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# THE INTERLEUKIN-10 FAMILY CYTOKINES GENE POLYMORPHISMS IN PLAQUE PSORIASIS

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# ABBREVIATIONS

| | | |
|---|---|---|
| AID | – | autoimmune disorder |
| ANOVA | – | analysis of variance |
| ARMS-PCR | – | amplification refractory mutation system polymerase chain reaction |
| CDSN | – | corneodesmosin |
| EDC | – | Epidermal Differentiation Cluster |
| GM-CSF | – | granulocyte-macrophage colony-stimulating factor |
| HCR | – | α-helix coiled-coil rod homolog |
| HIV-1 | – | human immunodeficiency virus 1 |
| HLA | – | human leukocyte antigen |
| HWE | – | Hardy-Weinberg equilibrium |
| IL | – | interleukin |
| IL-1Ra | – | interleukin-1 receptor antagonist |
| IL-10Rα | – | interleukin-10 receptor alpha chain |
| IL-10Rβ | – | interleukin-10 receptor beta chain |
| IL-22R | – | interleukin-22 receptor |
| IFN-γ | – | interferon-gamma |
| LD | – | linkage disequilibrium |
| LPS | – | bacterial lipopolysaccharide |
| MDA-7 | – | melanoma differentiation-associated gene 7 |
| MHC | – | major histocompatibility complex |
| OTF3 | – | octamer transcription factor-3 |
| PASI | – | psoriasis area and severity index |
| PBMC | – | peripheral blood mononuclear cell |
| PSORS1 | – | psoriasis susceptibility 1 |
| QRT-PCR | – | quantitative real-time polymerase chain reaction |
| RT-PCR | – | reverse transcription polymerase chain reaction |
| SNP | – | single nucleotide polymorphism |
| TCF19 | – | transcription factor-19 |
| TDT | – | transmission/disequilibrium test |
| Tc2 cell | – | cytotoxic T cell type 2 |
| Th2 cell | – | T helper cell type 2 |
| TNF-α | – | tumor necrosis factor-alpha |
| Trl cell | – | regulatory T cell type 1 |
| 5'-UTR | – | 5'-untranslated region |

# 1. INTRODUCTION

Psoriasis is a chronic inflammatory skin disease. All major human populations are affected with a peak prevalence of 2–3% observed in North-European Caucasians. The occurrence of psoriasis is due to an interaction between a genetically predisposed individual and environmental influences, but the exact cause of the disease remains unknown. Nevertheless, the principal clinical features of psoriasis (inflammatory infiltrate and epidermal hyperproliferation with abnormal differentiation of keratinocyte) appear to be driven mainly by various cytokines and chemokines released by the activated, skin-homing pathogenic T-cell population.

There is increasing evidence to suggest that cytokines of the interleukin-10 (IL-10) family, interleukin-10 (IL-10) itself and the three homologues – interleukin-19 (IL-19), interleukin-20 (IL-20) and interleukin-24 (IL-24) – have a role in the function of epidermis and in psoriasis. Genes encoding IL-10, IL-19, IL-20 and IL-24 form a cluster in the region q31-32 of chromosome 1. Functional relevance of the IL-10 gene polymorphisms has been demonstrated by their involvement in determining susceptibility and severity to a number of immune-inflammatory diseases. Moreover, IL-10.G polymorphism from the IL-10 promoter region has been identified to be protective against psoriasis. Descriptions of single nucleotide polymorphisms (SNPs) of IL-19, IL-20 and IL-24 genes are accessible in the NCBI dbSNP database. No polymorphisms of these genes have been investigated in complex diseases up to the present time.

The aim of this study was to elucidate the role of the genes of IL-10 cluster (IL-10, IL-19, IL-20 and IL-24) in predicting the risk for plaque-type psoriasis and to describe the linkage disequilibrium (LD) pattern of these genes. Association, linkage disequilibrium and haplotype analyses of the genes of IL-10 cluster were performed. To explain the functional role of the genes of IL-10 cluster in the pathogenesis of psoriasis we studied the whole blood IL-10 and IL-20 mRNA expressions in previously genotyped patients with plaque psoriasis and healthy controls.

# 2. REVIEW OF LITERATURE

## 2.1. Psoriasis

### 2.1.1. Epidemiology of psoriasis

Psoriasis is an ancient and universal inflammatory disease, initially described at the beginning of medicine in the *Corpus Hippocraticum*. Hippocrates used the term *psora*, meaning "to itch". For centuries psoriasis was described as a variant of leprosy and was regarded as contagious until Von Hebra identified it as a distinct disease entity (Glickman, 1986).

Psoriasis is considered to be a T-cell-mediated autoimmune disease with similarities to other complex autoimmune disorders (AIDs) such as rheumatoid arthritis, Crohn`s disease and diabetes. The resemblances of psoriasis to other autoimmune disorders are the distinct role of genetic and environmental factors, variable age of onset, the great variability of the tissue reaction with different degrees of activity, extent of body involvement and frequency of relapses.

Estimates of the prevalence of psoriasis vary from 0.4% to 4.7% with rates varying between countries and races (Christophers, 2001). Psoriasis tends to be more frequent at higher latitudes than lower latitudes and more frequent in Caucasians than in other races. In certain ethnic groups no psoriasis is seen, such as the American Indians and Eskimos. In contrast to other AIDs, which demonstrate an increasing prevalence rate, no significant changes in incidence rates are reported in psoriasis. This fact indicates that perhaps the environmental influence on the pathogenesis of psoriasis is less important than it is on the other autoimmune conditions mentioned above. Moreover, psoriasis is equally common in men and women, while one of the features of autoimmunity is that the proportion of females affected outweighs males significantly (Christophers, 2001).

Psoriasis can begin at any age, although epidemiological studies demonstrate that it most commonly appears for the first time between the ages of 15 and 25 years (Henseler *et al*., 1985). The triggering factors induce psoriasis in predisposed persons. The main triggering factors that initate or exacerbate psoriasis are bacterial pharyngitis, stress, HIV-1, various medications (lithium, ß-blockers), injury to the skin and alcohol abuse (Camp, 1992).

An important feature of psoriasis relates to the severity of disease. There are three parameters which appear as major determinants of disease severity: the extent of body involvement, lesional activity and frequency of relapses. The severity of psoriasis is determined largely by the type of psoriasis. Patients with early onset of psoriasis are more likely to show widespread and recurrent disease as compared with late onset patients.

Between 5% and 42% of patients with psoriasis have psoriatic arthritis that is usually seronegative for rheumatoid factor and is presented in several cha-

racteristic forms: oligoarticular disease, distal interphalangeal arthritis, arthritis mutilans, and spondylitis or sacroilitis (Lebwohl, 2003). Between 10% and 30% of patients with psoriasis have nail changes: pitted nails, onycholysis, subungual hyperkeratosis and discoloration of the nail plate (Nickoloff *et al*., 2004). Patients with more severe and long-standing disease are more likely to have nail changes and psoriatic arthritis than those with less severe or recent-onset disease.

Psoriasis is a lifelong disease with spontaneous remissions and exacerbations. The disease is rarely fatal, but in despite of this it clearly has a potentially devastating effect on the patient's quality of life. Psoriasis constrains a number of normal daily activities, such as use of hands, walking, sleeping, and sexual activity (Nevitt *et al.*, 1996). At least 30% of patients contemplate suicide, which places psoriasis on par with other major medical diseases such as depression, heart disease, and diabetes (Krueger *et al*., 2001).

### 2.1.2. Classification and diagnosis of psoriasis

Psoriasis may appear in several clinical forms. The most common variant is the plaque-type psoriasis that affects approximately 80…90% of the adult patients (Lebwohl, 2003; Fry, 1988). Less frequently occurring cutaneous manifestations are guttate psoriasis, pustular psoriasis and erythrodermic psoriasis.

There are two distinct patient cohorts with plaque-type psoriasis: one with early onset and another with late onset disease. Patients with disease onset below the age of 40 are nominated as early onset psoriasis, while patients with the onset of disease at the age of 40 and later years are referred to as the late onset psoriasis. In early onset of psoriasis the mean age of occuring the eruption are at the age of 16 years (women) and 22 years (men), and in late onset psoriasis at 60 years (women) and 57 years of age (men). Patients with early onset more likely demonstrate a history with affected parents compared to patients with late onset disease (44% in the early onset psoriasis, 0% in the late onset psoriasis) (Henseler *et al*., 1985).

Diagnosis of plaque-type psoriasis bases on typical clinical and histological features. Plaque psoriasis is defined by sharply demarcated, erythematous scaling papules and plaques that typically affect the elbows, knees, scalp and sacral region. The silvery scales that cover papules and plaques are removed by gentle scraping; fine bleeding points are usually seen, the so-called Auspitz sign. The histological picture is characterized by acanthosis with regular elongation of the rete ridges with a thickening in their lower portion, thinning of the suprapapillary epidermis with occasional the presence of small spongiform pustules of Kogoj, pallor of the upper layers of the epidermis, a diminished to absent granular layer, confluent parakeratosis, the presence of Munro microabscesses, elongation and edema of the dermal papillae, and dilated and tortuous capillaries (Elder *et al.*, 1999).

There are various possibilities to determine the severity of plaque psoriasis. One more usable is the psoriasis area and severity index (PASI score). PASI score is based on the degree of erythema, desquamation and infiltration of lesions, combined with surface extension and it varies from 0.0 to 72.0 (Fredriksson *et al*., 1978). The insufficiency of PASI score is that it does not consider the dynamic behaviour of psoriatic lesions. The dynamic behaviour of psoriatic lesions means that psoriasis relates to the great variability to relapse and sudden outbreak of lesions.

### 2.1.3. Pathogenesis of plaque-type psoriasis

The pathogenesis of psoriasis has three major aspects: (1) a polygenic genetic predisposition; (2) a strong microbial/environmental component involved in psoriasis onset; (3) a chronic T-cell-mediated inflammation of the skin and, in a proportion of patients, of joints, that after onset may persist lifelong.

T-cells form a major constituent of the inflammatory infiltrate in the papillary dermis and they are also present in the epidermis. The activated T-cell clones established from psoriatic skin lesions have a selective capacity to enhance keratinocyte proliferation *in vitro* (Prinz *et al*., 1994). The effect of the T-cell populations is mediated by the pro-inflammatory cytokine pattern. Innate mediators like IL-2, IFN-γ (Barker *et al*., 1991) and TNF-α (Lew *et al*., 2004) stimulate inflammation in the Type 1 pathway through activating effects on dendritic cells and increasing transcription of other T-helper 1 genes. Several findings suggest that anti-inflammatory cytokines and their receptors (IL-1Ra, IL-10) also play a critical part in the disease pathogenesis. In the psoriatic skin the deficiency of IL-1Ra (Kristensen *et al*., 1992) and the deficiency of IL-10 (Nickoloff *et al*., 1994; Mussi *et al*., 1994) are established. Furthermore, therapeutic application of the anti-inflammatory cytokines IL-4 (Ghoreschi *et al*., 2003) and IL-10 (Asadullah *et al*., 1998) alleviates psoriasis significantly.

### 2.1.4. Genes associated with plaque-type psoriasis

Independent genome-wide linkage scans have suggested the involvement of large numbers of chromosomal regions in the development of psoriasis. Replication has only been achieved for a limited number of regions, including chromosomes 1q21, 3q21, 4q34, 6p21, 17q25 and 19p13 (Capon *et al.*, 2000 and 2002; International Psoriasis Genetics Consortium, 2003).

The most potent genetic influence on susceptibility to AIDs is the major histocompatibility complex (MHC). The highest risk to susceptibility to psoriasis has also been reported for a region located on the short arm of chromosome 6. In contrast to other AIDs that are mostly linked to a certain class II

HLA alleles, psoriasis is the only known chronic inflammatory disease that has a strong association with a region encoding human leukocyte antigen-C (HLA-C) and specifically with the HLA-Cw6 allele (Trembath *et al*., 1997; Nair *et al*.; 1997). The susceptibility region in chromosome 6 (PSORS1 – psoriasis susceptibility 1) has refined to an approximately ~300 kb region around HLA-C gene, and contains several candidate genes including HLA-C, octamer transcription factor-3 (OTF3), transcription factor-19 (TCF19), corneodesmosin (CDSN) and α-helix coiled-coil rod homolog (HCR) genes (Balendran *et al*., 1999; Oka *et al*., 1999; Veal *et al*., 2002; Asumalahti *et al*., 2002; Orru *et al*., 2005). The HLA association of psoriasis affirms a pathogenetic link to the immune system and suggests that the pathogenic process is driven by autoantigens that may be presented by HLA-Cw6 in those patients who carry this allele.

In addition, several non-MHC psoriasis susceptibility loci (1q21, 16q and 17q) associate with different autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, Crohn's disease and diabetes. This provides support for the hypothesis that susceptibility to different AIDs may be based on same identical susceptibility genes and identical immunopathways (Wandstrat *et al*., 2001). Zhou *et al*. (2003) have established 1338 genes with potential roles in the pathogenesis of psoriasis by use of Affymertix Gene Chips. Many of the identified genes are known to be involved in immune response and proliferation.

## 2.2. IL-10 and the family of IL-10-related cytokines

### 2.2.1. Classification of IL-10 related cytokines

Several cytokines exist within structurally related families. This is well-recognized in the case of TNF family (Gruss *et al.*, 1995). A family of IL-10-related cytokines comprises a series of herpesviral and poxviral members and several human cellular paralogs of IL-10 (IL-19, IL-20, IL-22, IL-24, and IL-26) (Fickenscher *et al.*, 2002). The IL-10-related cytokines share limited primary sequence identity, structural homology and receptor subunits. Although IL-10 homologues are not functionally similar, they hold pleiotropic cell specific activities as IL-10 itself does and at least some of them are clearly involved in the regulation of inflammatory responses in various tissues.

Cellular human IL-10-related cytokines form additionally two subfamilies of more closely related ligands – IL-10 subfamily and IL-19 subfamily. IL-10 subfamily contains IL-10 itself, IL-22 and IL-26, while the IL-19 subfamily contains IL-19, IL-20 and IL-24 (Gallagher *et al*., 2004).

