conditioned on paternal inheritance (Karason et al. 2005). The susceptibility gene for Crohn's disease, CARD15 (Hugot et al. 2001; Ogura et al. 2001), overlaps with this region. Association with this gene has also been reported in a study on PsA patients (Rahman et al. 2003), although other studies have failed to confirm this association (Giardina et al. 2004; Lascorz et al. 2005).

OBJECTIVES

The comprehensive goal of this thesis was to identify chromosomal regions and genes involved in the development and progression of the inflammatory diseases psoriasis and psoriatic arthritis.

SPECIFIC AIMS

Paper I

The aim of this study was to analyze the association of several autoimmune disease susceptibility loci in patients from northern Sweden with psoriatic arthritis.

Paper II

The aim of this study was to investigate whether two candidate genes located within the PSORS5 region, CSTA and ZNF148, harbor the genetic cause of psoriasis at this locus.

Paper III

In this study we focused on a region on chromosome 5q previously reported to be a susceptibility locus in our genome-wide scan. The ambition was to refine the linkage analysis with a denser set of microsatellite markers. This study also aimed at performing an association analysis on three SNPs with reported functional activity in RA and CD, located within the genes SLC22A4 and SLC22A5.

Paper IV

The goal of this study was to investigate whether the SLC12A8 gene is differently expressed in skin biopsies from psoriatic plaque than in normal skin from a person without psoriasis and to find genetic variations within this gene that might be the functional variants contributing to psoriasis susceptibility within this population.

MATERIAL AND METHODS

SUBJECTS

Background of the psoriasis families

The psoriasis project started in 1992 in collaboration with the Swedish Psoriasis Association. In order to perform a population genetic study on a Swedish psoriasis data set, 22 000 questionnaires were sent to Swedish Psoriasis Association members, inquiring about age at onset and current disease status. Data from 11 366 probands was analyzed. Both parents of about two thirds of the affected probands did not suffer from psoriasis. In these families, the probability of siblings having psoriasis was close to 0.25 (Swanbeck et al. 1994). This data is compatible with a recessive mode of inheritance. Based on this theory, blood samples were only collected from families in which both parents were available and unaffected. Two family data sets were collected. The first data set was collected with the intention of performing a genome-wide linkage analysis; the families thus consisted of two unaffected parents and at least two affected children. To ensure a correct diagnosis of psoriasis, all participants in this study were examined by the same physician (Annica Inerot). The mean age of the affected individuals was 40.2 and the average age at onset was 20. A total of 114 nuclear families (481 individuals) were included in the studies. The other data set was collected as replicate material for association studies and contains 152 trios (456 individuals), with at least one parent born in southwestern Sweden. Unlike the previous data set, these patients were not examined by a dermatologist but were considered to probably have psoriasis, based on their membership in the Swedish Psoriasis Association and self-reported health status.

Paper I

120 patients with inflammatory joint disease and psoriasis or a history of psoriasis, were included in this study based at the outpatient clinic of the Department of Rheumatology at Umeå University Hospital. All patients were diagnosed with psoriasis of the skin by a dermatologist and one had PPP. The mean age of the affected individuals was 47.3, the average age at onset of the skin disease was 26.2, and the mean age at onset of the joint/axial disease was 34.0.

94 controls from the same part of Sweden and with the same ethnic background as the patients were randomly selected from a population register.

Paper II

Eleven healthy blood donors and 11 psoriasis patients from the first family data set were selected for the SNP discovery part of this study. The inclusion criteria were psoriasis with concomitant arthritic symptoms and carriage of the risk haplotype at the PSORS5 locus, previously shown to be associated with psoriasis (Hewett et al. 2002).

In accordance with the hypothesis of a founder mutation from southwestern Sweden causing the genetic susceptibility at the PSORS5 locus, only families originating from this region were selected for the association study. This included the whole family data set 2 and 40 families from family data set 1 with at least one parent born in southwestern Sweden.

Paper III

The linkage analysis was performed on the whole family data set 1. In the analysis, the families were also stratified for arthritic symptoms. The group with arthritic symptoms consisted of 55 families while the group without arthritic symptoms comprised 59 families.

The association analysis was supplemented with the second family data set, consisting of 152 trios, of which 47 had and 52 lacked arthritic symptoms. Arthritic symptom status was unclear in 53 families which were hence not used in the stratified analysis. The stratification criteria for arthritic symptoms were based on self-reporting. The association analysis was performed on a total of 264 families, of which 102 had arthritic symptoms.

Paper IV

Eight affected individuals were selected for the sequencing project, four patients who did not carry the associating haplotype and four who did.

The LD analysis of PSORS5 was performed on the whole family data set 2 and 40 families from family data set 1, in which at least one parent was born in southwestern Sweden.

Gene expression of SLC12A8 was performed on 14 involved skin biopsies from psoriatic patients and 10 control skin biopsies from healthy individuals with no family history of psoriasis. Psoriatic patients were from family set 1 with at least one parent born in southwest Sweden. Selection criteria included linkage to PSORS5 and presence of associating haplotype.

PCR

Polymerase chain reaction (PCR) is a rapid and flexible in vitro method for amplifying defined target DNA sequences within a source of DNA. Since it was invented in 1986 (Mullis et al. 1986), it has become a mainstay of molecular biology. A process previously requiring one to two weeks and entailing isolation of DNA, cloning the DNA into a viral or plasmid vector, growing the cloned DNA using living host cells (usually bacteria) and, finally, isolating the DNA again could now be performed in 2 to 3 hours. To permit such selective amplification, sequence information is needed on the regions flanking the target sequence, enabling the construction of two primer sequences.

Primers are usually 15-25 nucleotides long and require careful construction ensuring specific binding in the right temperature range without the generation of any hairpin or primer-dimer structures. Numerous software programs exist to simplify this procedure, of which we have used DNASTAR (LASERGENE) and Primer Express v. 2.0 (Applied Biosystems). To ensure specificity, each primer was screened against the entire human genome using the BLAST function at NCBI. Primers were ordered from the Invitrogen company.

The principle of PCR is to exponentially amplify target DNA in a series of about 30 cycles, each composed of three steps (Figure 6): (1) denaturation of genomic DNA and amplicons at 94-95 °C; (2) annealing of primers to single-stranded DNA at primer-specific annealing temperatures, usually between 50 and 65 °C; and (3) DNA synthesis by primer elongation at 72 °C. After 30 cycles of exponential DNA synthesis, a discrete band of a specific size can be visualized after the DNA has been stained with the DNA-binding chemical ethidium bromide and undergone agarose gel electrophoresis.

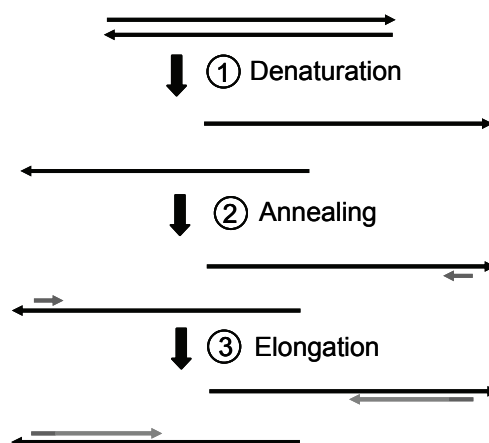


Figure 6. Schematic drawing of the PCR cycle. (1) Denaturing at 94-95 °C. (2) Annealing at 50-65 °C (3) Elongation at 72 °C.

Touchdown PCR

Touchdown PCR is a modification of conventional PCR that may result in higher specificity of the amplification product. It involves the use of a higher annealing temperature for the primers than the optimal temperature in early PCR cycles. The annealing temperature is then successively decreased by 1°C every other cycle until a specified or 'touchdown' annealing temperature is reached, usually starting from 65 °C and ending at 55 °C, over 20 cycles. The touchdown temperature is then used for the remaining (about 25) cycles. This allows for the enrichment of the correct product at the expense of any non-specific product, enables simultaneous amplification of different primer pairs (with different annealing temperatures) and does not require any temperature optimization. We used this technique in Paper IV when sequencing 30 kb of the SLC12A8 gene.

REAL-TIME RT-PCR

Gene expression of SLC12A8 has been measured with the real-time RT-PCR technique using the TaqMan assay. This method measures the relative amount of mRNA of a specific gene within a sample. The aim is to ascertain whether there are any differences in mRNA expression between different samples. In this case, we wanted to see whether SLC12A8 is differently expressed in psoriatic skin than in normal skin from a healthy control individual. The first step in this process is to isolate RNA from skin biopsies. Since human skin is rather tough and hard to homogenize the frozen biopsies were first disrupted by grinding with mortar and pestle. Further disruption and homogenization were performed using a TissueLyser (Qiagen) in Qiazol solution (Qiagen). RNA was then isolated using the RNeasy® Lipid Tissue Mini Kit (Qiagen).