### 2.2.2. IL-10 and its role in the skin functioning and in the pathogenesis of psoriasis

IL-10 is a multifunctional cytokine secreted and responded by a number of cell types. Not only certain T-cell subsets (Th2, Tc2, and Trl) synthesize IL-10 but also monocytes, macrophages, keratinocytes and several other cell types. IL-10 inhibits the formation of proinflammatory cytokines like TNF-α in T-cells and monocytes (Fiorentino *et al*., 1991), downregulates MHC class II expression in monocytes (Willems *et al*., 1994) and modifies chemokine receptor expression (Takayama *et al*., 2001). In B cells IL-10 stimulates the production of immunoglobulins and the expression of MHC class II antigens (Rousset *et al*., 1992). It was recently discovered that IL-10 promotes the activity of NK cells (Cai *et a*l., 1999). The pleiotropic activities of IL-10 are mediated by its specific cell surface receptor complex (IL-10Rα and IL-10Rβ). Due to the described biological activities IL-10 plays a dominant part in several immune reactions and its inadequate expression has been demonstrated in autoimmune diseases, infections and malignancies.

The overexpression of IL-10 has been reported in some inflammatory dermatoses like atopic dermatitis (Ohmen *et al*., 1995) as well as in various skin tumors like melanoma (Krüger-Krasagakes *et al*., 1994) and basal cell and squamous cell carcinoma (Kim *et al*., 1995). Low cutaneous IL-10 protein expression level (Nickoloff *et al*., 1994) and low IL-10 concentration in blister fluid (Mussi *et al*., 1994) have been observed in psoriatic lesions. In addition, the trend of lower IL-10 protein secretion capacities of peripheral blood leukocytes in response to endotoxin in psoriatic patients when compared with three groups – patients with cutaneous T-cell lymphoma, patients with atopic dermatitis and healthy controls – has been reported, but differences were not statistically significant (Asadullah *et al*., 1998).

### 2.2.3. IL-19 subfamily cytokines and their role in the skin functioning and in the pathogenesis of psoriasis

The IL-19 subfamily is characterized by their amino-acid homologies and by the fact that all the members of this subfamily (IL-19, IL-20 and IL-24) are able to bind and signal through the IL-20 receptor beta chain.

IL-20 has been found to be preferentially expressed in monocytes. Its main targets are keratinocytes where IL-20 binds type I IL-20R (IL-20Rα and IL-20Rβ) and type II IL-20R (IL-20Rβ and IL-22R) complexes. IL-19 has been detected in immune cells, such as LPS- or GM-CSF-activated and resting monocytes, and at lower level in resting and stimulated B cells. This cytokine binds to the type I IL-20R complex. IL-24 mRNA has been detected by RT-PCR in human monocytes and its expression is upregulated in monocytes

by LPS stimulation and in T-cells following activation by anti-CD3 monoclonal antibody. IL-24 binds to type I and type II IL-20 receptor complexes (Dumoutier *et al*., 2001; Wolk *et al*., 2002). Binding of the IL-19 subfamily members to the IL-20 receptor complexes activate the STAT pathway in cytokine responsive cells (Parrish-Novak *et al*., 2002).

The role of IL-19, IL-20 and IL-24 in the function of epidermis and in the pathogenesis of psoriasis has been verified, but the entire understanding of the pathogenic actions of above described cytokines in psoriasis is still unclear. Blumberg *et al.* (2001) have shown that overexpression of IL-20 under different promoters in transgenic mice caused neonatal lethality with skin abnormalities, similar to those found in human psoriatic skin. Microarray and RT-PCR analyses in HaCaT-cells have demonstrated that expressions of several genes involved in inflammation are increased in response to IL-20 and therefore this cytokine may modulate the inflammatory response in the skin (Rich *et al*., 2001). IL-19 induces IL-6 and TNF-α production and apoptosis in monocytes (Liao *et al*., 2002). The involvement of the cytokines like IL-6 and TNF-α has been clearly demonstrated in the pathogenesis of psoriasis. Romer *et al*. (2003) have confirmed the pathogenic role of IL-19 and IL-20 in psoriasis demonstrating the higher expression of IL-19 and IL-20 and their receptors IL-20Rα and IL-20Rβ in involved psoriatic skin in contrast to uninvolved psoriatic skin.

Recent studies indicate that also the third member of the IL-19 subfamily – IL-24 (MDA-7) – might have a pathogenic role in psoriasis. The rat ortholog of the human IL-24 named as c49a has been found in fibroblast-like cells during skin wound healing (Soo *et al*., 1999). Elevated expression of c49a gene before and during the proliferation phase of repair suggests that c49a might promote cell proliferation. Moreover, the expression of IL-24 mRNA has been found in mononuclear cells located in the papillae and in the dense inflammatory areas in the subpapillary dermis in psoriatic lesions (Romer *et al*., 2003). Production of IL-24 by the infiltrating monocytes in the dermis may initiate persistent activation of receptor and abnormal proliferation of keratinocytes in the epidermis. Abnormal proliferation of keratinocyte is crucial for the development of psoriasis.

### 2.2.4. Genetic polymorphisms in IL-10-related cytokines

Recent studies indicate that around 5% of the human genome can be attributed to segmental duplications that result in the formation of gene clusters (Bailey *et al*., 2002). Clusters of genes are defined as three or more paralogous genes or pseudogenes that are present within a 1Mb stretch (Horton *et al.*, 2004).

IL-10 gene cluster locates in a 200 kb region of chromosome 1 within the locus q31-32 and holds four genes: IL-10 (Kim *et al*., 1992), IL-19 (Gallagher *et al*. 2000), IL-20 (Blumberg *et al*. 2001) and IL-24 (Jiang *et al*., 1995). The

IL-19, IL-20 and IL-24 genes are positioned in a head-to-tail direction, whereas the IL-10 gene is transcribed in the opposite direction toward the telomere. The structures of the IL-10 and IL-20 genes consist of five exons and four introns. Genes that encode IL-19 and IL-24 have additional exons positioned upstream of their first coding exons. For that reason the exons that encode the 5'-UTRs of the IL-19 and IL-24 mRNAs are alternatively spliced (Kotenko *et al.*, 2002).

Single nucleotide polymorphisms (SNPs) are the typically bi-allelic base-pair substitutions and there are approximately 6 million common SNPs in the human genome (Crawford *et al*., 2005; Hafler *et al.*, 2005). SNPs in cytokine IL-10 gene within the promoter region have extensively been investigated (Eskdale *et al*., 1995; 1997; D'Alfonso *et al*., 2000; Donger *et al*., 2001). Three SNPs of the IL-10 gene promoter region at positions -1082 G/A, -819 C/T and -592 C/A are closely linked and occur mainly as three haplotypes – GCC, ACC and ATA (Turner *et al*., 1997). The fourth potential haplotype GTA is extremely rare in Caucasians (Eskdale *et al*., 1999).

The results from twin and family studies have demonstrated that ~50…75% of the variation in IL-10 production is genetically determined (Reuss *et al*., 2002; Westendorp *et al*., 1997). The polymorphisms at the promoter region of the IL-10 gene are in close proximity to several transcription factors that may interfere with gene transcription (Kube *et al*., 1996). An association between IL-10 promoter SNPs and IL-10 expression levels has been shown, but different results have been conflicting. A possible reason for the incompatible results is that the regulation of IL-10 differs in different cell types (T cells, B cells and macrophages) and with different stimuli. Moreover, the transcription level and protein level of IL-10 may vary due to posttranscriptional regulation of IL-10 gene expression.

The relevance of IL-10 promoter SNPs has been demonstrated by their involvement in determining susceptibility and/or severity for a number of immune-inflammatory diseases including rheumatoid arthritis (Crawley *et al*., 1999), primary Sjogren's syndrome (Hulkkonen *et al*., 2001), inflammatory bowel disease (Tagore *et al*., 1999), systemic lupus erythematosus (Gibson *et al*., 2001) and severe asthma (Lim *et al*., 1998). At the present time only two studies have analyzed the association of IL-10 SNP at position -1082 with susceptibility to psoriasis (Craven *et al*., 2001; Reich *et al*., 1999). An increased frequency of the heterozygous G/A genotype has been described in the subset of patients with late onset of disease (Craven *et al*., 2001).

Moreover IL-10.G microsatellite from the promoter region has been identified to be protective against familial type of disorder (Hensen *et al*., 2003). Microsatellite IL-10.G locates at position -1.1kb of the IL-10 promoter region that is close to SNPs at positions -1082 and -819.

Descriptions of SNPs of IL-19, IL-20 and IL-24 genes are accessible in the NCBI dbSNP database (www.ncbi.nlm.nih.gov/SNP/). No polymorphisms of these genes have been investigated in complex diseases up to the present time.

The above described results suggested that genes of IL-10 cluster IL-10, IL-19, IL-20 and IL-24 may have effects on psoriasis. Therefore during the initial study we decided to perform association and haplotype analyses of the IL-10, IL-19, IL-20 and IL-24 genes in purpose to find potential link between polymorphic genes of IL-10 cluster and plaque-type psoriasis. Genomic locus containing IL-10, IL-19, IL-20 and IL-24 genes are shown in Figure 1. Alterations in expression levels of IL-10, IL-19, IL-20 and IL-24 have been demonstrated in the pathogenesis of plaque-type psoriasis. The variations in expression levels may be related to individual polymorphisms, haplotypes and extended haplotypes of IL-10, IL-19, IL-20 and IL-24 genes. For that reason during the second step of the study we investigated the functional significance of these genes to susceptibility and severity of plaque-type psoriasis.

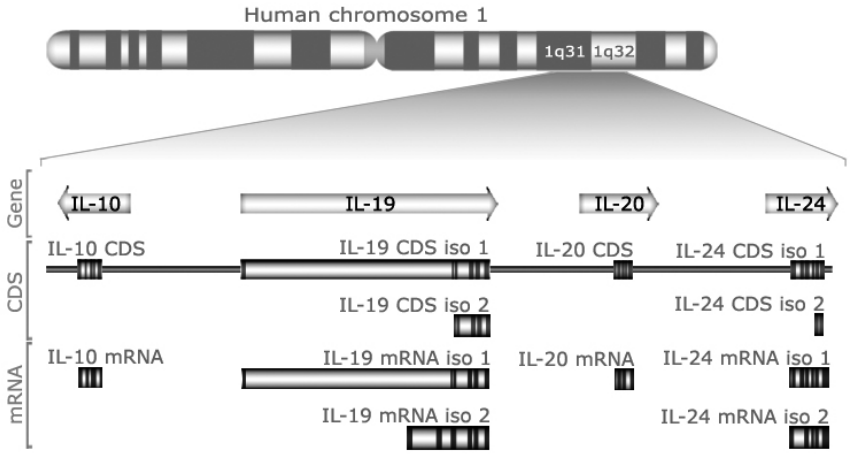



**Figure 1.** Genomic locus containing IL-10, IL-19, IL-20 and IL-24 genes. Coding regions (CDS) and mRNAs of respective genes are also shown in the illustration.

# 3. MAIN GOALS OF THE PROJECT

The general aim of the present work was to elucidate the role of the genes of IL-10 cluster (IL-10, IL-19, IL-20 and IL-24) in predicting the risk for plaque-type psoriasis.

Based on this the specific aims of our study were as follows:

1. To describe the linkage disequilibrium (LD) pattern emerging from the genes of IL-10 cluster.
2. To detect possible associations between polymorphisms of the IL-10, IL-19, IL-20 and IL-24 genes and plaque-type psoriasis.
3. To examine IL-10 and IL-20 gene expression variations between healthy controls and patients with plaque psoriasis.
4. To assess the functional significance of various genotypes and haplotypes of the IL-10 and the IL-20 genes in determining the susceptibility and severity of plaque-type psoriasis.

# 4. MATERIALS AND METHODS

## 4.1. Ethical considerations

The Ethics Review Committee on Human Research of University of Tartu approved the study protocols and the informed consent forms. All participants signed the written informed consent.

## 4.2. Characteristics of study participants

All the subjects who participated in this study – patients and control subjects – were Caucasians living in Estonia. Unrelated patients with plaque psoriasis from the Department of Dermatology, University of Tartu, were divided into subgroups according to the age of disease onset and family history of psoriasis. The age of disease onset was defined as the time at which the patient first became aware of psoriatic skin lesions. Patients with disease onset below the age of 40 were assigned as early onset psoriasis, while patients with onset of disease at the age of 40 and later years were referred to as the late onset disease. Patients were considered to have familial psoriasis if they had at least one first- or second-degree relative with psoriasis, and considered to have sporadic disease if they had no relatives with psoriasis. Patients were regarded to have type I psoriasis if they had the early onset of disease and familial psoriasis and type II psoriasis, if they had the late onset and sporadic form of disease (according to Henseler *et al*., 1985).

The severity of psoriasis was quantified by the Psoriasis Area and Severity Index (PASI) (by Fredriksson *et al*., 1978). The PASI scores were calculated on the basis of the degree of erythema, desquamation and infiltration of lesions combined with the surface extension. According to the PASI scores the patients were divided into two groups – PASI score equal or below 20 and PASI score above 20. The higher PASI score represents a greater degree of psoriatic severity. Disease severity was also scored solely in terms of the extent of the skin lesions at the time of the examination. On the basis of the extent the patients were divided into three groups: the extent of eruption equal or below 10%, the extent between 11% and 30% and the extent above 30% of psoriatic involvement of the body. The course of psoriasis was qualified as intermittent if periodically an eruption became better during one year and persistent if rash remained permanent (or progressed) for the whole previous year. We excluded the psoriasis patients who were treated with systemic anti-psoriasis therapy at the time of the examination of the severity of skin eruption and the course of psoriasis.

**Table 1.** Characteristics of study participants of the analysis of IL-10 promoter polymorphisms.

| | *Total number of individuals* | *Sex female/male* | *Patients with family history* | *Age range (year)* | *Mean onset of psoriasis (year)* |
|---|---|---|---|---|---|
| Psoriasis patients | 248 | 118/130 | 99 | 18–89 | 30 |
| Early onset psoriasis patients | 177 | 86/91 | 85 | 18–73 | 20 |
| Late onset psoriasis patients | 71 | 32/39 | 14 | 43–89 | 53 |
| Healthy controls | 148 | 91/57 | – | 18–71 | – |

**Table 2.** Characteristics of study participants of the analysis of IL-19, IL-20 and IL-24 genes.

| | *Total number of individuals* | *Sex female/male* | *Patients with family history* | *Age range (year)* | *Mean onset of psoriasis (year)* |
|---|---|---|---|---|---|
| Psoriasis patients | 254 | 119/135 | 101 | 18–89 | 30 |
| Early onset psoriasis patients | 180 | 86/94 | 85 | 18–73 | 20 |
| Late onset psoriasis patients | 74 | 33/41 | 16 | 43–89 | 53 |
| Healthy controls | 148 | 91/57 | – | 18–71 | – |

Healthy volunteers, free from the positive family history of psoriasis, served as a control group. Individuals with a history of other dermatoses were not included in the control group. Control subjects were recruited from among medical students, health care personnel and patients presenting at the dermatological outpatient clinic with either facial teleangiectasis or skin tags.