The “RT” in RT-PCR stands for the reverse transcription of RNA into cDNA, which is the next step in the procedure. This step must be optimized for the amount of RNA put into the reaction. Too much RNA can have an inhibitory effect on the reaction, resulting in the produced cDNA failing to be comparable to the original amount of RNA. cDNA synthesis was performed for each sample on 250 ng RNA with the Applied Biosystems High Capacity cDNA Reverse Transcription Kit.

The real-time PCR reaction is similar to a conventional PCR reaction but the amount of PCR product is measured at every cycle throughout the reaction. This real time measurement is possible due to a specially designed non-extendable TaqMan probe, containing a reporter dye at the 5' end and a quencher dye at the 3' end. As long as the probe is intact, fluorescence energy transfer occurs, that is, the fluorescence emission of the reporter dye is absorbed by the quenching dye (FRET). During the extension phase of the PCR, the Taq polymerase exploits its 5'→3'-exonuclease activity to degrade the probe (Figure 7). During degradation, the reporter and

quencher dyes are separated and the accumulation of PCR products is directly detected by monitoring the increase in fluorescence of the reporter dye.

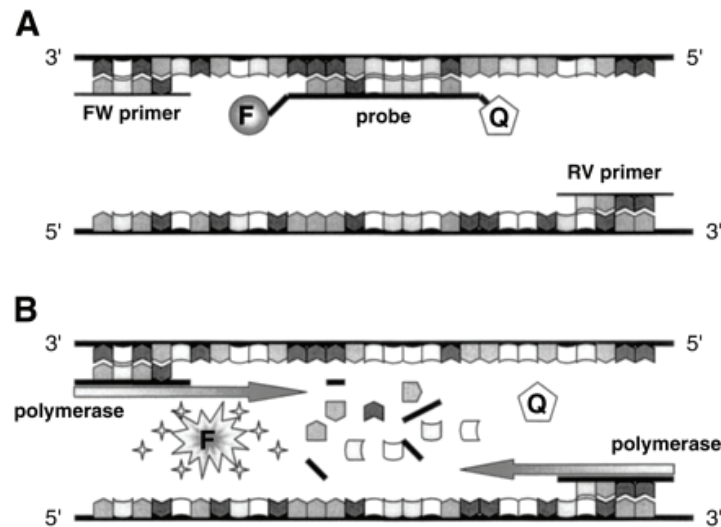


Figure 7. Schematic representation of the TaqMan principle. **A:** Primers and probe anneal to the target gene. Fluorescence emission does not occur because the probe is still intact. **B:** During the extension phase of the PCR reaction, the probe is cleaved by the 5'-3' exonuclease activity of the Taq polymerase, allowing fluorescence emission. FW: forward; RV: reverse; F: fluorophore; Q: quencher dye. (Overbergh et al. 2003)

One of the most important aspects of gene expression studies is the selection of a good control gene. An invariant endogenous control corrects for sample-to-sample variations in RT-PCR efficiency and errors in sample quantification. For a long time, GAPDH and β -actin were widely used as control genes. As housekeeping genes, they code for proteins with essential cell functions and should therefore be evenly expressed in all cell types. However, the expression of common housekeeping genes has been reported to vary considerably as a result of different treatments (Oliveira et al. 1999). We used the TaqMan Human Endogenous Control Plate (Applied Biosystems) in order to select a stable internal control gene in our study. The expression of the 11 included controls was studied in eight skin biopsies, four of which were from psoriatic skin and four were from healthy individuals. Human PGK1 was selected as an appropriate control gene for our study.

For the reactions to be specific for cDNA without the simultaneous amplification of any possible contaminating DNA, the primers are designed in different exons and the probe within the exon-exon boundary. In this way, only the amplification of cDNA can be measured. In order to cover all exons within the 30-kb region showing association with psoriasis, two assays were selected for SLC12A8 (Figure 14). These and the control PGK1 assay were ordered from Applied Biosystems. The real-time PCR analysis was performed on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems). Each sample was analyzed in triplicate to account for any possible differences between wells caused, for example, by pipetting variations. Fluorescence emission is measured continuously during the PCR reaction and ΔRn (increase in fluorescence emission, from which the background fluorescence signal is

subtracted) is plotted against the cycle number (Figure 8). The threshold line is set in the exponential phase of the amplification curves, where the reaction reaches a fluorescent activity above background levels (Figure 8). The C_T value is the cycle value obtained for each sample from the point where the threshold line crosses the amplification curve.

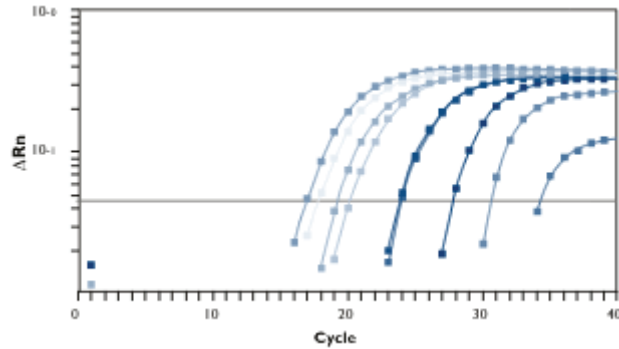


Figure 8. PCR amplification plot. Increase in fluorescence emission is plotted against the cycle number.

Relative Standard Curve Method and Comparative C_T Method ($\Delta\Delta C_T$) are two experimental design and analysis methodologies for relative quantification of gene expression. In Paper IV, we selected the second method. The advantage of this method is that it does not require a standard curve to be run on each plate and thus results in reduced reagent and RNA usage. This method relies on equal PCR efficiencies for all target genes/assays and the endogenous control, which is not an issue since all TaqMan Gene Expression Assays ordered from Applied Biosystems are designed to have amplification efficiencies of 100 %. A calibrator sample must be defined for the calculations. A calibrator is a sample used as the basis for comparing results. In this case, it consisted of a randomly selected non-psoriatic skin biopsy. The amount of target, normalized to an endogenous control and relative to a calibrator (fold difference), is given by:

$$2^{-\Delta\Delta C_T}$$

where the ΔC_T is calculated by:

$$\Delta C_T = C_{T \text{ target}} - C_{T \text{ control}}$$

and $\Delta\Delta C_T$ is calculated by:

$$\Delta\Delta C_T = \Delta C_T (\text{test sample}) - \Delta C_T (\text{calibrator sample})$$

Fold differences are usually expressed as a range, a result of incorporating the standard deviation into the calculations.

It is very important to treat every sample in the exact same way, otherwise false positive results could be produced.

GENOTYPING SNPs

SNPs are DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered. They make up about 90 % of all human genetic variations and occur at every 100 to 1000 bases along the three-billion-base human genome. SNPs are good markers for association studies because of their high frequency and evolutionary stability (they do not change much from generation to generation).

In this thesis, SNPs have been used in association studies using three different genotyping methods, i.e. SNaPshot, TaqMan and Sequenom in Papers II, III and IV, respectively.

SNaPshot

This method relies on a one-nucleotide extension reaction and can be multiplexed for several SNPs. We used this method for SNP genotyping in Paper II. It starts with a PCR reaction amplifying the target SNP. The PCR product is then cleaned using EXOSAP-IT (USB Corp) to digest unincorporated dNTPs and dephosphorylate excess PCR primer so that it cannot take part in the SNaPshot reaction. The SNaPshot primer targets a sequence immediately upstream of the SNP site. During the SNaPshot reaction, a single base extension occurs by the addition of a complementary dye-labeled ddNTP to the annealed primer. Each of the four ddNTPs is fluorescently labeled with a different color dye. After primer extension, the products are cleaned with SAP to remove any unincorporated fluorescently labeled ddNTP. At this stage, the multiplexing of 5 different primer extension products was performed. In order for the different SNPs to be separated during the run on the ABI3100 Sequence Analyzer (Applied Biosystems), extension primers were designed with different lengths, ranging from 22 nt to 56 nt. Length was adjusted by the addition of a non-specific tail (A_n) to the primer's 5' end. SNP analysis was performed using Genotyper v3.7 software (Applied Biosystems). An example of a resulting electropherogram of four SNPs is seen in Figure 9.

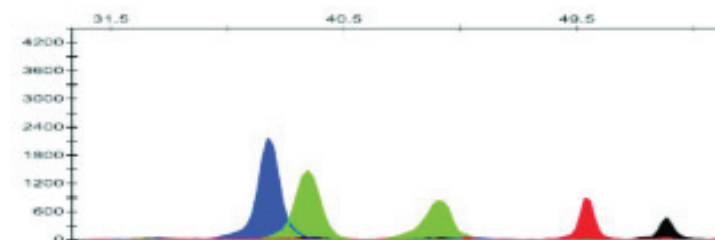


Figure 9. An electropherogram of a SNaPshot reaction

TaqMan

The TaqMan allele discrimination assay was used for the SNPs genotyped in Paper III. Each assay contains one primer pair, that amplifies the SNP, and two Taqman probes. Each probe has a fluorescent label on one side and a quencher on the other. They differ in that one is labeled with a VIC dye and is complementary to one allele of the SNP and the other is labeled with 6-Fam and is complementary to the other allele of the SNP. During the annealing phase of the PCR, only the probe that is 100 % complementary to the DNA will bind. During the extension phase of the PCR, Taq-polymerase will degrade that probe, releasing the fluorophore from the quencher and generating a permanent assay signal of only one dye color (Figure 10). When a heterozygote sample is analyzed, both probes will be able to bind, generating signals from both VIC and 6-Fam fluorophores.

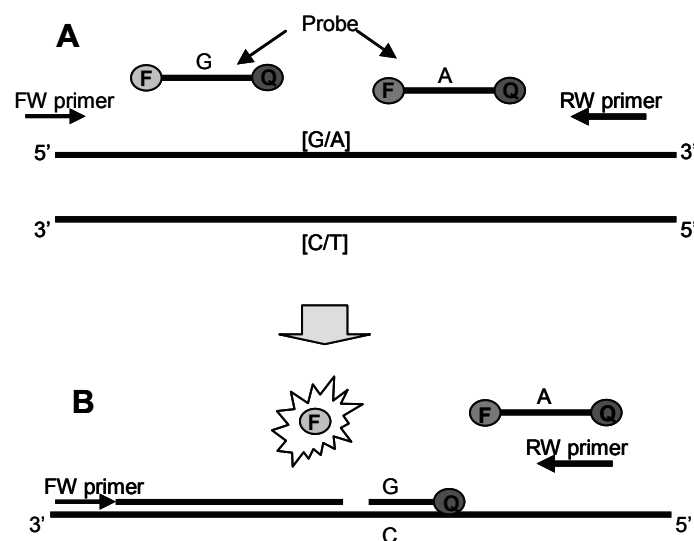


Figure 10. TaqMan chemistry enables allele discrimination by selective annealing of TaqMan probes.

A: Denatured template and TaqMan assay components.

B: Polymerization of PCR product with cleavage of the annealed probe which releases the fluorophore to generate a signal.

Q = Quencher, F = Fluorophore (6-Fam or VIC), FW = forward, RW = reverse

Genotyping of our SNPs was performed either with TaqMan SNP Genotyping Assays or Custom TaqMan SNP Genotyping Assays (Applied Biosystems) and run on an ABI 7900HT sequence detection system instrument (Applied Biosystems).

The major benefit of this genotyping technology is that it is a one-step procedure not requiring any optimization. On the other hand, there is no possibility to multiplex. This assay is therefore a first choice for smaller studies not involving too many SNPs.

MALDI-TOF

Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) is a relatively new SNP analysis technique based on mass spectrometry. This is a very high-throughput system providing the possibility of multiplexing up to 40 SNPs. This technology is similar to the SNaPshot in that they both involve PCR amplification of the region containing the SNP of interest and a primer extension reaction to generate allele-specific DNA products. The difference lies in the separation of the primer extension products; where SNaPshot uses fluorescence detection and gel separation, the MALDI-TOF products are separated by chip-based mass spectrometry.

PCR assays and associated extension reactions for the Sequenom genotyping were designed using the SpectroDESIGNER software (Sequenom) and amplified using iPLEX assays (Sequenom). After PCR amplification, the allele-specific extension reactions were performed using the Mass EXTEND Reagent Kit. Reaction products were cleaned using SpectroCleaner (Sequenom). Products were then loaded on a 384-element chip nanoliter pipetting system (Sequenom) and were analyzed on a MassARRAY mass spectrometer (Bruker Daltonik). SpectroTYPER RT 3.3.0 software (Sequenom) was used for genotype calling.

GENOTYPING MICROSATELLITES

Microsatellite genotyping is a widely used tool for a variety of applications such as linkage mapping studies and association studies. Microsatellites are tandemly repeated sequences, in which the repeating unit is 1 to 6 nucleotides long. The number of these repeats in a given microsatellite can be highly variable, a characteristic which makes them useful as genetic markers. The majority of microsatellites occur in introns or other non-coding regions of the genome. The microsatellite analysis will make use of the differences in the number of repeats between the different alleles. This variation in number of repeats affects the overall length of the microsatellite. The length of the microsatellite is determined by PCR using fluorescent dye-labeled primers that flank both ends of the microsatellite sequence, and thus generate a DNA fragment the length of which depends on the number of repeats in the microsatellite. It is these fluorescently labeled DNA fragments which are analyzed on a Genetic Analyzer with the fragment analysis technique.

In this thesis we used this technique for the association analysis in Paper I and for the linkage analysis in Paper III. Multiple markers may be run in the same lane if they are separated either by size or by the fluorescent label color. Using this strategy, our markers were divided into different groups based on their length and labeled with one of the dyes in either dye set D (6-FAM (Blue), HEX (Green), NED (Yellow)) or dye set G5 (6-FAM, VIC (Green), NED, PET (Red)). Multiplexing of up to 5 markers was usually done post-PCR. However, in some cases we succeeded in multiplexing the PCR reactions as well. In order to achieve adequate size resolution,

a size standard “ladder” must be run concurrently in each pooled sample to create a standard curve of sufficient precision. The size standard is labeled with a different colored fluorescent dye from the fragments to be analyzed. Before the multiplexed PCR reactions were analyzed on either an ABI 377, ABI 3100 or ABI 3700 Genetic analyzer (Applied Biosystems), the PCR reactions were diluted in a formamide solution and denatured at 95 °C for 5 minutes. The resulting analysis was performed with either Genotyper or Genemapper software (Applied Biosystems) (Figure 11). For correct genotyping, a small group of individuals was represented on each plate and in family typing, the genotypes were checked for errors in the expected pattern of Mendelian inheritance using the PEDCHECK program.

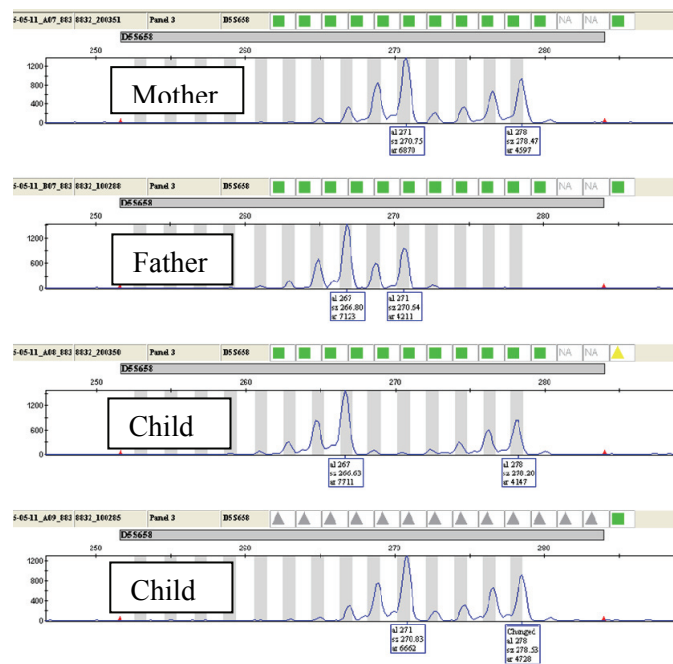


Figure 11. A di-nucleotide microsatellite marker labeled with 6-FAM, analyzed with ABI PRISM® GeneMapper™ Software Version 3.0. Each individual is heterozygous and the children have inherited one allele from their father and one from their mother.