Main characteristics of study participants are shown in Table 1 and Table 2.

## 4.3. The criteria of selection of SNPs

For mapping disease loci we used SNP markers because of their high variability, abundance and their low mutation rate. The selection of IL-10 gene SNPs was based on previously published studies on associations between IL-10 promoter SNPs and complex diseases. The selected SNPs of the IL-10 gene promoter region were rs1800896 (IL-10 -1082 or -1087), rs1800871 (IL-10 -819 or -824) and rs1800872 (IL-10 -592 or -597). The dbSNP database search (on reference sequence NT_021877) was performed to find out the SNPs of the IL-19 gene, IL-20 gene and IL-24 gene. The selection criteria for SNP inclusion were the following: frequency of minor allele higher than 10% and distance between SNPs at least 1000 bp. Only validated SNPs were included in the study. The selected SNPs were rs2243158, rs2243168, rs2243191, rs2073186, rs2243174, rs2243188 and rs2243193 of the IL-19 gene; rs2981572, rs2981573, rs2232360 and rs1518108 of the IL-20 gene and rs3762344, rs291111, rs1150253, rs1150256 and rs1150258 of the IL-24 gene (Figure 2).

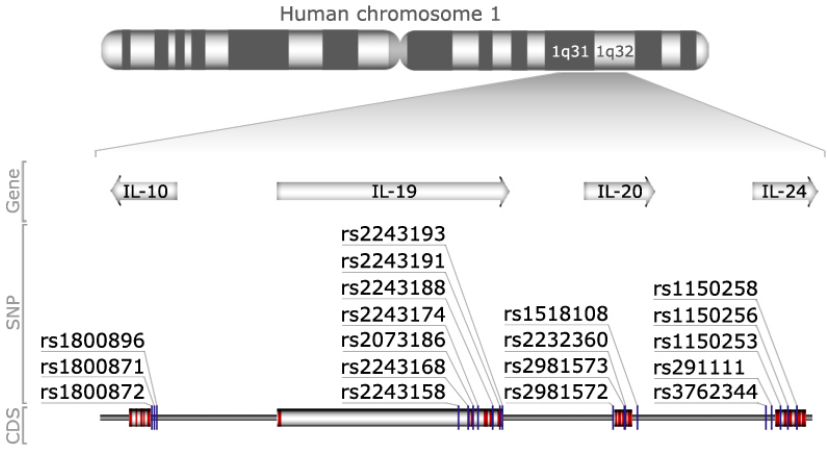



**Figure 2.** Selected SNPs of IL-10, IL-19, IL-20 and IL-24 genes.
128498bp fragment of human chromosome 1 locus 1q31-32. The selected SNPs are represented on the illustration by their cluster ID numbers in the public SNP database. Coding regions (CDS) of the IL-10, IL-19, IL-20 and IL-24 genes are shown as a box on a horizontal bar whereas the horizontal bar represents the noncoding region of the illustrated fragment.

## 4.4. DNA isolation

Peripheral blood was obtained from psoriasis patients and healthy controls. Genomic DNA was extracted from the whole blood. The standard protocol was used by which firstly the cells were lysed, then the nuclei were lysed and thereafter DNA was precipitated by ethanol. Formed precipitate was dissolved after the washing of DNA with 70% ethanol in Tris/EDTA.

## 4.5. Genotyping of polymorphisms

For the detection of SNPs in IL-10 gene the Amplification Refractory Mutation System-PCR (ARMS-PCR) method was applied. In ARMS-PCR method the reverse primer contains SNP specific substitution at 3'-terminal base (in general there are two reverse primers with different substitutions). The forward primer is similar to both reactions. For each SNP two reactions were applied (each with different reverse primer). One nucleotide mismatch at 3' end is sufficient to suppress exponential amplification. Described method has been widely used for screening multiple SNPs. Also, for IL-10 SNPs this method was published (Howell *et al*., 2001). Primers used for the detection of SNPs of IL-10 gene are shown in Table 3.

Novel tetra-primer ARMS-PCR method was applied for genotyping of polymorphisms of IL-19, IL-20 and IL-24 genes (based on Ye *et al*., 2001). The tetra-primer ARMS-PCR method employs two primer pairs to amplify, respectively, the two different alleles of an SNP in a single PCR reaction. Either the allele-specific amplicons are generated using one allele-specific inner primer and one non-allele-specific outer primer. Outer primers are also used to generate a non-allele-specific control product. Sets of four primers, specific to each SNP, were designed using an online program available at *cedar.genetics.soton.ac.uk/public_html/primer1.html*. Each PCR reaction was carried out in a total volume of 20 µl containing 100 ng of template DNA, 20 pmol of each inner primer, 2 pmol of each outer primer, 0.2 mM dNTP, 1x reaction buffer (75 mM TRIS-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 0.1 mg/ml Tartrazine, 1% Sucrose; produced by Naxo, Tartu, Estonia) and 0.5 U Smart-Taq DNA polymerase (also by Naxo, Tartu, Estonia). Sequences of the primers which were used for genotyping the respective polymorphisms of IL-19, IL-20 and IL-24 genes, optimized concentrations of $MgCl_2$ and annealing temperatures in respective reactions are shown in Tables 4, 5 and 6. To increase the specificity of a PCR reaction we applied touchdown cycles: initial denaturation at 95°C for 2 min followed by 10 cycles of 1 min denaturation at 95°C, annealing at 10°C higher than annealing temperature (in Table 4) for 1 min (decreasing by 1°C per cycle) and extension at 72°C for 1 min.

**Table 3.** Sequences and melting temperatures of primers, annealing temperatures for specific PCR reactions and optimized concentrations of $Mg^{2+}$ used for genotyping of SNPs of IL-10 gene.

| *Genetic polymorphism* | *Primer sequence (5' – 3')* | $T_m$ | *Annealing temperature* | $Mg^{2+}$ | *Amplicon size* |
|---|---|---|---|---|---|
| IL-10 -592 or -597 C/A rs1800872 | IL-10 -592 common primer: TAACTTAGGCAGTCACCTTAGG | 48°C | 65.5°C | 2.75 mM | 151 bp |
| | IL-10 -592 A primer: ACATCCTGTGACCCCGCCTGTA | 60°C | | | |
| | IL-10 -592 C primer: ACATCCTGTGACCCCGCCTGTC | 62°C | | | |
| IL-10 -819 or -824 C/T rs1800871 | IL-10 -819 common primer: GCTGTCCCCCACCCCAACTGTG | 64°C | 65.5°C | 2.75 mM | 166 bp |
| | IL-10 -819 T primer: ACCCTTGTACAGGTGATGTAAT | 47°C | | | |
| | IL-10 -819 C primer: ACCCTTGTACAGGTGATGTAAC | 48°C | | | |
| IL-10 -1082 or -1087 G/A rs1800896 | IL-10 -1082 common primer: CTTGGATTAAATTGGCCTTAGA | 50°C | 65.0°C | 4.8 mM | 210 bp |
| | IL-10 -1082 A primer: ACTACTAAGGCTTCTTTGGGAA | 49°C | | | |
| | IL-10 -1082 G primer: ACTACTAAGGCTTCTTTGGGAG | 49°C | | | |
| Control Primers | 63 TGCCAAGTGGAGCACCCAA | 57°C | as per specific SNP conditions | | 796 bp |
| | 64 GCATCTTGCTCTGTGCAGAT | 50°C | | | |

**Table 4.** Sequences and melting temperatures of primers, annealing temperatures for specific PCR reactions and optimized concentrations of $Mg^{2+}$ used for genotyping of SNPs of IL-19 gene.

| *Genetic polymorphism* | *Primers (5' – 3')* | $T_m$ | *Annealing temperature* | $Mg^{2+}$ | *Amplicon size* |
|---|---|---|---|---|---|
| rs2243158 G/C | Forward inner primer (C allele):<br>GGT GGA TCC ACC CAG CAA ACC TTC AC | 72˚C | 68˚C | 2,5 mM | 487 bp<br>– control |
| | Reverse inner primer (G allele):<br>TTT TAT TCA GGT GGA TAA GAG GAA ATG GTC | 66˚C | | | 290 bp<br>– G allele |
| | Forward outer primer:<br>GCC ACA GCT CTC AGG AAA GTG ACC TAA G | 69˚C | | | 253 bp<br>– C allele |
| | Reverse outer primer:<br>CCA GCA TCT GGA ACA TCA TAG CCA TAC A | 69˚C | | | |
| rs2243168 A/T | Forward inner primer (T allele):<br>GGA AGT TGC CAA GCT GCC CTC TAT CT | 69˚C | 60˚C | 3,5 mM | 328 bp<br>– control |
| | Reverse inner primer (A allele):<br>CAA TAA GGA GCT AGG GGA AGA AGC CGA T | 69˚C | | | 215 bp<br>– T allele |
| | Forward outer primer:<br>AGA AGG GTA AGA GAA TGA GAA GCG GTG G | 69˚C | | | 167 bp<br>– A allele |
| | Reverse outer primer:<br>TGG TTT TTG ATG TTT GCC CCT GAA ATA A | 69˚C | | | |
| rs2073186 T/C | Forward inner primer (C allele):<br>AGG TGC TCA GAG GGG ACA GGA TTG CC | 73˚C | 65˚C | 2,5 mM | 295 bp<br>– control |
| | Reverse inner primer (T allele):<br>ACC TTC CAA AAT TAC CCC CAA GCC CCA | 73˚C | | | 209 bp<br>– C allele |
| | Forward outer primer:<br>TGT TAG GGC ACG CTA GTG TCC CAG GGA TA | 73˚C | | | 139 bp<br>– T allele |
| | Reverse outer primer:<br>TCC TTT GGG TCA CAA ACC TGG TCA CCT C | 73˚C | | | |

**Table 4.** (Continuation)

| *Genetic polymorphism* | *Primers (5' – 3')* | $T_m$ | *Annealing temperature* | $Mg^{2+}$ | *Amplicon size* |
|---|---|---|---|---|---|
| rs2243174 A/G | Forward inner primer (A allele):<br>TGG TGC TGT TCT TAC AAT GGA CAC CA | 68˚C | 60˚C | 2,5 mM | 407 bp<br>– control |
| | Reverse inner primer (G allele):<br>GAT CTT GTC ACT GGC TTT CCT GCC TAA C | 68˚C | | | 272 bp<br>– G allele |
| | Forward outer primer:<br>TTG GAA TCC CTG GGA GGA ATT AAA GAA G | 68˚C | | | 189 bp<br>– A allele |
| | Reverse outer primer:<br>AGA ATA CTT CCC AGG ACT GGA GGA GCT C | 68˚C | | | |
| rs2243188 C/A | Forward inner primer (C allele):<br>TGG GGA AGA TGG AAG ATG AGA GGT AGA CCC | 72˚C | 65˚C | 2,5 mM | 366 bp<br>– control |
| | Reverse inner primer (A allele):<br>AGG AGG CTG AAG GCC TGG CAC CCT TCT | 76˚C | | | 238 bp<br>– A allele |
| | Forward outer primer:<br>AAT GTC ACT TCT CAT GTG GGG AGG CAG GA | 74˚C | | | 185 bp<br>– C allele |
| | Reverse outer primer:<br>AAT GAT GGA GAT GGG GAG CCC AGG AGA T | 74˚C | | | |
| rs2243191 C/T | Forward inner primer (C allele):<br>CTG GAT TAA TAA GAA TCA TGA AGT AAT TTC | 59 ˚C | 60˚C | 2,5 mM | 436 bp<br>– control |
| | Reverse inner primer (T allele):<br>ATA CAG GTT CCT TGT CAT CAA GCT GCG A | 70˚C | | | 279 bp<br>– T allele |
| | Forward outer primer:<br>CCT GCG AGG AAA ATA ATA TTG AGT CTG T | 64˚C | | | 215 bp<br>– C allele |
| | Reverse outer primer:<br>CAC AGT AGA CTT TTT GGC TAT TTT CAA GC | 64˚C | | | |
| rs2243193 G/A | Forward inner primer (A allele):<br>AAG GGC TGC CTT CCC ATC TAA TTT ATT TTA | 67˚C | 60˚C | 2,5 mM | 414 bp<br>– control |
| | Reverse inner primer (G allele):<br>TCA CAT CAC AGA CAT GGA CTA TAT GAC GTC | 67˚C | | | 281 bp<br>– G allele |
| | Forward outer primer:<br>GGA ACC TGT ATA GTG ATC CAG GGA TGA A | 67˚C | | | 193 bp<br>– A allele |
| | Reverse outer primer:<br>CTG TCA GAA ACA CCC TGT CCT CAG TCT T | 67˚C | | | |

**Table 5.** Sequences and melting temperatures of primers, annealing temperatures for specific PCR reactions and optimized concentrations of $Mg^{2+}$ used for genotyping of SNPs of IL-20 gene.