DNA SEQUENCING

DNA sequencing is the process of determining the nucleotide order of a given DNA fragment. The chain termination method of DNA sequencing in use today was initially developed by Frederick Sanger and coworkers in 1975 (Sanger et al. 1975). The key principle of the Sanger method was the use of dideoxynucleotide triphosphates (ddNTPs) as DNA chain terminators. When this method was invented the DNA fragments were visualized by radiolabelling. Since then, the technique has developed into a high-throughput sequencing technology, mostly by the development of fluorescence-labeled ddNTPs. This is also the “dye-terminator sequencing” technique which has been used in Papers II and IV in this thesis.

The initial step in DNA sequencing is to amplify the target DNA with PCR. This PCR product is then used as a template in the sequencing reaction. The sequence reaction resembles the PCR reaction but the DNA is not exponentially increased as in the PCR reaction since only one primer (either forward or reverse) is added to the reaction. As only one strand is synthesized, the reaction becomes linear. Another difference, characteristic for the sequence reaction, is the addition of four fluorescence-labeled ddNTPs to the normal nucleotide precursors. These nucleotides are analogs of the normal dNTPs but differ in that they lack a hydroxyl group at the 3' carbon position. As these molecules are incorporated into growing DNA, they terminate the DNA chain elongation because they cannot form a phosphodiester bond with the next dNTP. If the ddNTP concentration is set very much lower than that of its normal dNTP analog, chain termination will occur randomly, producing fragments of all possible sizes. These fragments are then separated on a polyacrylamide gel in an automated sequencer. A laser constantly scans the bottom of the gel, detecting the different nucleotides by their different fluorescence. The subsequent sequence is presented as peaks in an electropherogram (Figure 12).

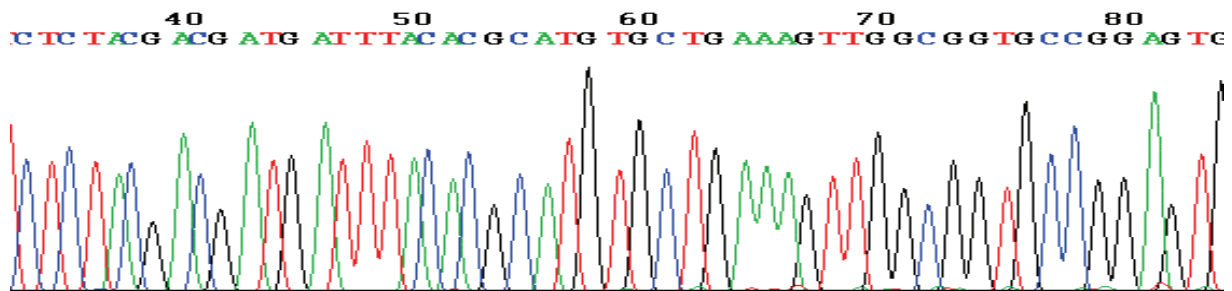


Figure 12. A DNA sequence as it is presented from an automated sequencer

In this thesis we have used the Big Dye Terminator chemistry (Applied Biosystems) on PCR products purified with ExoSac-I (USB). After the sequencing reaction, the products were precipitated with ethanol and NaAc and re-suspended in a denaturing formamide solution. The separation of DNA fragments and the detection and recording of dye fluorescence was performed on an ABI PRISM 3100 Genetic Analyzer or on the ABI PRISM 3730 Genetic Analyzer. DNA sequences were analyzed using the SeqScape (Applied Biosystems) or Vector NTI (InforMax) software.

Current methods can directly sequence only relatively short DNA fragments (about 100-800 bp) in a single reaction. The main obstacle to sequencing DNA fragments above this size limit is insufficient power of separation for distinguishing large DNA fragments that differ in length by only one nucleotide. However, high-throughput methods have parallelized the sequencing process so that many sequences can be produced and detected simultaneously. There are also other sequencing techniques on the market today including, for example, pyrosequencing and genome sequencing (Hanna et al. 2000; Margulies et al. 2005).

BIOINFORMATICS

Up-to-date annotated DNA and protein data from three continents is provided by the collaboration between NCBI/GeneBank-GenPept in the USA (Benson et al. 2002), European Bioinformatics Institute/European Molecular Biology Laboratory in Europe (Stoesser et al. 2002) and the National Institute of Genetics/DNA Database of Japan (Tateno et al. 2002). All the annotated work by scientists worldwide can be seen by scrolling and zooming over chromosomes in two genome browsers at the University of California, Santa Cruz (UCSC) (<http://genome.ucsc.edu/>) and Ensemble (<http://www.ensembl.org/index.html>). The UCSC browser was especially used in the conservation analysis of DNA across different species and for the search of regulatory and conserved transcription binding sites within the SLC12A8 gene in Paper IV.

Transcription Element Search System (TESS) (<http://www.cbil.upenn.edu/tess>) is a web-based service that searches DNA sequence for transcription factor-binding sites. We used this database to search for potential effects of SNPs on motifs involved in transcriptional regulation. The same search was also performed using the Motif search on GenomNet, a Japanese database which uses the TRANSFAC library (<http://motif.genome.jp/>).

In order to find (new) sequences, we also used the BLAST program at NCBI's website (<http://www.ncbi.nlm.nih.gov/BLAST/>). This program provides alignments of short sequences to other sequences in the database.

STATISTICAL ANALYSES

There are two commonly used techniques used for identifying genetic risk factors for complex disorders such as psoriasis, i.e. positional cloning and candidate gene approach.

Positional cloning

Linkage analysis

No information about the physiology or biology of the studied disease is needed for this technique. The search for the disease genes is performed by systematically scanning the entire DNA contents. The positional cloning of a disease gene can be divided into two steps; the first involves the localization of the chromosomal region on which the disease gene is located and the second involves identifying the correct gene within this region and finding the correct disease-causing mutation. Linkage analysis is the statistical tool used in the first step. It is based on the recombination phenomenon of homologous chromosomes during meiosis. A marker located beside an inherited disease gene will more often than not be inherited together with the gene. The closer the two are, the less likely they are to be separated during meiosis.

Thus, linkage analysis exploits the phenomenon of recombination to localize disease genes in the genome relative to the known position of genetic markers. Microsatellites are the genetic markers that have been most widely used for linkage analysis. They are short tandem repeats (STR) of 1-6-nucleotide DNA segments evenly spaced in the genome at a frequency of about 1 per 30 kb (for the most common dinucleotide repeat) (Hearne et al. 1992). Their polymorphic nature makes them very informative and well suited for linkage analysis, as the inheritance patterns can often be deciphered. Today, as sequencing of the human genome has identified hundreds of thousands of SNPs, whole-genome scans can be performed with high density SNPs, genotyping between 10 000 and 1 800 000 SNPs using the microarray technique. Linkage analysis requires a large collection of families with multiple affected individuals and can be divided into parametric (or model-based) and nonparametric (or model-free) methods.

Parametric linkage analysis

In parametric linkage analysis the mode of inheritance, such as autosomal dominant, autosomal recessive, x-linked dominant and x-linked recessive, must be specified in addition to other genetic parameters, such as penetrance, disease-allele frequency, phenocopy and mutation rates. The main quantity of interest in parametric linkage analysis is the recombination fraction θ (the probability of recombination between two loci at meiosis). The unit for parametric linkage is the LOD score, which is a function of the recombination fraction θ . Two loci are linked if the recombination fraction is less than 0.5, and a recombination fraction of 0.5 signifies an absence of linkage. The higher the LOD score, the greater the evidence of linkage. A score of 3 has traditionally been regarded as significant evidence of linkage (Chotai 1984). This is equivalent to $p=0.0001$. However, later work by Lander and Kruglyak showed that this value corresponds to a genome-wide significance level of about 0.09; the threshold for significance should be at least $\text{LOD}=3.3$ to obtain a genome-wide type I error of 0.05 (Lander et al. 1995). Another threshold $-\text{LOD}=2-$ was set to define suggestive linkage. Such linkage should also be reported as regions like this can be confirmed by other studies.

Heterogeneous LOD score

For diseases caused by inherited mutations in more than one gene, a heterogeneous LOD score (hLOD) can be calculated. This method is used for diseases for which it can be assumed that mutations in the disease genes are so rare that each family will be linked to only one such gene. At each position, the heterogeneity LOD score is maximized with respect to another parameter, α , representing the proportion of families showing linkage to the specified region. If the genetic component of a disease is due to a few major genes, this method might still be feasible but the power to detect linkage is reduced. Reduction of locus heterogeneity can be attempted by stratifying the families into sub-phenotypes of the diseases or by using families from isolated populations in which the number of original founder mutations may be low. When it comes to diseases for which genetic heterogeneity exists, it is difficult to estimate the model parameters. It is therefore common for genome scans to be done for a range of parametric models.