| *Genetic polymorphism* | *Primer sequence (5' – 3')* | $T_m$ | *Annealing temperature* | $Mg^{2+}$ | *Amplicon size* |
|---|---|---|---|---|---|
| IL-20 -1053 T/G rs2981572 | Forward inner primer (T allele): TTGTCATAAGCTTTTTAATTCATTCTT | 58°C | 55°C | 4.5 mM | 156 bp – T allele |
| | Reverse inner primer (G allele): CAAGATAAAAATATTTTAGTGCAATGTC | 58°C | | | 219 bp – G allele |
| | Forward outer primer: ACTCATCAATAATATTTTCATCATATGCT | 58°C | | | 320 bp – control |
| | Reverse outer primer: AGTTTTAAGATAAAATAATAATGGGCTG | 58°C | | | |
| IL-20 1380 A/G rs2981573 | Forward inner primer (A allele): CCTCTCCTAGCTGATGATGAACTGAA | 64°C | 58°C | 2.5 mM | 181 bp – A allele |
| | Reverse inner primer (G allele): CTCTTTCAGACCTCACATTTGGAATAAC | 64°C | | | 255 bp – G allele |
| | Forward outer primer: TCTGAATAGGACCTAGGAATTCAATTCTTT | 64°C | | | 382 bp – control |
| | Reverse outer primer: ATGCTGAAAAGGACCCAAAGAATAATAG | 64°C | | | |
| IL-20 1462 A/G rs2232360 | Forward inner primer (G allele): AGGGTTGTGGGTGAAAGAGTAGAGTTTATG | 66°C | 60°C | 2.5 mM | 200 bp – G allele |
| | Reverse inner primer (A allele): GATTTTTTGCCATTGACATGAAGCATGT | 68°C | | | 273 bp – A allele |
| | Forward outer primer: GGGATAGAGCTCCTAGACTACAGCTGGG | 67°C | | | 415 bp – control |
| | Reverse outer primer: CAATCAATGCCAAAGTCGCATATACCTT | 67°C | | | |
| IL-20 3978 T/C rs1518108 | Forward inner primer (T allele): CTTCTTCCCCCACCTCACTCTGACGT | 70°C | 60°C | 2.5 mM | 171 bp – T allele |
| | Reverse inner primer (C allele): AGAAAACTAAGCAACTAAATTGGGGGTTCG | 68°C | | | 249 bp – C allele |
| | Forward outer primer: AGACAGGTGTATGAGCCCCCTTTTGAG | 69°C | | | 364 bp – control |
| | Reverse outer primer: GTCATTGAAACTATATCAGGGCCCAGGC | 69°C | | | |

**Table 6.** Sequences and melting temperatures of primers, annealing temperatures for specific PCR reactions and optimized concentrations of $Mg^{2+}$ used for genotyping of SNPs of IL-24 gene.

| *Genetic polymorphism* | *Primers (5′ – 3′)* | $T_m$ | *Annealing temperature* | $Mg^{2+}$ | *Amplicon size* |
|---|---|---|---|---|---|
| rs3762344 G/A | Forward inner primer (A allele):<br>GTT CTC TCC TTG ACC TTC CTT CTG CAA | 67˚C | 60˚C | 2,5 mM | 357 bp<br>– control |
| | Reverse inner primer (G allele):<br>CCT CCG ATG AGC TTA TCA TAG CAT TAT GAC | 67˚C | | | 225 bp<br>– G allele |
| | Forward outer primer:<br>CAG GTC ATC ACA TCC TCA TGG TCT TAT G | 67˚C | | | 189 bp<br>– A allele |
| | Reverse outer primer:<br>AAC AGA GGC ATG AGG TCA TGG ATA CAC | 67˚C | | | |
| rs291111 T/C | Forward inner primer (T allele):<br>AGT CAC AAC TAC TCA TCT CTG CCT CGG T | 67˚C | 60˚C | 3,5 mM | 401 bp<br>– control |
| | Reverse inner primer (C allele):<br>GAC ATA TTG TTG CTA ATT GCT TTC GTG ATG | 67˚C | | | 264 bp<br>– C allele |
| | Forward outer primer:<br>GTG CCA GAG AGA TTG GGA AGA GTC TG | 67˚C | | | 195 bp<br>– T allele |
| | Reverse outer primer:<br>AGA ATG GCT TAA CCT TGC GAC CTA AGA G | 67˚C | | | |
| rs1150253 A/G | Forward inner primer (G allele):<br>TTG AGG GAG ATG CTA AAG CAT AGC CCA CG | 73˚C | 63˚C | 2,5 mM | 399 bp<br>– control |
| | Reverse inner primer (A allele):<br>CCC CTG GGT TAT GGG TCA CGG GGT AT | 73˚C | | | 249 bp<br>– G allele |
| | Forward outer primer:<br>GCA TGA GCA TCA CTT GAA CCT GGT AGG CA | 73˚C | | | 205 bp<br>– A allele |
| | Reverse outer primer:<br>TCC TTC TTT CCT CTC TCG CTC CCT CCC T | 73˚C | | | |

**Table 6.** (Continuation)

| | | | | | |
|---|---|---|---|---|---|
| rs1150256 A/G | Forward inner primer (A allele):<br>AGA GAG CTA GAT TCA TCC AGG CTG CA | 67˚C | 60˚C | 2,5 mM | 381 bp<br>– control |
| | Reverse inner primer (G allele):<br>AGA TTT AGA GCC GAA GAC TCT ATT CGG TAC | 65˚C | | | 239 bp<br>– G allele |
| | Forward outer primer:<br>GAG GGG ATG CTA TTT TAT GAT TCT GGA GT | 66˚C | | | 198 bp<br>– A allele |
| | Reverse outer primer:<br>TTT AAG AAC CAC TAT GGG CAG GAT TTT G | 66˚C | | | |
| rs1150258 C/T | Forward inner primer (C allele):<br>CTA CTT GAA AAC TGT TTT CAA AAC CC | 60˚C | 60˚C | 2,5 mM | 412 bp<br>– control |
| | Reverse inner primer (T allele):<br>CCT GAC TTC AAC TGT TCT ATT GTG TTA | 60˚C | | | 249 bp<br>– C allele |
| | Forward outer primer:<br>CAC CTT CTA GAA GAT CCC TAT CTC TG | 60˚C | | | 219 bp<br>– T allele |
| | Reverse outer primer:<br>ATC CCC ATT TTA CAG ATA ACA AAC TG | 60˚C | | | |

The following 25 cycles were performed at an appropriate annealing temperature, followed by the final extension at 72°C for 10 min. PCR products were separated on gel electrophoresis using 2% agarose gel and visualized by ethidium bromide staining.

The direct sequencing of incidental DNA samples was performed using ABI Genetic Analyzer 310 (Applied Biosystems, Foster City, CA, USA) for verification of the tetra-primer ARMS-PCR results and validation of the polymorphisms. The results of the tetra-primer ARMS-PCR were completely consistent with the results of direct sequencing.

## 4.6. Gene expression analysis

TaqMan-QRT-PCR method with gene specific primers was applied to analyze the gene expression levels (Ginzinger *et al*., 2002). For that purpose blood samples from previously genotyped psoriasis patients and healthy controls were collected.

Pax Gene Blood collecting tubes and RNA kit (Qiagen, Hilden, Germany) were used to collect and extract RNA. The blood was collected in the morning between 8.00 and 12.00 to minimize the effect of circadian variation in cytokine production (Westendorp *et al*., 1997). The RNA expression was determined in whole blood as this method represents a more physiological situation than examination of isolation PBMC (Crawley *et al*., 1999). cDNA was synthesized applying reverse transcriptase reaction with oligo(dT)18 primers using RevertAid$^{TM}$ First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania) in pursuance of manufacturer's protocol. Synthesized cDNA was analyzed using gene specific primers, TaqMan® probes and TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The sequences and concentrations of primers and 6-carboxy-fluorescein (FAM)/6-carboxytetramethyl-rhodamine (TAMRA) double-labelled probes were analogous as described by Wolk *et al.* (2002) and are shown in Table 7. IL-10 and IL-20 expression levels were calculated in relation to the housekeeping gene HPRT-1 (hypoxanthine phosphoribosyl-transferase-1).

**Table 7.** The sequences and concentrations of primers and 6-carboxy-fluorescein (FAM)/6-carboxytetramethyl-rhodamine (TAMRA) double-labelled probes.

| *Gene* | *Oligonucleotide Sequences* | *Conc. (nM)* | *Product size (bp)* |
|---|---|---|---|
| IL-10 | 5'-GGCAACCTGCCTAACATGCTT-3' (exon 1)<br>5'-CAAGTTGTCCAGCTGATCCTTCAT-3' (exon 2)<br>FAM-5'-AAAGAAAGTCTTCACTCTGCTGAAGGCATCTCG-3'-TAMRA | 900<br>50<br>200 | 90 |
| IL-20 | 5'-GGAGGACTGAGTCTTTGCAAGAC-3' (exon 2)<br>5'-CCGGAGAGTATAATGGTCAGGG-3' (exon 3)<br>FAM-5'-CAAAGCCTGCGAATCGATGCTGC-3'-TAMRA | 900<br>900<br>200 | 125 |
| HPRT-1 | 5'-GACTTTGCTTTCCTTGGTCAGG-3' (exon 6)<br>5'-AGTCTGGCTTATATCCAACACTTCG-3' (exon 7)<br>FAM-5'-TTTCACCAGCAAGCTTGCGACCTTGA-3'-TAMRA | 300<br>300<br>200 | 101 |

## 4.7. Statistical analysis

Statistical analysis of the genotype-phenotype associations was performed using population genetics software package GENEPOP version 3.3 (Raymond *et al*., 1995). This program computes a range of different population genetic parameters for co-dominant markers. These parameters include exact tests for Hardy-Weinberg equilibrium, population differentiation etc.

Haplotype-based association analysis of individuals was performed using the THESIAS program. This program is based on the maximum likelihood model described in Tregouet *et al.* (2002) and is linked to the SEM algorithm (Tregouet *et al*., 2004) allowing the simultaneous estimation of haplotype frequencies and of their associated effects on the phenotype of interest. The pairwise LD matrix was computed using the same program. The extent of disequilibrium was expressed in terms of standardized D'characteristic. An absolute value of D' of 1 indicates complete LD and 0 corresponds to no LD.

Expression levels of IL-10 and IL-20 mRNA were calculated in relation to the housekeeping gene HPRT-1. For quantification of mRNA we used the comparative Ct method, where the amount of the target gene was normalized to the level of endogenous reference (ΔCt value). The values of expressions of IL-10 and IL-20 distribute with an approximately normal distribution. Biostatistic and graphing program GraphPad Prism was used for further statistical analysis where unpaired Student's t-test and one way ANOVA were applied.

The significance level of the tests for declaring a probability value as significant was set to 0.05.

# 5. RESULTS

## 5.1. Association, linkage disequilibrium and haplotype analyses of the IL-10 gene

An association study with three SNPs in the IL-10 promoter region - IL-10 -1082 G/A (rs1800896), IL-10 -819 C/T (rs1800871) and IL-10 -592 C/A (rs1800872) – was performed where 248 unrelated patients suffering from plaque-type psoriasis were compared with 148 healthy controls. Genotype distributions of examined groups (cases and controls) were in Hardy-Weinberg equilibrium (HWE). We found that 54.7% of the control population had the allele G and 45.3% had the allele A at position -1082, 70.9% had the allele C at positions -819 and -592 and 29.1% had the allele T at position -819 and the allele A at position -592 in the IL-10 gene. On comparing psoriasis patients with control group the SNPs of IL-10 promoter did not associate with susceptibility to psoriasis. However, the differencies of distributions of alleles and genotypes at positions -592 and -819 between patients with intermittent and persistent eruption were statistically significant with allelic p-value 0.001 and genotypic p-value 0.001. The over-representation of the allele -592 A and the allele -819 T and over-representation of the genotype -592 A/A and the genotype -819 T/T in the subgroup of persistent eruption was found (Table 8).

The measure of LD (denoted as D') was calculated for all pairs of IL-10 promoter SNPs on pooled samples. The pairwise LD matrix presented that the nearly complete linkage disequilibrium ($|D'|$ between 0.95 and 1.00) existed between the polymorphisms within the IL-10 gene. The presence of three haplotypes (HT1 GCC, HT2 ACC and HT3 ATA) with a frequency ≥1% was estimated in the pooled samples. These haplotypes accounted for 99.5% of all possible haplotypes. The frequencies of haplotypes were alike with those published previously by Koss *et al.* (2000) about Caucasians. The results of comparison of haplotype frequencies for IL-10 promoter SNPs between psoriasis patients and controls are presented in Table 9. No significant effects were observed about these haplotypes in susceptibility to plaque-type psoriasis. Comparison of controls and different psoriasis subgroups indicated that there were no significant differences in the distribution of haplotypes depending on the age of onset and family history of psoriasis. Nevertheless, the ACC haplotype was significantly over-represented in patients with PASI score equal or below 20 ($p<0.05$, OR 1.463, 95% CI 1.008 – 2.123) and also in patients with limited eruption ($p<0.05$, OR 1.634, 95% CI 1.040 – 2.566) when compared with controls (Table 10). Comparing the distribution of IL-10 haplotypes between patients with intermittent and persistent eruption we found that more patients with persistent eruption had the haplotype ATA ($p<0.01$, OR 2.445, 95% CI 1.384 – 4.318) (Table 11).

**Table 8.** Frequencies of genotypes and alleles of the -819 C/T and -592C/A polymorphisms of the IL-10 gene in controls and in the whole group of psoriasis patients and in subgroups of intermittent and persistent course of psoriasis (%).

| *-819C>T/ -592C>A* | *Genotypes* | | | *Alleles* | |
|---|---|---|---|---|---|
| | $1^a/1^a$ | $1^a/2^b$ | $2^b/2^b$ | $1^a$ | $2^b$ |
| Controls n=148 | 51.3 | 39.2 | 9.5 | 70.9 | 29.1 |
| Psoriasis patients n=248 | 56.4 | 38.0 | 5.6 | 75.4 | 24.6 |
| Patients with intermittent course of disease n=188 | 60.3 | 36.6 | 3.1 | 78.6 | 21.4 |
| Patients with persistent course of disease n=41 | 39.0 | 43.9 | 17.1* | 61.0 | 39.0* |

* – $p<0.05$ allelic and genotypic differences compared the patients with persistent course of disease to the patients with intermittent course of disease
$1^a$ – most frequent allele
$2^b$ – least frequent allele

**Table 9.** Results of haplotype analysis of the SNPs of IL-10 promoter region in patients with plaque-type psoriasis. The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values by comparison with the reference haplotype in psoriasis patients compared with controls are indicated. Haplotype frequencies and haplotype-phenotype associations were estimated using maximum likelihood method.

| *Haplotype* | *IL-10 -1082* | *IL-10 -819* | *IL-10 -592* | *Controls (n=148)* | *Psoriasis patients (n=248)* | *Haplotypic OR (95% CI)* | *p-value* |
|---|---|---|---|---|---|---|---|
| HT1 | G | C | C | 44.9 | 42.6 | * | |
| HT2 | A | C | C | 26.4 | 32.7 | 1.277 (0.907–1.796) | 0.16 |
| HT3 | A | T | A | 28.3 | 24.2 | 0.889 (0.629–1.254) | 0.50 |

* – the haplotype combining the most frequent alleles at each position is chosen as the reference haplotype (GCC).