Non-parametric linkage analysis

In the case of multifactorial diseases, for which several genes and environmental factors contribute to disease risk, there is no clear mode of inheritance. The advantage of a NPL analysis is that it does not require any specification of a genetic disease model. It is also more robust than parametric linkage analysis when the disease model is unknown, but it is less powerful than a correctly specified parametric linkage analysis. The most common NPL design is the ASP analysis, which is based on the identity by descent (IBD) sharing of marker alleles among affected sib pairs. When there is no linkage between a marker and the diseases, the expected allele sharing between two affected siblings is 0.25, 0.5 and 0.25 for 0, 1 and 2 alleles, respectively. However, if a marker is linked to a susceptibility gene, affected sib pairs will share alleles at that marker more often than expected as a result of random segregation. If parents of the affected sib pairs are unavailable, the markers might not be sufficiently polymorphic for IBD sharing to be distinguished. In such cases, the proportions of IBD sharing can only be estimated. In ASP studies, significant linkage requires a LOD score of 3.6. We used the NPL analysis when analyzing the 5q region with a denser set of microsatellite markers in Paper III.

Linkage Disequilibrium

When an interesting chromosomal region has been defined by linkage analysis, the next step in positional cloning is to finally identify the correct disease-causing gene, requiring that the chromosomal region be further narrowed down. This is usually achieved by association analysis which relies on the phenomenon of LD. LD is a condition in which alleles at two loci or genes are found together in a population at a greater frequency than that predicted simply by the product of their individual allele frequencies. Alleles at markers near disease-causing genes tend to be in LD in the affected individuals since there is a lack of ancestral recombination events between them. This is particularly the case in isolated, homogeneous populations, in which it can be assumed that most affected individuals carry the same mutation. A set of alleles of closely linked loci on a chromosome that tend to be inherited together is commonly referred to as a haplotype. A haplotype is more informative than a single SNP which is why haplotype analysis is used in fine-mapping studies to obtain greater power. Using haplotype analysis, the disease mutation can be localized between two predicted historical crossover points. We have used this method in Paper IV.

Candidate gene approach

In contrast to linkage mapping which is a screening of the entire genome without any prior knowledge about the role of certain genes, the candidate gene approach directly tests the effects of genetic variants within candidate genes identified by for example the knowledge of the genes function, the position of the gene within a DNA region linked to the disease or identified through animal studies. This approach involves assessing the association between particular alleles within the candidate gene and the disease itself. An advantage of these studies is that they do not require large families with both affected and unaffected members, but can also be performed with unrelated

cases and control subjects. Furthermore, candidate gene studies are better suited for detecting genes underlying common and more complex diseases where the risk associated with any given candidate gene is relatively small (Risch et al. 1996; Collins et al. 1997). The major problem associated with the case-control design is that it may result in false positive results if the controls are not appropriately matched to the cases with respect to ethnicity or other factors that influence an individual's genetic composition. This approach was used in paper I.

TDT test

In order to avoid this kind of error, the transmission disequilibrium test (TDT) can be used. This test is a family-based association test most commonly requiring two parents and one affected child. For a marker locus with two alleles, the TDT compares the number of heterozygous parents transmitting one allele with the number of heterozygous parents transmitting the other allele to the affected offspring. The null hypothesis is that each allele has a 50 % chance of being transmitted from parent to child. Any deviation from this expected value indicates association with the overtransmitted allele. This test was used on our psoriasis family material in Papers II, III and IV.

RESULTS AND DISCUSSION

PAPER I

In paper I, we have investigated the association of earlier suggested regions for psoriasis, PsA, rheumatoid arthritis (RA), and ankylosing spondylitis (AS) with PsA. In this paper we studied a cohort from northern Sweden including 120 patients with psoriasis and defined joint disease and 94 controls. Patients and controls had the same ethnic background. Association was performed on 6 chromosomal regions and allele frequencies were compared between cases and controls using the chi-square test. A total of 40 markers were analyzed. Microsatellite markers are more polymorphic than SNPs and are thus more informative. When choosing which marker to genotype we therefore tried to focus on the microsatellite markers. We examined a homogeneous population with the same ethnic background comprising patients and controls from northern Sweden. This population is very well suited for genetic association studies since it derives from a founder population and has been fairly isolated throughout history.

As mentioned in the genetic section, PSORS5 have shown linkage and association in our Swedish family material, especially when the families were stratified regarding to joint involvement (Samuelsson et al. 1999; Hewett et al. 2002). Additionally, this chromosomal region has been identified, by a genome scan, as a susceptibility locus for RA in European families (Cornelis et al. 1998). The aim for this locus was therefore to analyze whether it confers susceptibility to an arthritic phenotype. In this study we included 8 markers, 2 SNPs, which had previously associated with psoriasis, and six microsatellite markers. Four of the microsatellite markers gave significant result in our previous TDT study and one were the microsatellite marker which gave the highest NPL value in the genome scan. The sixth microsatellite marker was the one that gave the highest p-value in the genome scan on RA families. The lack of association of this locus with PsA implies that PSORS5 is not a susceptibility locus for PsA in a population from northern Sweden. This is in line with the hypothesis that PSORS5 is a psoriasis locus in patients originating from southwest Sweden.

A genetic link between psoriasis and psoriatic arthritis was first suggested by Moll and Wright in 1973 (Moll et al. 1973). There is a continuing debate on whether psoriasis and psoriatic arthritis are truly manifestations of the same parent disease or whether PsA is a discrete variant of inflammatory arthritis occurring more frequently in patients with psoriasis, like the associations seen with other immune-mediated diseases (Scarpa et al. 2006). Either way, it is likely to assume that both diseases share at least some common inflammatory mediators and pathways caused by a common genetic variant. For that reason we were interested in analyzing whether two psoriasis susceptibility regions on chromosome regions, 1q21 (PSPRS4) (Capon et al. 1999) and 8q24 (Trembath et al. 1997) would be susceptibility loci shared also

with PsA. We selected 13 microsatellite markers within the 1q21 region and 10 microsatellite markers within the 8q24 region for association analysis. The markers were analyzed both as single markers and as part of constructed haplotypes. Three markers at the PSORS4 locus within a region of 2.05 Mb, and 2 markers at the 8q24 locus within a region of 2.9 Mb, showed p values < 0.05 . However when corrections for multiple analyses were applied, these markers did not reach significance. To exclude these regions from involvement in the pathogenesis of PsA, another confirmatory study would be needed, analyzing a denser set of markers.

The MHC region on chromosome 6 has been the major focus of most research in autoimmune diseases (Bowcock 2005). In psoriasis, the strongest and most reproducible association is with the Cw6 allele at the HLA-C locus (Tiilikainen et al. 1980). The situation in PsA is considerably more complex and genetic studies have shown associations with several HLA antigens including HLA-B13, B17, B27, B38, B39, Cw6, DR4, DR7 and DQ3 (Murray et al. 1980; Gladman et al. 1986; McHugh et al. 1987; Salvarani et al. 1989; Torre Alonso et al. 1991). Association with PsA has also been found with the MICA-A9 triplet repeat polymorphism and with polymorphisms in the TNFA region (Gonzalez et al. 2001; Hohler et al. 2002). Disease association with the TNF region has also been established for RA, AS and psoriasis (Mulcahy et al. 1996; Fraile et al. 1998; Hohler et al. 1998; Martinez et al. 2000; Vasku et al. 2000; Gonzalez et al. 2001; Gonzalez et al. 2001; Cvetkovic et al. 2002; Hohler et al. 2002; Kim et al. 2003). In this study we selected two microsatellite markers within the TNFB region for the association study. We received association with the 123 allele of the TNFB marker. In a former study by Alenius et al., association was received with the HLA antigens HLA-B17, B37, B44 and B62 (Alenius et al. 2002). To be able to decide which of the HLA antigens that is the most reliable marker for disease susceptibility of PsA, we performed an LD analysis in a subgroup of 83 patients that had previously been HLA typed (Alenius et al. 2002). HLA-B27 and HLA-Cw*0602 are antigens that often show association with PsA and were thus also included in the LD analysis. Since TNFB123 allele showed linkage disequilibrium to at least 4 different B antigens it is more likely that TNFB123 is a more reliable marker for disease susceptibility or disease association in PsA than any of the HLA-B antigens.

The first whole-genome scan of PsA families identified a peak of linkage on chromosome 16q21. Conditioning the linkage analysis on paternal transmission to affected individual increased the LOD score from 2.17 to 4.19 (Karason et al. 2003). This LOD score was further increased to 5.69 in a following study by addition of markers to this region, further confirming the importance of this locus (Karason et al. 2005). In an attempt to confirm a role of this locus in our population we selected 6 microsatellite markers for association analysis. However, we could not detect association to any of the markers. To completely rule out a role of the 16q21-locus a study is needed where a denser set of SNPs are analyzed.

The cytotoxic T lymphocyte antigen 4 (CTLA4) is a member of the immunoglobulin superfamily and is a costimulatory molecule expressed by activated T cells. By binding to B7-1 and B7-2 on antigen-presenting cells it transmits an inhibitory signal to T cells. T cells are believed to play an important role in the pathogenesis of

PsA and have been located in psoriatic skin lesions as well as in the synovium of patients with PsA (Marker-Hermann et al. 2000; Hohler et al. 2001). As a T cell regulatory gene, expressed at the site of disease, CTLA4 is a good candidate for susceptibility to PsA. Previous studies have also shown association of this gene with several autoimmune diseases including RA (Yanagawa et al. 1995; Marron et al. 1997; Ahmed et al. 2001; Rodriguez et al. 2002; Ueda et al. 2003). The effect of CTLA4 together with its shown association with several different autoimmune diseases implies that intragenic variants could induce a generalized susceptibility to autoimmunity. Our intention was to investigate whether CTLA4 could be a susceptibility factor for psoriatic arthritis. We choose to analyze the microsatellite marker which did associate with RA. The length of this marker had also been associated with the stability of the CTLA4 mRNA. The shortest (AT)_n allele leads to an elevated amount of CTLA4 mRNA compared to the longer alleles. So the mRNA with longer (AT)_n have shorter half-lives and, are more unstable. Our lack of association with this marker indicates that CTLA4 is not involved in the susceptibility to PsA. This lack of association have also been obtained by other studies (Matsushita et al. 1999; Vigano et al. 2005; Caputo et al. 2007). However, to exclude CTLA4 from the involvement in susceptibility to PsA confirmatory studies are needed on different populations which also includes other SNPs within this gene that has shown association in the different autoimmune diseases.

Development of an autoimmune disease depends on the inheritance of several predisposing variants. Some of these variants are expected to be common for different autoimmune diseases, including pathways involved in the general immune mechanisms, while others are disease specific. In this study association was seen between PsA and the MHC locus. This is in accordance with the fact that the MHC frequently plays a major role in autoimmunity.

PAPER II

Although the PSORS5 region on chromosome 3q21 had been narrowed down to a small region within the SLC12A8 gene, no coding SNPs are present in our study population. As other immune-mediated complex diseases, like RA (Cornelis et al. 1998) and atopic dermatitis (Lee et al. 2000; Kurz et al. 2005), also have reported linkage with the 3q21 region, we could not rule out the possibility that the overlapping linkage reports might be due to a mutation in a common gene with a function in immune response regulation. A co-localization of susceptibility loci for psoriasis and atopic dermatitis have also been found on chromosomes 1q21, 17q25 and 20p12 (Cookson et al. 2001; Giardina et al. 2006). For this purpose we choose a candidate gene approach and focused on two genes, CSTA and ZNF148. The main reason for choosing CSTA was that it had an increased expression level in psoriatic skin compared to healthy skin in a global gene expression study (Bowcock et al. 2001). It belongs to a superfamily of cysteine proteinase inhibitors. The position of this gene within the PSORS5 region together with its expressed level in psoriatic plaque made it a strong candidate gene for psoriasis susceptibility. ZNF148 was selected for two reasons. Firstly it is located 5' of the SLC12A8 gene, less than 100kb

from the nearest SNP in the associated haplotype. Secondly, it is a zinc finger binding protein that binds GC-rich DNA elements. Transcriptional regulatory proteins containing tandemly repeated zinc finger domains are thought to be involved in both normal and abnormal cellular proliferation and differentiation. ZNF148 has been shown to be involved in the regulation of epithelial cell growth (Bai et al. 2002) and the regulation of T cell receptors (Wang et al. 1993). The cohort used in the study included families with at least one parent born in the southwest part of Sweden. In order to find every coding SNPs within these genes we sequenced every exon and 400 bp of the CSTA promoter for 11 probands carrying the associated haplotype and 11 healthy blood donors. 3 SNPs were found in each gene of which two were new SNPs in each gene respectively. All three SNPs were included in the genotype study of ZNF148. One SNP were in exon 9, but did not cause any amino acid exchange, and two SNPs were 3' of exon 2 and 3 respectively. In CSTA, one SNP were in the promoter region, the second were in exon 3 and gave the amino acid exchange, threonine to methionine, and the third SNP were intronic and hence not included in the genotype analysis. Association was calculated using the TDT analysis both in the total family set and with stratification for families transmitting the associated haplotype to their affected children. The results obtained gave no significant associations with all the included SNPs regardless of using the whole family set or the stratified family set. This result shows that no coding variants within these genes are responsible for the genetic cause of psoriasis at the PSORS5 locus.

PAPER III

In this study we focused on a region on chromosome 5q, an interesting candidate region for psoriasis susceptibility for many reasons. The primary reason for analyzing this region in more detail was previous results from our genome scan (Samuelsson et al. 1999). Marker D5S816 gave a NPL value of 2.22 in the total family material and increased to 2.45 in a subgroup stratified for joint complaint (Samuelsson et al. 1999). When applying the information content term *prediction score* (Knutsson 2002) to the whole genome scan, chromosome 5q was also the region that received one of the highest prediction score values. Prediction score is an evaluation tool that combines the informativeness of a region with its obtained (NPL) score. The information in a region can be low due to low heterozygosity, failed genotyping or low marker density. Such regions could harbour interesting loci, but are often easily overlooked due to their low LOD score values. Prediction score extrapolates the outcome of incomplete data on the basis of observed data, to be able to compare the levels of information in a linkage context. Linkage with this region has also been reported in an Icelandic linkage study of female psoriasis patients (Karason et al. 2005). This region has also been reported to harbour susceptibility loci for other autoimmune diseases such as CD, RA and asthma (Grunig et al. 1998; Kauppi et al. 2001; Rioux et al. 2001; Tokuhira et al. 2003; Peltekova et al. 2004). An altered gene function of two genes, SLC22A4 and 5, within this region has also been assigned to three SNPs in RA and CD (Tokuhira et al. 2003; Peltekova et al. 2004). Additionally, this region harbours a cytokine gene cluster and other immunologically active genes that are good candidate genes for psoriasis.

The aim of this study was therefore to refine the linkage analysis in this region with a denser set of microsatellite markers. In addition, we wanted to perform an association analysis on the three SNPs with reported functional activity in RA and CD.

By slightly increasing the family material and analyzing a denser set of microsatellite markers, we obtained an increase in the NPL score from 2.45 to 3.1 in the subgroup with joint complaint (Figure 13). This NPL value is not surprising considering the size of the family material and the fact that this probably is a minor psoriasis locus compared with the MHC locus. The 95 % confidence interval for the peak position spanned between marker rs1050152 and D5S2112. This region is approximately 25 Mb and contains over 100 genes. This NPL score together with the significant difference in NPL value between the two subgroups indicate an arthritic involvement of this region around the peak marker D5S436. Thus our findings indicate that a careful characterization of sub-phenotypes may facilitate the localization of genes that contribute to complex genetic diseases.

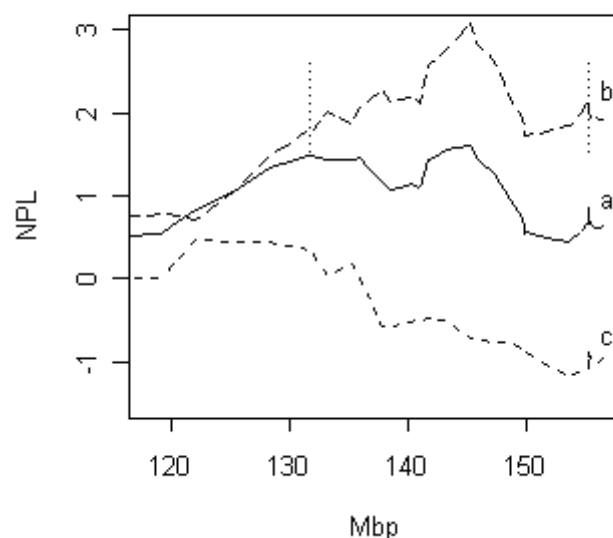


Figure 13. Result of linkage analysis of chromosome region 5q31-32. (a) Total family material (114 families), (b) families with joint complaints (55 families), (c) families without joint complaint (59 families)

As the PSORS5 locus on chromosome 3q21 also is associated with joint complaint, we wanted to investigate a possible epistasis between these two loci. We found a negative correlation between the NPL values indicating possible locus heterogeneity between the two loci. To inquire into the female linkage theory of this locus as proposed by Karason et al. 2005, we performed an NPL analysis including only affected females. However, this theory could not be confirmed in our population.