**Table 10.** The ACC haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values by comparison with the reference haplotype in the whole group of psoriasis patients and in subgroups of patients according to the severity and the course of disease compared with controls are indicated.

| *Case/control status* | *HT2 ACC haplotype frequency (%)* | *OR (95% CI)* | *p-value* |
|---|---|---|---|
| Controls n=148 | 26.4 | | |
| Psoriasis patients n=248 | 32.7 | 1.277 (0.907–1.796) | 0.16 |
| Patients with extent of eruption equal or below 10% n=65 | 39.5 | 1.637 (1.040–2.566) | 0.03 |
| Patients with extent of eruption between 11% and 30% n=75 | 31.2 | 1.216 (0.762–1.940) | 0.41 |
| Patients with extent of eruption above 30% n=89 | 28.5 | 1.113 (0.722–1.714) | 0.63 |
| Patients with PASI equal or below 20 n=147 | 37.9 | 1.463 (1.008–2.123) | 0.04 |
| Patients with PASI above 20 n=82 | 26.2 | 0.968 (0.612–1.530) | 0.89 |
| Patients with intermittent course of psoriasis n=188 | 33.9 | 1.242 (0.868–1.778) | 0.23 |
| Persistent course of psoriasis n=41 | 28.6 | 1.319 (0.745–2.336) | 0.34 |

**Table 11.** The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values by comparison with the reference haplotype in subgroup of patients with persistent course of psoriasis compared with subgroup of patients with intermittent course of psoriasis are indicated. Haplotype frequencies and haplotype-phenotype associations were estimated using maximum likelihood method.

| *Haplo-type* | *IL-10 -1082* | *IL-10 -819* | *IL-10 -592* | *Intermittent course of psoriasis (n=188)* | *Persistent course of psoriasis (n=41)* | *Haplotypic OR (95% CI)* | *p-value* |
|---|---|---|---|---|---|---|---|
| HT1 | G | C | C | 45.4 | 33.8 | * | |
| HT2 | A | C | C | 33.9 | 28.6 | 1.134 (0.651–1.975) | 0.66 |
| HT3 | A | T | A | 21.3 | 37.4 | 2.445 (1.384–4.318) | 0.002 |

*– the haplotype combining the most frequent alleles at each position is chosen as the reference haplotype (GCC).

## 5.2. Association, linkage disequilibrium and haplotype analyses of the IL-20 gene

We analyzed the frequencies of SNPs at positions -1053 T/G (rs2981572), 1380 A/G (rs2981573), 1462 A/G (rs2232360) and 3978 T/C (rs1518108) of the IL-20 gene involving 254 patients with plaque-type psoriasis and 148 unrelated healthy volunteers. Genotype distributions for the four analyzed IL-20 gene polymorphisms had no deviation from HWE. The results of association analysis represented that 71% of the control population had the allele T and 29% had the allele G at position -1053; 76% had the allele A and 24% had the allele G at position 1380; 75% had the allele A and 25% had the allele G at position 1462 and 54% had the allele T and 46% had the allele C at position 3978 in the IL-20 gene.

The higher frequency of the -1053 allele G ($p<0.05$) in the patient group with plaque psoriasis compared to the control group was observed. Additionally, over-representation of the IL-20 -1053 G allele of patients with disease onset earlier than 40 years ($p<0.05$) and of patients with sporadic disease ($p<0.05$) was found. The frequency of the -1053 heterozygous genotype T/G was higher and the frequency of the homozygous genotype T/T was lower in psoriasis patients when compared with the control group, but the difference of frequencies was not statistically significant ($p=0.08$) (Table 12).

The association analysis of the 1380 A/G and the 1462 A/G polymorphisms resulted in negative findings for both allele and genotype distributions (Table 13 and Table 14). Although there appeared to be fewer persons possessing the

IL-20 1380 G/G homozygous genotype in the psoriasis group, the difference was not statistically significant (p=0.09) (Table 13). We suppose that the allele G at position 1380 might reduce the risk of psoriasis but further studies with large numbers of G/G subjects would be warranted to support this evidence.

At position IL-20 3978 the frequencies of both genotypes T/T and C/C were higher and the frequency of the heterozygous genotype T/C was lower in the psoriasis group in comparison with the control group ($p<0.05$). However, the differencies of frequencies of alleles T and C were not statistically significant (p=0.9) (Table 15).

The pairwise LD matrix demonstrated that the nearly complete LD ($|D'|$ between 0.879 and 0.985) existed between the polymorphisms of positions -1053, 1380 and 1462 within the IL20 gene. The presence of five haplotypes (HT1 TAA, HT2 GGG, HT3 GAA, HT4 GAG and HT5 TGG) each with a frequency ≥1% was found. These haplotypes covered 98.9 % from all possible haplotypes in the pooled samples. Frequencies of common haplotypes of IL-20 SNPs were compared between psoriasis patients and controls and the results are present in Table 16.

The frequency of the haplotype HT3 GAA in the group of psoriasis patients was significantly higher ($p<0.01$, OR 2.341, 95%CI 1.346-4.074) compared with the control group. Similarly the haplotype HT3 GAA was over-represented in the subgroup of early onset psoriasis ($p<0.01$, OR 2.305, 95%CI 1.285-4.132) and in the subgroup of late onset of disease ($p<0.01$, OR 2.542, 95% CI 1.266-5.102); in the subgroup of familial psoriasis ($p<0.02$, OR 2.220, 95%CI 1.249-3.945) and in the subgroup of sporadic disease ($p<0.01$, OR 2.523, 95%CI 1.390-4.580). No significant associations were observed concerning the other common haplotypes.

**Table 12.** Frequencies of genotypes and alleles of the -1053 T/G polymorphism of the IL-20 gene in controls and patients with psoriasis (%).

| *-1053 T>G* | *Genotype* | | | *Alleles* | |
|---|---|---|---|---|---|
| | *T/T* | *T/G* | *G/G* | *T* | *G* |
| Controls n=148 | 52.0 | 37.9 | 10.1 | 70.9 | 29.1 |
| Psoriasis patients n=254 | 40.6 | 46.8 | 12.6 | 64.0 | 36.0* |
| Early onset psoriasis n=180 | 39.5 | 48.3 | 12.2 | 63.6 | 36.4* |
| Late onset psoriasis n=74 | 43.25 | 43.25 | 13.5 | 64.9 | 35.1 |
| Familial psoriasis n=101 | 42.6 | 46.5 | 10.9 | 65.8 | 34.2 |
| Sporadic psoriasis n=153 | 39.2 | 47.1 | 13.7 | 62.7 | 37.3* |

* – $p<0.05$ allelic differences compared to the controls

**Table 13.** Frequencies of genotypes and alleles of the 1380 A/G polymorphism of the IL-20 gene in controls and patients with psoriasis (%).

| *1380 A>G* | *Genotype* | | | *Alleles* | |
|---|---|---|---|---|---|
| | *A/A* | *A/G* | *G/G* | *A* | *G* |
| Controls n=148 | 60.1 | 31.8 | 8.1 | 76.0 | 24.0 |
| Psoriasis patients n=254 | 63.4 | 33.5 | 3.1 | 80.1 | 19.9 |
| Early onset psoriasis n=180 | 62.2 | 33.9 | 3.9 | 79.2 | 20.8 |
| Late onset psoriasis n=74 | 66.2 | 32.4 | 1.4 | 82.4 | 17.6 |
| Familial psoriasis n=101 | 64.3 | 33.7 | 2.0 | 81.2 | 18.8 |
| Sporadic psoriasis n=153 | 62.8 | 33.3 | 3.9 | 79.4 | 20.6 |

**Table 14.** Frequencies of genotypes and alleles of the 1462 A/G polymorphism of the IL-20 gene in controls and patients with psoriasis (%).

| *1462 A>G* | *Genotype* | | | *Alleles* | |
|---|---|---|---|---|---|
| | *A/A* | *A/G* | *G/G* | *A* | *G* |
| Controls n=148 | 58.8 | 33.1 | 8.1 | 75.3 | 24.7 |
| Psoriasis patients n=254 | 60.3 | 35.4 | 4.3 | 78.0 | 22.0 |
| Early onset psoriasis n=180 | 57.8 | 37.2 | 5.0 | 76.4 | 23.6 |
| Late onset psoriasis n=74 | 66.2 | 31.1 | 2.7 | 81.8 | 18.2 |
| Familial psoriasis n=101 | 60.4 | 37.6 | 2.0 | 79.2 | 20.8 |
| Sporadic psoriasis n=153 | 60.1 | 34.0 | 5.9 | 77.1 | 22.9 |

**Table 15.** Frequencies of genotypes and alleles of the 3978 T/C polymorphism of the IL-20 gene in controls and patients with psoriasis (%).

| *3978 T>C* | *Genotypes* | | | *Alleles* | |
|---|---|---|---|---|---|
| | *T/T* | *T/C* | *C/C* | *T allele* | *C allele* |
| Controls n=148 | 25.0 | 58.1 | 16.9 | 54.1 | 45.9 |
| Psoriasis patients n=254 | 31.1 | 44.9* | 24.0 | 53.5 | 46.5 |
| Early onset psoriasis n=180 | 31.7 | 45.0 | 23.3 | 54.2 | 45.8 |
| Late onset psoriasis n=74 | 29.7 | 44.6 | 25.7 | 52.0 | 48.0 |
| Familial psoriasis n=101 | 31.7 | 44.5 | 23.8 | 54.0 | 46.0 |
| Sporadic psoriasis n=153 | 30.7 | 45.1 | 24.2 | 53.3 | 46.7 |

* – $p<0.05$ genotypic differences compared to the controls

**Table 16.** Results of haplotype analysis of the IL-20 gene. The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values in comparison with the reference haplotype are indicated. Haplotype-phenotype associations were estimated using maximum likelihood method.

| *Haplo-type* | *IL20 -1053* | *IL20 1380* | *IL20 1462* | *Controls (n=148)* | *Psoriasis patients (n=254)* | *Haplotypic OR (95% CI)* | *p-value* |
|---|---|---|---|---|---|---|---|
| HT1 | T | A | A | 68.1 | 62.7 | * | |
| HT2 | G | G | G | 21.5 | 19.3 | 0.975 (0.682 – 1.394) | 0.92 |
| HT3 | G | A | A | 6.9 | 15.1 | 2.341 (1.346 – 4.074) | 0.002 |
| HT4 | G | A | G | 0.3 | 1.4 | 3.454 (0.264 – 45.094) | 0.344 |
| HT5 | T | G | G | 2.1 | 0.4 | – | – |

*– the haplotype combining the most frequent alleles at each site is chosen as the reference haplotype (TAA).
– – the haplotypic OR by comparison to the reference with its 95% CI was not estimated

## 5.3. Association, linkage disequilibrium and haplotype analyses of the IL-19 gene

An association study with seven SNPs of IL-19 gene – rs2243158, rs2243168, rs2243191, rs2073186, rs2243174, rs2243188 and rs2243193 – was performed using study population similar with the one used for IL-20 gene investigation.

Genotype distributions of the seven analyzed IL-19 gene polymorphisms had no deviation from HWE. Allele frequencies of IL-19 SNPs of controls and cases are reported in Table 17. The patients with psoriasis had a lower frequency of the SNP rs2243188 minor allele A (19.5% versus 26.0%, $p<0.05$) when comparing the psoriasis group with controls. Therefore the rs2243188 minor allele presumedly has a protective effect against susceptibility to psoriasis. Likewise the lower representation of the allele A at this position in patients with late onset psoriasis (16.0% versus 26.0% in controls, $p<0.02$) and in sporadic psoriasis (19.3% versus 26.0% in controls, $p<0.05$) was observed. Although there appeared to be fewer persons possessing the SNP rs2243188 allele A in the early onset and familial psoriasis group, the differences were not statistically significant. The prevalence of the minor allele at position rs2243158 was significantly higher in controls than in the subgroup of patients with type II phenotype (10.1% versus 4.1%, $p<0.05$) and the prevalence of the minor allele at position rs2243168 was significantly higher in controls than in the subgroup of patients with late onset disease (8.8% versus 3.3%, $p<0.05$) and in the subgroup of patients with type II phenotype (8.8% versus 2.5%, $p<0.02$). Due to those reasons rs2243158 and rs2243168 are represented as potential subtype specific markers.

LD and haplotype analysis of the IL-19 gene was executed to test whether the individual protective effect of the IL-19 polymorphisms that was observed in the single-marker association analysis depends on the haplotypic background. The pairwise LD matrix showed that the nearly complete LD ($|D'|$ between 0.88 and 0.99) existed between the polymorphisms within the IL-19 gene. We excluded SNP rs2243158 and SNP rs2243168 from the further haplotype analysis because the frequencies of minor alleles of these polymorphisms were lower than 0.10. The presence of five common haplotypes (HT1 CACCG, HT2 TGATA, HT3 CACTA, HT4 TAATA and HT5 TACCG) with a frequency $\geq 1\%$ was established. The common haplotypes covered 97.7% of all haplotypes in the pooled samples. The control group had a higher frequency of the haplotype HT2 TGATA than the psoriasis group, but the difference was not statistically significant ($p=0.09$, OR 0.737, 95% CI 0.516-1.053). Nevertheless, the haplotype HT2 TGATA was significantly more frequent in controls when compared with the late onset psoriasis subgroup ($p=0.05$; OR 0.58, 95% CI 0.335-1.00). The frequencies of the IL-19 gene haplotypes and haplotype effects are presented in Table 18.