Our association analysis data indicate that the three functional SNPs that associate with RA and CD are not involved in psoriasis susceptibility in our population. The lack of association is in line with several studies on psoriatic arthritis, RA, CD and diabetes (Barton et al. 2005; Butt et al. 2005; Newman et al. 2005; Martinez et al.

2006; Smyth et al. 2006). A substantial ethnic difference has also been reported for these autoimmune-disease associated SNPs, particularly between the Caucasian and the Japanese populations (Yamazaki et al. 2004; Mori et al. 2005). This was also seen in our study. Rs3792876 in SLC22A4 was extremely rare in our population compared with the Japanese population in which the association was originally identified. The low degree of allelic heterogeneity of this SNP made it difficult to detect any possible association. These different results obtained by different studies indicate that the SNPs previously reported as functional are population specific or may not themselves be causative, but rather in linkage disequilibrium with another causative variation.

PAPER IV

This paper is a sequel to a previously published paper on chromosome 3q21, in which an associated haplotype was identified within the SLC12A8 gene (Hewett et al. 2002). This haplotype consisted of five SNPs of which four (rs816154, rs1554241, rs702045, rs531740) were located within a 25-kb region and the fifth (rs2228674) was about 64 kb from the nearest of the other four. As the region spanned by the four SNPs also overlapped one of the haplotype blocks found to associate with psoriasis in a German association study (Huffmeier et al. 2005), we considered it the most likely region to harbor a psoriasis susceptibility mutation. In order to identify all, including potential population- and disease-specific, DNA variations, we set out to re-sequence a 30-kb region spanning the four SNPs in eight psoriatic individuals, half of whom were carrying the associated haplotype. Identified DNA variations were evaluated using an *in silico* bioinformatics approach with respect to a possible function. Comparative analysis between different species and prediction of changes in transcription sites were undertaken. The degree of evolutionary conservation (and therefore intolerance to change) was regarded as a predictor of the likelihood of clinical significance of a particular substitution (Ureta-Vidal et al. 2003). Based on that outcome, as well as the results of the confirmative study (Huffmeier et al. 2005), 71 SNPs were selected and genotyped in our cohort of psoriatic families originating from the southwestern part of Sweden.

Association was tested for both single SNPs and for haplotypes, using a sliding window approach including up to six consecutive markers. Significant associations were found for three SNPs (rs2993637, rs1554241 and rs2942812), with P-values of 0.008, 0.006 and 0.009, respectively. The association was caused by an overtransmission of the most common allele. Interestingly, rs1554241 was also one of the singly associating SNPs in the German case control study. We also found significant association with several haplotypes in a total of six different regions. Two of the singly associated SNPs were also included in the associating haplotypes in two of these six regions.

Analysis of the expression pattern of the only known gene within this most associated region, SLC12A8, was another goal of this study. Since all associations found with this region are within the introns of this gene, it is most likely that the risk factor at the PSORS5 locus affects regulatory or splicing functions. Four of the

exons of SLC12A8 are situated within the most associating 30-kb region. In order to also be able to identify possible splicing variation between these exons, we selected two TaqMan expression assays with one primer in each exon (Figure 14).

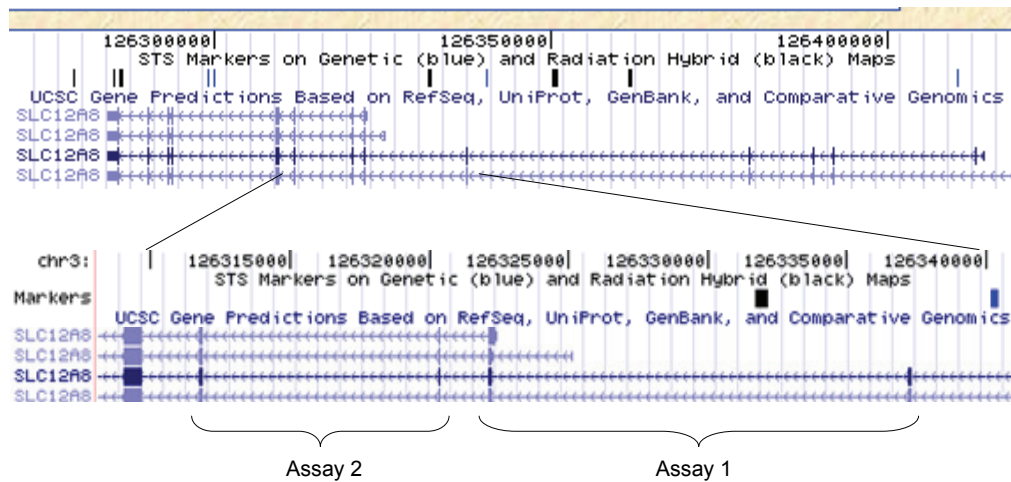


Figure 14. Schematic representation of the 30-kb region and the position of the two assays used for gene expression.

The gene expression study found a medium fold difference of 0.43-0.74 ($P < 0.0004$) and 0.49-0.87 ($P < 0.002$) in patients, compared to controls, in the two different assays, respectively. These values correspond to a decrease in gene expression by 0.3-1.3 times in assay 1 and by 0.15-1 times in assay 2.

In summary, in this study we have shown that SLC12A8 is significantly downregulated in skin biopsies from psoriatic plaque, compared with healthy skin from control individuals. We also found association with six haplotype regions and three single SNPs. In our opinion, those three SNPs and any associating SNPs within these six haplotype blocks are the most probable disease-predisposing variants and are hence the primary candidates for functional analyses.

GENERAL DISCUSSION AND FUTURE PROSPECTS

The studies within this thesis have confirmed an association of PsA with the HLA region on chromosome 6. The association was with the TNFB123 allele. This allele also turned out to be the most reliable marker for PsA disease susceptibility in the LD study of associating antigens in the northern Swedish population. No association could be confirmed between PsA and a microsatellite marker with a function in mRNA stability of the CTLA4 gene, with which association had been found in RA patients. Nor was any association found with the PSORS5 region, consistent with the theory that this region comprises a psoriasis susceptibility variant caused by a founder mutation in the southwestern part of Sweden. This region has been further analyzed in a psoriasis family material from southwestern Sweden. Two genes, CSTA and ZBP148, within the linkage region of this locus, were excluded from involvement in psoriasis after sequencing and genotyping coding and splice site SNPs. The association of the SLC12A8 gene was further investigated by resequencing the 30-kb region with the strongest association. Intronic association was found with three SNPs and six haplotype regions. The disease-predisposing variant is likely to reside within one of these regions. A slight variation has also been shown between the mRNA expression of SLC12A8 in psoriatic and control skin biopsies. The 5q31-32 region was also analyzed in a psoriasis family material. Linkage was confirmed in this region in a group with arthritic symptoms, with a 95 % confidence interval for the peak position between markers rs1050152 and D5S2112. However, our association analysis data indicate that the three functional SNPs (previously found to be) associated with RA and/or CD are not involved in psoriasis susceptibility in our population.

The next step concerning the 5q locus is to fine-map this 25-Mb region, in an attempt to get closer to the disease gene. LD using constructed haplotypes is a valuable tool for this purpose. Haplotype analysis allows the inference of likely historical crossover points which localize the disease mutation.

Research on the PSORS5 region will continue with an examination of the associating haplotype blocks. In order not to miss any potential disease-predisposing SNP, any existing SNPs in our population that have not already been genotyped will undergo this process. Furthermore, the intention is to relate the associating genotypes to the results of the expression study of SLC12A8, requiring genotyping of all the associating SNPs in the controls from the gene expression study. The associating SNPs in cases and controls can be compared in order to ascertain whether any of them can explain the difference in SLC12A8 expression. Potential disease-predisposing SNPs can then be analyzed for functionality. The electrophoretic mobility shift assay (EMSA) can be used for assessing whether the SNP actually causes a difference in protein binding, for example by a transcription factor, to DNA. The verification of a disease-predisposing SNP will hopefully help to reveal a contributing pathway in the pathogenesis of psoriasis.

The identification of genes involved in human diseases is the main focus of molecular genetics research. Today, the genetic background of nearly every Mendelian disease has been identified, largely by positional cloning (Dean 2003). In the case of common complex diseases, the identification of causative gene variants has proven much more difficult, mainly because the connection between phenotype and genotype is much weaker due to the different mechanisms underlying complex diseases. A main feature distinguishing a Mendelian disease from a complex disease is the relative risk conferred by the disease-predisposing alleles. Mendelian diseases are often caused by high relative-risk alleles, while moderate-to-low relative-risk alleles are assumed to underlie complex disease phenotypes. This is a result of the involvement of several genes in a complex disease, making the contribution of each gene smaller. Genetic heterogeneity, i.e. two or more sets of genes, sometimes overlapping, that can lead to a specific phenotype in the same or different disease populations, is another confounding factor. This heterogeneity decreases the ability to detect linkage in linkage studies. Incomplete penetrance is also an obstacle in linkage analysis since not all mutation carriers develop the expected phenotype. This is often the case as complex disease-causing variants are expected to be common variants with a high frequency in the healthy population as well (Reich et al. 2001). Phenotypic expression of complex diseases also varies, possibly due to environmental trigger factors, age, gender and modifier genes. Infectious diseases (such as streptococcal infection in psoriasis) often play an important role in complex diseases. A complex disease can also depend on non-Mendelian inheritance, such as imprinting, causing a gene expression difference. A careful classification of sub-phenotypes has been proposed in order to strengthen the connection between genotype and phenotype in complex diseases, thus helping to identify the disease genes (Karason et al. 2005).

Once a small associated region has proven to associate to a specific phenotype, the LD phenomenon can make the identification of a disease-causing allele more difficult. If there is a high degree of LD, all variations in the region will associate equally strongly. The psoriasis susceptibility locus with the strongest effect, PSORS1, is an example of such a region. In circumstances such as these, functional studies are a valuable tool for identifying the correct mutation. We used expression analysis of the SLC12A8 gene at PSORS5 as a first step in a functional approach.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Vår kropp är uppbyggd av miljarder celler. Inuti varje cell finns en cellkärna och inne i den våra kromosomer. Människan har normalt 46 kromosomer som delas in i 23 par. Den ena kromosomen i varje par har vi ärvt från vår mamma och den andra från pappa. De första 22 kromosomparen (kromosom 1-22) kallas autosomer och finns hos alla människor. Det sista paret är könskromosomerna, X och Y. Kvinnor har normalt två X-kromosomer, medan män har en X- och en Y-kromosom. Kromosomerna består av deoxyribonukleinsyra (DNA). DNA-molekylen är i sin tur uppbyggd av 4 olika nukleotider (eller baser som de också kallas), adenine (A), cytosin (C), tymine (T) och guanin (G). Dessa utgör det "genetiska alfabetet" och kodar för mellan 20 000 och 25 000 gener (anlag). Generna kodar för proteiner (äggviteämnen) som är de molekyler som utför alla viktiga funktioner i cellen. Eftersom vi har två kromosomer av varje slag så har vi också en dubbel uppsättning gener, en från mamma och en från pappa. DNA-sekvensen som kodar för generna varierar lite från person till person, det är det som gör oss alla olika. Vissa variationer kan dock också orsaka sjukdom. Sådana variationer i DNA:t kallas för mutationer. Sjukdomar som beror på mutationer i gener är ärftliga. Sjukdomens symptom avgörs av vilken eller vilka gener som är förändrade samt på vilket sätt mutationen förändrar genen och dess protein. Sjukdomar som orsakas av en mutation i endast en gen kallas för monogena sjukdomar. Många vanliga sjukdomar som t.ex. cancer, hjärt-kärlsjukdomar, diabetes och celiaki (glutenintolerans) beror på förändringar i flera olika gener i samverkan med omgivningsfaktorer eller livsstil. Dessa sjukdomar brukar kallas multifaktoriella sjukdomar, polygena eller komplexa sjukdomar. Till denna grupp av sjukdomar hör även psoriasis och psoriasis artrit.

Psoriasis är en kronisk hudsjukdom som drabbar ca 2-3 % av den svenska befolkningen. Den kan yttra sig på många sätt. Den vanligaste formen heter *psoriasis vulgaris* och yttrar sig som röda fjällande torra fläckar, sk. psoriasisplack (se figur 1). Placken beror på en inflammation i huden som ökar blodcirkulationen, vilket gör att huden rodnar och blir varm. Cellerna i psoriasis-huden omsätts snabbare än vanligt. Ett normalt hornlager hinner inte bildas och cellerna lossnar inte längre en och en utan flagar av i stora sjok, vilket orsakar fjällningen. Placken brukar vara mellan en och sex centimeter stora och sitter oftast på armbågarna, knäna, underbenen, i hårbotten och på nedre delen av ryggen. Psoriasis går i skov och varierar mycket i intensitet och utbredning mellan olika individer.

Cirka 30 % av psoriatikerna får även den associerade sjukdomen psoriasis artrit. Det är en kronisk inflammatorisk ledsjukdom som orsakar smärta, stelhet och i sin allvarligaste form, rörelsehinder. Inflammationen kan vara lokaliserad till en eller ett fåtal leder men vanligtvis är många leder engagerade. Lederna kan angripas och brytas ned av sjukdomen, så kallad leddestruktion, och utan behandling kan detta leda till funktionsnedsättning och handikapp.

Beroende på formen av psoriasis finns det ett antal olika behandlingsmetoder att tillgå, dock ingen som kan bota sjukdomen. Alla behandlingar är också förknippade med mer eller mindre obehagliga biverkningar. Många patienter upplever därför att de har en nedsatt livskvalitet. Detta gör psoriasis till en allvarlig sjukdom där det krävs mer forskning.

I den här avhandlingen har jag använt mig av DNA insamlat från 266 psoriasisfamiljer. Den främsta metoden har varit associationsanalys av olika markörer i gener till psoriasis. (Om en gen associerar till en sjukdom så är det också troligt att den är inblandad i sjukdomens uppkomst.) Målet med projektet har varit att försöka identifiera och verifiera en gen eller mutation som bidrar till uppkomsten av psoriasis. Tyngdpunkten lades på ett område på kromosom 3 (3q21) som i tidigare studier har pekats ut som troligt att innehålla in sjukdomsorsakande mutation.

I arbete II undersöktes om två gener som ligger i detta område, CSTA och ZNF148, är associerade till sjukdomen psoriasis. CSTA-genen var intressant att studera eftersom tidigare studier hade visat högre genaktivitet i psoriasis hud jämfört med vanlig frisk hud. ZNF148 valdes eftersom den låg väldigt bra till i regionen samt kan ha en funktion i regleringen av tillväxten av celler. Association kunde inte detekteras till dessa två gener, varför det inte är troligt att de har någon påverkan på uppkomsten av sjukdomen psoriasis.

I arbete IV analyserades en gen, SLC12A8, som i tidigare studier har visat sig associera till psoriasis. Genens funktion är att transportera joner över cellmembran. Man vet ännu inte hur en förändring i en sådan funktion skulle kunna orsaka psoriasis. Vi hittade tre basparsvariationer (SNP:er) och sex små regioner (haplotyp-block) som associerar till sjukdomen och skulle kunna vara/innehålla den sjukdomsorsakande mutationen. Vidare kunde vi konstatera att det fanns lite mindre av genen SLC12A8 i psoriasisbiopsier jämfört med kontrollbiopsier. Fortsatta studier behövs för att koppla ihop denna förändring i gennivå med en mutation i DNA-sekvensen.

I arbete III tittade vi på en region på kromosom 5 som också har pekats ut i en tidigare screening av hela genomet (alla kromosomer). Genom att analysera flera markörer så har vi minskat ner området och därmed också närmast oss en eventuell sjukdomsorsakande mutation i den här kromosomregionen. Vi har även fastställt att två gener, SLC22A4 och SLC22A5, troligtvis inte associerar till psoriasis i vår population.

Vi har även genom ett samarbete med en grupp i Umeå fått tillgång till DNA från 120 individer med psoriasis artrit och 94 matchade kontroller. Dessa användes till att, i arbete I, undersöka association mellan 6 olika kromosomala regioner och sjukdomen psoriasis artrit. Vi fann association till en markör i HLA-regionen på kromosom 6. Detta område kodar för HLA-molekyler som binder antigen och aktiverar immunförsvaret. Däremot fann vi ingen association till området 3q21 och inte heller till en markör i CTLA4-genen, vilket tyder på att dessa inte har någon påverkan på psoriasis artrit.

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