**Table 17.** Results of association analysis of IL-19 gene SNPs in plaque-type psoriasis. Allele frequencies of the seven studied polymorphisms of the IL-19 gene were compared between the psoriatis patients (n=254) and the controls (n=148), p-values for allelic association were calculated using Fisher's exact test.

| *SNP ID* | *Polymorphism* | | *Allele frequency in controls (%)* | | *Allele frequency in cases (%)* | | *p-value* |
|---|---|---|---|---|---|---|---|
| | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | |
| rs2243158 | G | C | 89.9 | 10.1 | 91.9 | 8.1 | 0.36 |
| rs2243168 | A | T | 91.2 | 8.8 | 93.3 | 6.7 | 0.33 |
| rs2073186 | C | T | 73.0 | 27.0 | 78.3 | 21.7 | 0.08 |
| rs2243174 | A | G | 76.7 | 23.3 | 81.1 | 18.9 | 0.14 |
| rs2243188 | C | A | 74.0 | 26.0 | 80.5 | 19.5 | 0.029* |
| rs2243191 | C | T | 74.0 | 26.0 | 78.0 | 22.0 | 0.23 |
| rs2243193 | G | A | 73.0 | 27.0 | 77.0 | 23.0 | 0.21 |

* – $p<0.05$ allelic differences compared to the controls
1[a] – most frequent allele
2[b] – least frequent allele

**Table 18.** Frequencies of common haplotypes in the IL-19 gene according to the case/control status. The haplotypic OR-s with their 95% CI-s and p-values in comparison with the reference haplotype are also indicated. Haplotype-phenotype associations were estimated using maximum likelihood method.

| *Haplo-type* | *rs2073186* | *rs2243174* | *rs2243188* | *rs2243191* | *rs2243193* | *Controls (n=148)* | *Psoriasis patients (n=254)* | *Haplotypic OR (95% CI)* | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| HT1 | C | A | C | C | G | 70.7 | 75.0 | * | |
| HT2 | T | G | A | T | A | 22.1 | 17.4 | 0.737 (0.516 – 1.053) | 0.09 |
| HT3 | C | A | C | T | A | 1.0 | 2.5 | 2.255 (0.616 – 8.256) | 0.22 |
| HT4 | T | A | A | T | A | 2.7 | 1.4 | 0.430 (0.142 – 1.297) | 0.13 |
| HT5 | T | A | C | C | G | 1.4 | 1.4 | 1.244 (0.332 – 4.664) | 0.74 |

* – the haplotype combining the most frequent alleles at each position is chosen as the reference haplotype (CACCG).

## 5.4. Associations of combined IL-19 and IL-20 haplotypes with psoriasis

Since IL-19 gene maps close to the IL-20 gene on human chromosome 1q32, therefore the LD analysis for all pairs of studied IL-19 SNPs and IL-20 SNPs was implemented. The pairwise LD matrix of the IL-19 and IL-20 polymorphisms pointed that IL-19 SNPs and IL-20 SNPs (excluding rs1518108) were in significant LD with each other (|D'| between 0.78 and 0.99).

Thereafter eight-marker haplotype analysis with five SNPs across the IL-19 gene (rs2243191, rs2073186, rs2243174, rs2243188 and rs2243193) and with three SNPs across the IL-20 gene (rs2981572, rs2981573 and rs2232360) was performed. We established five major haplotypes (HT1 CACCGTAA, HT2 TGATAGGG, HT3 CACCGGAA, HT4 CACTAGGG and HT5 TAATAGGG) each with a frequency ≥1% that in sum covered 91.86% of all possible marker combinations in the pooled samples. The frequencies of these haplotypes and haplotype effects are presented in Table 20. We found that patients with plaque psoriasis had a higher frequency of the haplotype HT3 CACCGGAA ($p<0.01$, OR 2.548, 95%CI 1.379-4.706) comparing with the control group.

In addition, the haplotype HT3 CACCGGAA was associated with an increased risk of different subtypes of plaque psoriasis. The haplotype HT3 CACCGGAA had a positive association with early onset ($p<0.02$, OR 2.225, 95%CI 1.175-4.213) and late onset of psoriasis ($p<0.05$, OR 2.467, 95% CI 1.1258-5.405), familial ($p<0.02$, OR 2.424, 95%CI 1.199-4.903) and sporadic psoriasis ($p<0.01$, OR 2.877, 95%CI 1.478-5.601). This association mainly reflects a significant individual effect of allele G at position -1053 of IL-20 ($p<0.01$, OR 2.548, 95% CI 1.379-4.706).

Comparing psoriasis patients with controls no significant association was established in relation to the other common haplotypes (HT1 CACCGTAA, HT2 TGATAGGG, HT4 CACTAGGG and HT5 TAATAGGG). Moreover, the protective effect of the IL-19 haplotype TGATA on late onset psoriasis did not withstand after the combined haplotype analysis of the IL-19 and IL-20 genes. The frequency of haplotype TGATAGGG did not differ significantly ($p=0.34$, OR 0.722; 95% CI 0.367-1.422) in the subgroup of late onset psoriasis. Detailed results of the haplotype analysis of the IL-19 gene and also results of the extended haplotype analysis of the IL-19 and IL-20 gene between patients with late onset disease and the control group are presented in Table 21.

**Table 20.** Results of extended haplotype analysis of the IL-19 and IL-20 genes in patients with plaque-type psoriasis. The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values in comparison with the reference haplotype in psoriasis patients compared with controls are indicated. Haplotype frequencies and haplotype-phenotype associations were estimated using maximum likelihood method.

| *Haplo-type* | *rs2073186* | *rs2243174* | *rs2243188* | *rs2243191* | *rs2243193* | *rs2981572* | *rs2981573* | *rs2232360* | *Cont-rols (n=148)* | *Psori-asis patients (n=254)* | *Haplotypic OR (95% CI)* | *p-value* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HT1 | C | A | C | C | G | T | A | A | 64.6 | 60.3 | * | |
| HT2 | T | G | A | T | A | G | G | G | 17.4 | 15.5 | 0.947 (0.639–1.403) | 0.78 |
| HT3 | C | A | C | C | G | G | A | A | 5.6 | 13.5 | 2.548 (1.379–4.706) | 0.003 |
| HT4 | C | A | C | T | A | G | G | G | 1.4 | 2.0 | 1.597 (0.420–6.073) | 0.49 |
| HT5 | T | A | A | T | A | G | G | G | 2.4 | 0.8 | 0.342 (0.100–1.172) | 0.09 |

* – the haplotype combining the most frequent alleles at each position is chosen as the reference haplotype (CACCGTAA).

**Table 21.** Results of IL-19 haplotype analysis and extended haplotype analysis of the IL-19 and IL-20 genes in patients with late onset psoriasis. The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values in comparison with the reference haplotype in late onset psoriasis patients compared with controls are indicated. Haplotype frequencies and haplotype-phenotype associations were estimated using maximum likelihood method.

| *IL-19 haplotypes* | *Controls (n=148)* | *Late onset psoriasis patients (n=74)* | *Haplotypic OR (95% CI)* | *p-value* |
|---|---|---|---|---|
| CACCG | 70.7 | 77.3 | * | |
| TGATA | 21.8 | 13.4 | 0.581 (0.335–1.000) | 0.05 |
| TAATA | 2.7 | 0.6 | 0.182 (0.022–1.501) | 0.11 |
| CACTA | 1.0 | 3.2 | 2.690 (0.549–13.17) | 0.22 |
| TACCG | 1.4 | 1.3 | 1.580 (0.277–8.999) | 0.61 |
| *IL-19 and IL-20 combined haplotypes* | | | | |
| CACCGTAA | 63.5 | 61.1 | * | |
| TGATAGGG | 16.7 | 11.8 | 0.722 (0.367–1.422) | 0.34 |
| CACCGGAA | 5.9 | 14.2 | 2.467 (1.126–5.405) | 0.02 |
| TAATAGGG | 2.7 | 0.7 | 0.260 (0.031–2.146) | 0.21 |
| TACCGTAA | 2.4 | 0.7 | 0.318 (0.035–2.869) | 0.31 |

* – the haplotype combining the most frequent alleles at each position is chosen as the reference haplotype.

## 5.5. Association, linkage disequilibrium and haplotype analyses of the IL-24 gene

An association study with five SNPs of the IL-24 gene (rs3762344, rs291111, rs1150253, rs1150256 and rs1150258) was performed. Genotype distributions of the analyzed IL-24 gene polymorphisms were in HWE. Allele frequencies, allelic and genotypic p-values of IL-24 SNPs of healthy control subjects and patients with psoriasis are presented in Table 22. Statistically significant associations with susceptibility to psoriasis were not found with any analyzed SNPs of the IL-24 gene.

Association analysis of SNPs of the IL-24 gene between different subgroups of psoriasis and controls revealed statistically significant difference of frequencies of allele and genotype distributions of rs1150253 in the psoriasis subgroup with limited eruption (extent of body involvement ≤10%). The higher frequency of allele G ($p<0.05$) and of genotype G/G ($p<0.05$) was found (Table 23). Similarly the higher frequencies of minor alleles of rs3762344, rs1150256 and rs1150258 were found in the group of psoriasis patients with the extent of the skin lesions equal or below 10% of body involvement, but the differences in comparison with healthy controls were not statistically significant (data not shown).

The pairwise LD matrix demonstrated that polymorphisms of the IL-24 gene were in nearly complete LD (|D'| between 0.94 and 0.99). We excluded SNP rs291111 from the further haplotype analyses while its minor allele frequency was lower than 0.10. Four common haplotypes (HT1 AAAC, HT2 GGGT, HT3 GAGT, and HT4 AGAC) each with the frequency ≥ 1% in IL-24 gene were revealed and in sum these haplotypes covered 98.24% of all possible marker combinations in the pooled samples. The frequencies of the main haplotypes and the haplotype effects are presented in Table 24. No significant association was observed concerning these major haplotypes in susceptibility to psoriasis.

However, we found that patients with limited eruption (extent equal or below 10%) had a higher frequency of the HT2 GGGT haplotype ($p=0.05$, OR 1.548, 95% CI 1.007-2.380) in comparison with the group of controls. The rs1150253 allele G effect on this phenotype within the GGGT haplotypic background was marginally significant ($p=0.05$, OR 3.508, 95% CI 0.978-12.582). The HT2 GGGT haplotype frequencies and haplotypic OR-s with their 95% CI-s and p-values by comparison with the reference haplotype in the whole group of psoriasis patients and in subgroups of patients according to the severity of disease compared with controls are indicated in Table 25.

The results of association and haplotype analyses of the IL-24 gene suggest that SNP rs1150253 may represent a potential subtype specific marker. As the susceptibility effects of rs1150253 allele G and haplotype GGGT were not observed in comparison with patients who had PASI score equal or below 20, the founded association seems not to be essential (Table 23 and Table 25). The

determination of skin involvement only by the extent of eruption is not sufficient for discrimination for psoriasis severity.

**Table 22.** Results of association analysis of IL-24 gene SNPs in plaque-type psoriasis. Allele frequencies of the five studied polymorphisms of the IL-24 gene were compared between the psoriatic patients (n=254) and the controls (n=148), p-values for allelic and genotypic association were calculated using Fisher's exact test.

| *SNP ID* | *Polymorphisms* | | *Allele frequencies (%)* | | | | *p-values* | |
|---|---|---|---|---|---|---|---|---|
| | | | *Controls (n=148)* | | *Cases (n=254)* | | *Allelic* | *Geno-typic* |
| | *1[a]* | *2[b]* | *1[a]* | *2[b]* | *1[a]* | *2[b]* | | |
| rs3762344 | G | A | 51.4 | 48.6 | 47,2 | 52,8 | 0.28 | 0.27 |
| rs291111 | T | C | 96.6 | 3.4 | 98.0 | 2.0 | 0.24 | 0.29 |
| rs1150253 | A | G | 57.8 | 42.2 | 51.6 | 48.4 | 0.08 | 0.12 |
| rs1150256 | A | G | 51.7 | 48.3 | 47.4 | 52.6 | 0.24 | 0.28 |
| rs1150258 | C | T | 51.4 | 48.6 | 47.2 | 52.8 | 0.28 | 0.28 |

1[a] – most frequent allele
2[b] – least frequent allele

**Table 23.** Frequencies of genotypes and alleles of the rs1150253 of the IL-24 gene in controls and in whole group of patients and in subgroups of patients according to the severity of psoriasis (%).

| *rs1150253 A>G* | *Genotypes* | | | *Alleles* | |
|---|---|---|---|---|---|
| | *A/A* | *A/G* | *G/G* | *A allele* | *G allele* |
| Controls n=148 | 35.1 | 45.3 | 19.6 | 57.8 | 42.2 |
| Psoriasis patients n=254 | 30.7 | 41.7 | 27.6 | 51.6 | 48.4 |
| Patients with PASI equal or below 20 n=151 | 31.2 | 40.1 | 28.6 | 51.3 | 48.7 |
| Patients with PASI above 20 n=85 | 29.9 | 44.3 | 25.8 | 52.1 | 47.9 |
| Patients with extent of eruption equal or below 10% n=66 | 23.5 | 44.1 | 32.4* | 45.6 | 54.4* |
| Patients with extent of eruption between 11% and 30% n=76 | 33.3 | 37.2 | 29.5 | 51.9 | 48.1 |
| Patients with extent of eruption above 30% n=94 | 33.3 | 43.5 | 23.1 | 55.1 | 44.9 |

* – $p<0.05$ allelic or genotypic differences compared to the controls.

**Table 24.** Results of haplotype analysis of the IL-24 gene in group of patients with plaque-type psoriasis in comparison with controls. The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values in comparison with the reference haplotype between psoriasis patients and controls are indicated. Haplotype frequencies and haplotype-phenotype associations were estimated using maximum likelihood method.

| *Haplotype* | *rs3762344* | *rs1150253* | *rs1150256* | *rs1150258* | *Controls (n=148)* | *Psoriasis patients (n=254)* | *OR (95% CI)* | *p-value* |
|---|---|---|---|---|---|---|---|---|
| HT1 | A | A | A | C | 48.2 | 46.2 | * | |
| HT2 | G | G | G | T | 39.4 | 47.6 | 1.241 (0.910 – 1.693) | 0.17 |
| HT3 | G | A | G | T | 8.3 | 4.2 | 0.604 (0.311 – 1.173) | 0.14 |
| HT4 | A | G | A | C | 2.8 | 0.03 | – | – |

* – the haplotype combining the most frequent alleles at each site is chosen as the reference haplotype (AAAC).

– – the haplotypic OR by comparison to the reference with its 95% CI was not estimated.

**Table 25.** The HT2 GGGT haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values in comparison with the reference haplotype in subgroups of patients according to the severity of psoriasis compared with controls are indicated.

| *Case/control status* | *HT2 GGGT haplo-type frequency (%)* | *OR (95% CI)* | *p-value* |
|---|---|---|---|
| Controls n=148 | 39.4 | | |
| Patients with extent of eruption equal or below 10% n=66 | 53.7 | 1.548 (1.007–2.380) | 0.046 |
| Patients with extent of eruption between 11% and 30% n=76 | 46.0 | 1.214 (0.815–1.808) | 0.34 |
| Patients with extent of eruption above 30% n=94 | 45.1 | 1.161 (0.787–1.716) | 0.45 |
| Patients with PASI equal or below 20 n=151 | 47.0 | 1.213 (0.865–1.700) | 0.26 |
| Patients with PASI above 20 n=85 | 49.3 | 1.390 (0.925–2.088) | 0.11 |

## 5.6. Associations of combined IL-20 and IL-24 haplotypes with psoriasis

The LD analysis of the IL-20 gene indicated that the polymorphisms rs2981572, rs2981573 and rs2232360 were in nearly complete LD (|D'| between 0.879 and 0.985). Inversely the IL-20 rs1518108 polymorphism, which is located in the 3' end of the IL-20 gene, was not in LD with the other polymorphisms of the IL-20 gene. As in the human genome the IL-24 gene follows the IL-20 gene, the LD analysis between the IL-20 polymorphism rs1518108 and IL-24 polymorphisms was additionally performed. The pairwise LD matrix showed that rs1518108 of the IL-20 gene was in significant LD (|D'| between 0.61 and 0.79) with all studied polymorphisms of the IL-24 gene.

The presence of five haplotypes (HT1 TAAAC, HT2 CGGGT, HT3 TGGGT, HT4 CGAGT and HT5 CAAAC) each with frequencies ≥1% was found in the extended haplotype analysis of IL-20 rs1518108 and polymorphisms of the IL-24 gene. These haplotypes covered 96.39% of all possible haplotypes in the pooled samples. The frequencies of these haplotypes and haplotype effects in psoriasis and control groups are presented in Table 26.

The extended haplotype analysis pointed out that the control group had a significantly higher frequency of the haplotype HT5 CAAAC ($P<0.001$, OR 0.154, 95% CI 0.059-0.411) compared with psoriasis patients. Respectively, the haplotype HT5 CAAAC was negatively associated with the early onset and the late onset of disease, familial and sporadic disease. Haplotype HT5 CAAAC was significantly lower-represented in the subgroups of patients with the extent of eruption between 11% and 30% and with the extent above 30%. Inversely the distribution of haplotypes in the subgroup of patients with the extent of eruption equal or below 10% of the body involvement compared to controls did not differ significantly. The lower frequency of haplotype HT5 CAAAC in the subgroups of patients with PASI equal or below 20 and PASI above 20 was found. However, when comparing the subgroup of patients with PASI above 20 with controls the difference of frequencies of haplotype HT3 CAAAC was not statistically significant. The detected protective effect of the HT5 CAAAC mainly reflect an individual allele C effect of IL-20 rs1518108 ($p=0.000186$, OR 0.154, 95% CI 0.058-0.411). The HT5 CAAAC haplotype frequencies and haplotypic OR-s, with their 95% CI-s and p-values by comparison with the reference haplotype in patients with different subtypes of psoriasis compared with controls are indicated in Table 27.

The control group had a higher frequency of the HT3 TGGGT ($p<0.05$, OR 0.591, 95% CI 0.356-0.981) compared with the psoriasis group. Comparing the subgroups of psoriasis the HT3 TGGGT was more frequent in the control group only in comparison with the group of early onset psoriasis ($p<0.05$, OR 0.548, 95% CI 0.316-0.951) and sporadic psoriasis ($p<0.05$, OR 0.523, 95% CI 0.295-

**Table 26.** Results of extended haplotype analysis of the IL-20 and IL-24 genes in patients with plaque-type psoriasis. The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and P-values by comparison with the reference haplotype in psoriasis patients compared with controls are indicated. Haplotype frequencies and haplotype-phenotype associations were estimated using maximum likelihood method.

| *Haplo-type* | *rs1518108* | *rs3762344* | *rs1150253* | *rs1150256* | *rs1150258* | *Controls (n=148)* | *Psoriasis patients (n=254)* | *OR (95% CI)* | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| HT1 | T | A | A | A | C | 38.4 | 44.7 | * | |
| HT2 | C | G | G | G | T | 28.0 | 40.6 | 1.292 (0.887–1.884) | 0.18 |
| HT3 | T | G | G | G | T | 11.4 | 7.0 | 0.591 (0.356–0.981) | 0.04 |
| HT4 | C | G | A | G | T | 6.8 | 3.5 | 0.457 (0.215–0.974) | 0.04 |
| HT5 | C | A | A | A | C | 10.1 | 1.5 | 0.154 (0.059–0.411) | 0.000186 |

* – the haplotype combining the most frequent alleles at each position is chosen as the reference haplotype (TAAAC).

**Table 27.** The HT5 CAAAC haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values by comparison with the reference haplotype in patients with different subtypes of psoriasis compared with controls are indicated.

| *Case/control status* | *HT5 CAAAC haplotype frequency (%)* | *OR (95% CI)* | *p-value* |
|---|---|---|---|
| Controls n=148 | 10.1 | | |
| Early onset psoriasis n=180 | 1.2 | 0.121 (0.038 – 0.385) | 0.0003 |
| Late onset psoriasis n=74 | 1.5 | 0.152 (0.033 – 0.711) | 0.0167 |
| Familial psoriasis n=101 | 0.5 | 0.059 (0.007 – 0.477) | 0.0079 |
| Sporadic psoriasis n=153- | 1.8 | 0.178 (0.061 – 0.525) | 0.0017 |
| Patients with extent of eruption equal or below 10% | 3.4 | 0.382 (0.099 – 1.471) | 0.16 |
| Patients with extent of eruption between 11% and 30% | 1.4 | 0.148 (0.031 – 0.715) | 0.0174 |
| Patients with extent of eruption above 30% | 0.6 | 0.056 (0.007 – 0.453) | 0.0068 |
| Patients with PASI equal or below 20 | 1.7 | 0.169 (0.058 – 0.495) | 0.0011 |
| Patients with PASI above 20 n=85 | 0.3 | 0.336 (0.002 – 46.148) | 0.66 |

0.925). A protective effect against psoriasis was also observed with haplotype HT4 CGAGT. The frequency of haplotype HT4 CGAGT was higher in control group in comparison with the whole psoriasis group ($p<0.05$, OR 0.457, 95% CI 0.215-0.974) and with subgroups of early onset psoriasis ($p=0.05$, OR 0.430, 95% CI 0.185-0.998) and sporadic psoriasis ($p<0.05$, OR 0.390, 95% CI 0.154-0.962).

The higher frequency of other two haplotypes – HT1 TAAAC and HT2 CGGGT – in the psoriasis group was observed; but the differences compared to controls were not statistically significant.

The effect of the IL-24 haplotype GGGT to psoriasis with the extent of eruption equal or below 10% of the body involvement did not withstand after combined haplotype analysis of the IL-20 and IL-24 polymorphisms (Table 28).

**Table 28.** Results of IL-24 haplotype analysis and extended haplotype analysis of the IL-20 and IL-24 genes in patients with extent of eruption equal or below 10% of body involvement. The haplotype frequencies (%) and haplotypic OR-s with their 95% CI-s and p-values by comparison to the reference haplotype in limited form of psoriasis compared with controls are indicated. Haplotype frequencies and haplotype-phenotype associations were estimated using maximum likelihood method.

| *IL-24 haplotypes* | *Controls (n=148)* | *Psoriatic patients with extent of the skin lesions ≤10% (n=66)* | *Haplotypic OR (95% CI)* | *p-value* |
|---|---|---|---|---|
| AAAC | 48.1 | 40.8 | * | |
| GGGT | 39.3 | 53.7 | 1.548 (1.007 – 2.380) | 0.046 |
| GAGT | 8.3 | 2.3 | 0.441 (0.119 – 1.641) | 0.22 |
| AGAC | 2.5 | 0.06 | – | – |
| *IL-20/IL-24 extended haplotypes* | | | | |
| TAAAC | 38.0 | 37.4 | * | |
| CGGGT | 27.5 | 43.5 | 1.618 (0.975 – 2.685) | 0.06 |
| TGGGT | 11.7 | 10.2 | 0.930 (0.467 – 1.852) | 0.84 |
| CAAAC | 10.5 | 3.4 | 0.383 (0.099 – 1.471) | 0.16 |
| CGAGT | 6.8 | 2.3 | 0.393 (0.097 – 1.591) | 0.19 |

* – the haplotype combining the most frequent alleles at each position is chosen as the reference haplotype.

– – the haplotypic OR by comparison to the reference with its 95% CI was not estimated

## 5.7. The LD analysis of the region comprising the IL-10 gene and genes of IL-19 subfamily

The pairwise linkage disequilibrium was determined for all 16 genotyped loci of the IL-10, IL-19, IL-20 and IL-24 genes to describe the LD pattern emerging from the genes of the IL-10 cluster. The existence of three haplotype blocks within the 128498 bp fragment of human chromosome 1q31-32 was established. The first haplotype block (0.5 kb) includes three SNPs from the IL-10 promoter region (rs1800896, rs1800871 and rs1800872). The second haplotype block (30 kb) includes five SNPs across the IL-19 gene (rs2243191, rs2073186, rs2243174, rs2243188 and rs2243193) and three SNPs from the IL-20 gene (rs2981572, rs2981573 and rs2232360). The third haplotype block (31.7 kb) includes one SNP (rs1518108) from the IL-20 gene and four SNPs across the IL-24 gene (rs3762344, rs1150253, rs1150256, rs1150258)

There was a breakdown of LD between rs1800872 of the IL-10 gene and SNPs of the IL-19 subfamily genes and also between rs1800871 of the IL-10 gene and SNPs of the IL-19 subfamily genes (|D'| between 0.02 and 0.14). The IL-10 SNP rs1800896 was in marginal significant LD (|D'| between 0.62 and 0.65) with the SNPs of the IL-19 gene (rs2073186, rs2243174, rs2243188, rs2243191 and rs2243193) and also with the SNPs rs2981573 and rs2232360 of the IL-20 gene (|D'| between 0.60 and 0.66). Nevertheless, there was a breakdown of LD between IL-10 rs1800896 and IL-20 rs2981572 (|D'| 0.19) and also between IL-10 rs1800896 and IL-20 rs1518108 (|D'| 0.01). We didn't observe LD between rs1800896 of the IL-10 gene and the markers across the IL-24 gene (rs3762344, rs1150253, rs1150256, rs1150258) (|D'| 0.01…0.02).

The pairwise LD matrix of the IL-19, IL-20 and IL-24 polymorphisms pointed that IL-19 SNPs (rs2243191, rs2073186, rs2243174, rs2243188 and rs2243193) and three SNPs from the IL-20 gene (rs2981572, rs2981573 and rs2232360) were in significant LD with each other (|D'|between 0.78 and 0.99). Additionally the LD was established between rs1518108 of the IL-20 gene and four SNPs across the IL-24 gene (rs3762344, rs1150253, rs1150256, rs1150258) (|D'| between 0.61 and 0.99). The breakdown of LD was observed between SNPs rs2232360 and rs1518108 of IL-20 gene (|D'| 0.24). The extent of LD including IL-10, IL-19, IL-20 and IL-24 genes and formed haplotype blocks are presented in Figure 3.

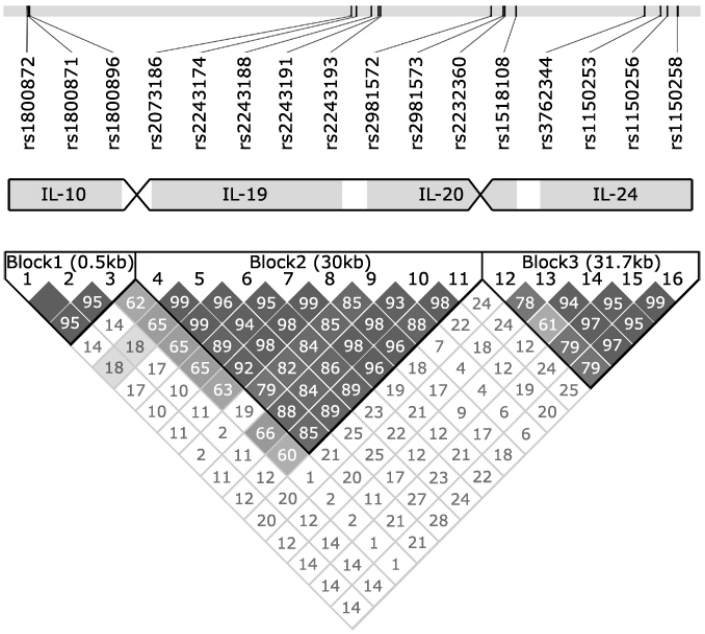



**Figure 3.** The extent of LD and formed haplotype blocks within the 128498 bp fragment of human chromosome 1 q31-32, in a region of IL-10 cluster genes are indicated. Vertical stripes on the upper band represent SNPs (SNP ID shown below the upper band) with their proportional localization in human genome. The broad band below the SNP IDs shows in which of the genes the SNPs occur (gene areas on the broad band do not reflect actual lengths of the genes in the human genome) and the narrowness of the band represents a breakdown of LD. In order to establish the LD structure and for defining haplotype blocks within the region Haploview software was used. For better visibility the D' values shown on the squares have been multiplied by 100.

## 5.8. IL-10 and IL-20 genes expression analyses

### 5.8.1. IL-10 gene expression analysis

Previously genotyped fifteen healthy volunteers (age range 21–59) and twenty-one patients with plaque-type psoriasis (age range 35–79) were examined. Psoriasis patients presented significantly higher levels of IL-10 expression in comparison with healthy controls ($p<0.01$) (Figure 4). No differences were found between values of IL-10 mRNA in different subgroups of disease severity.

Moreover, healthy controls with the allele G at position -1082 had higher IL-10 mRNA levels than those with the allele A ($p<0.05$) (Figure 5A). A similar trend was found comparing the G (+) haplotype (GCC) and A (+) haplotypes (ACC and ATA), but the differences of the IL-10 mRNA values were not

statistically significant (p>0.05) (Figure 5C). In psoriasis patients IL-10 -1082 G allele was related to significantly higher IL-10 mRNA levels than IL-10 -1082 A allele (p<0.001) (Figure 5B). Similarly, patients with the G (+) haplotype (GCC) had higher IL-10 mRNA levels than those with IL-10 A (+) haplotypes (ACC and ATA) (p<0.01) (Figure 5D). Furthermore, IL-10 -1082 G (+) haplotype (GCC) was associated with a higher IL-10 level in psoriasis patients compared to controls with the same haplotype (GCC) (p<0.01).

We were not able to confirm the expression of IL-10 mRNA in whole blood being significantly affected by allelic variants at positions -819 C/T and -592 C/A as described by Temple *et al*. (2003). However, healthy controls with genotypes -819 C/C and -592 C/C had a trend of higher values of IL-10 expression than those with genotypes -819 T/T and -592 A/A (p=0.13).

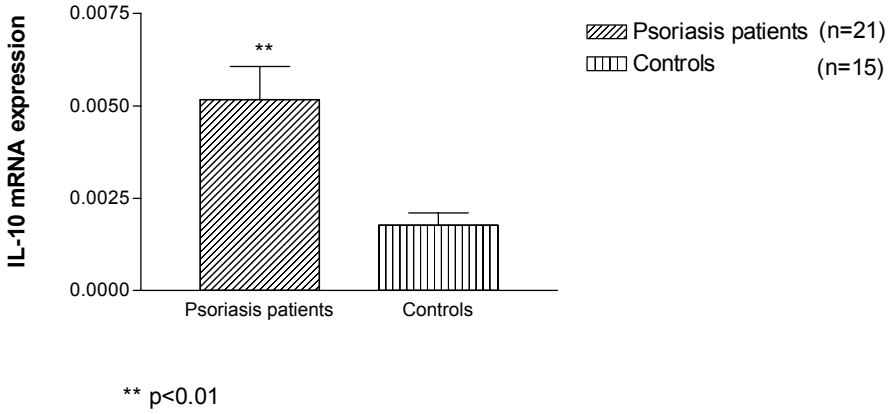



**Figure 4.** The IL-10 mRNA expression in patients with plaque-type psoriasis in comparison with healthy controls. Expression data relative to those of the housekeeping gene from four independent assays are given as mean ± SEM.

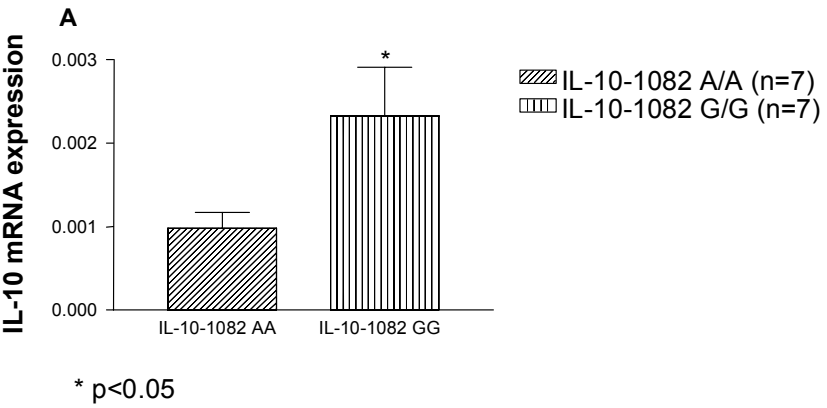



**Figure 5.**

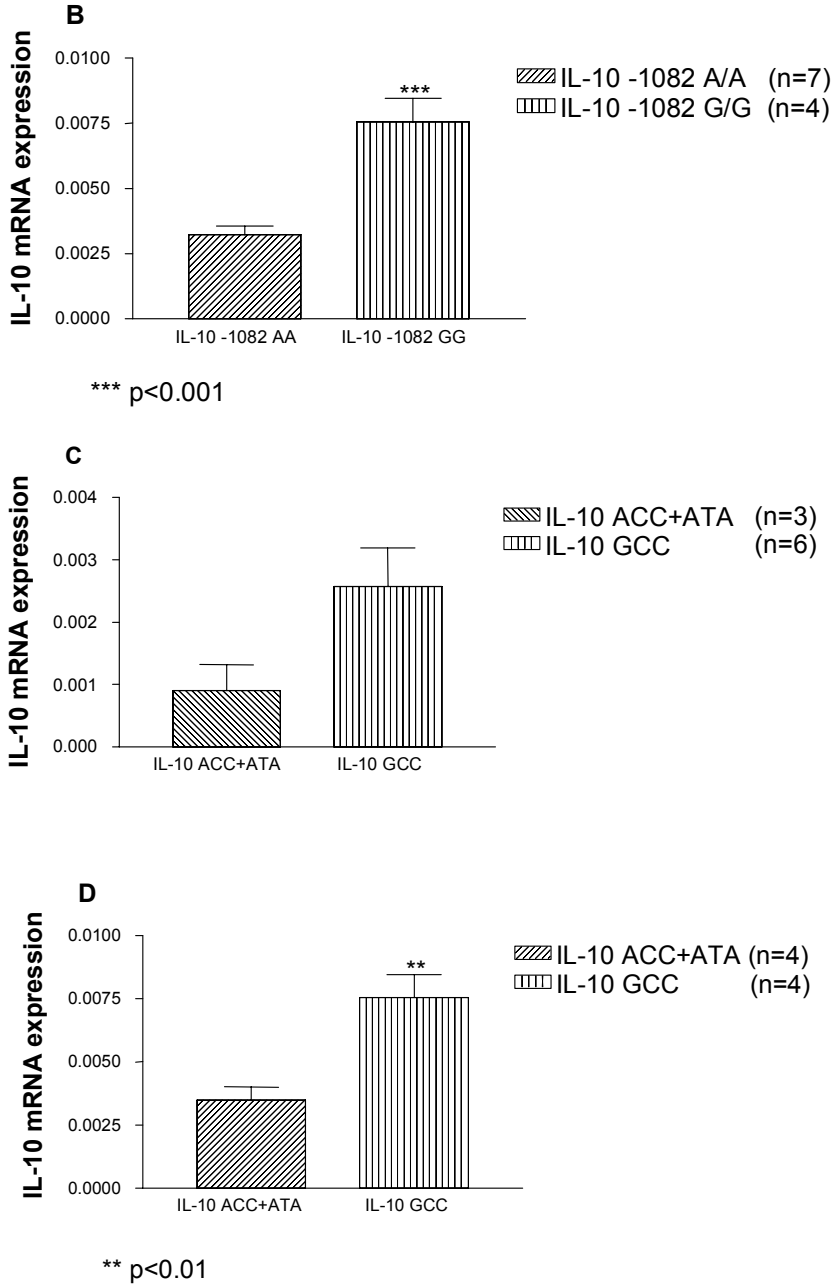
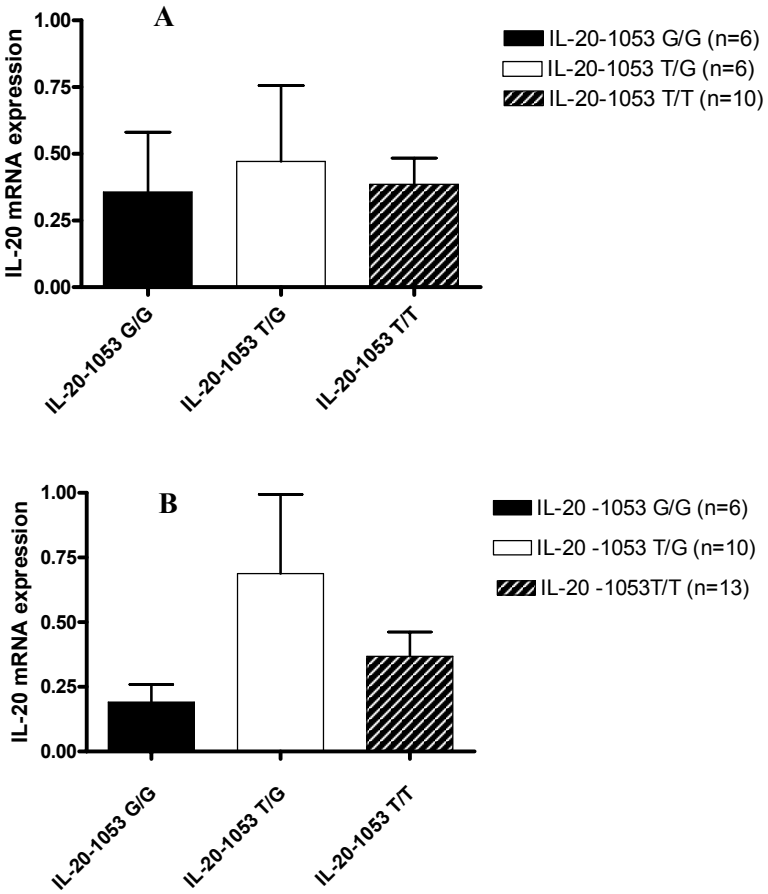



**Figure 5.** Whole blood IL-10 mRNA levels in healthy controls (A) and psoriasis patients (B) with homozygous IL-10 -1082 A/A and IL-10 -1082 G/G genotypes. Whole blood interleukin-10 mRNA levels in healthy controls (C) and psoriasis patients (D) by haplotypes are demonstrated. Expression data relative to those of the housekeeping gene from four independent assays are given as mean ± SEM.

### 5.8.2. IL-20 gene expression analysis

Whole blood IL-20 mRNA levels in 29 patients with plaque-type psoriasis (age range 23–79) and 22 healthy controls (age range 21-59) were analyzed to evaluate the expression of IL-20 gene. No differences were found between IL-20 mRNA values in psoriasis patients and in healthy controls. Similarly no differences were observed between IL-20 mRNA values in different subgroups of disease severity.

Thereafter, the effect of variations at positions -1053 and 3978 of the IL-20 gene upon IL-20 mRNA expression was assessed. Both SNPs are located in the potential regulatory regions in the IL-20 gene. Moreover, IL-20 promoter SNPs -1053 and 3'-UTR region SNP 3978 had a significant individual effect in the context of extended IL-19 subfamily genes haplotypes and appear to influence the general risk of plaque-type psoriasis. We could not establish expression of IL-20 mRNA being affected by IL-20 -1053 T/G and IL-20 3978 T/C polymorphisms in healthy controls (Figure 6A and 6C).



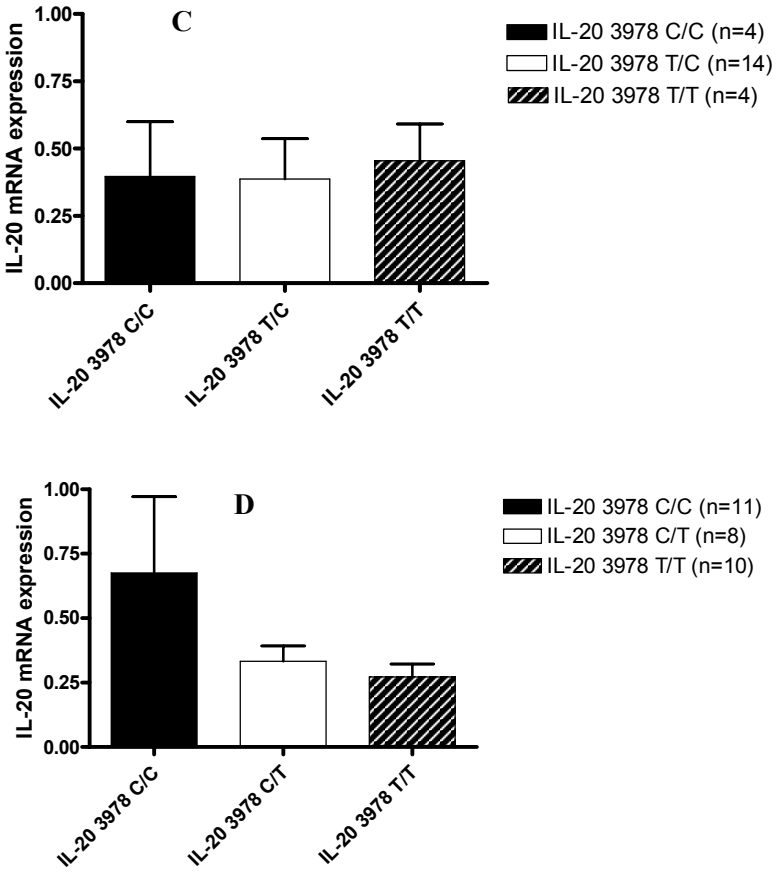



**Figure 6.** Whole blood IL-20 mRNA levels in healthy controls (A) and psoriasis patients (B) with different IL-20 -1053 genotypes. Whole blood IL-20 mRNA levels in healthy controls (C) and psoriasis patients (D) by IL-20 3978 genotypes. Expression data relative to those of the housekeeping gene from four independent assays are given as mean ± SEM.

However, psoriasis patients with heterozygous IL-20 -1053 T/G genotype tended to have higher IL-20 mRNA levels than those of homozygous genotypes (Figure 6B). This finding fits well with our results of the association analysis with the IL-20 gene where the trend of higher frequency of IL-20 -1053 heterozygous T/G genotype and lower frequency of T/T genotype in psoriasis patients compared with controls was established.

Measurement of levels of IL-20 expression in patients with certain IL-20 3978 genotypes indicated that psoriasis patients with homozygous genotype C/C at position 3978 had a trend of higher values of IL-20 expression than the

persons with heterozygous T/C and homozygous T/T genotypes, but the differences of the IL-20 mRNA values were not statistically significant ($p>0.05$) (Figure 6D). These data indicate that polymorphism IL-20 3978 in the 3'-UTR region might be involved in IL-20 mRNA destabilization. However, as both HT5 CAAAC and HT3 TGGGT had protective effect against psoriasis, we conclude that the contribution of nucleotide IL-20 3978 alone is not sufficient to determine the functional differences in psoriasis patients. This conclusion is supported by the fact that allele C at position 3978 had a significant individual protective effect (OR 0.154) in the context of HT5 CAAAC and a significant individual susceptibility effect (OR 2.186) in the context of HT2 CGGGT. Psoriasis patients had a higher frequency of the CGGGT haplotype compared to healthy controls, but the difference was not statistically significant (Table 26).

# 6. DISCUSSION

## 6.1. Associations between SNPs, haplotypes and extended haplotypes of the genes of IL-10 cluster and plaque-type psoriasis

The long arm of chromosome 1 has been of interest for mapping a susceptibility locus for psoriasis since the scan of one of the largest Italian pedigrees with psoriasis revealed evidence for linkage to the 1cen-q21 markers (Capon *et al*., 1999). This locus has been termed as PSORS4 susceptibility locus. Additional linkage disequilibrium analysis with cohort of 79 Italian triads (Capon *et al*., 2001) proved association between psoriasis and chromosome 1q21 markers lying in the region of the Epidermal Differentiation Cluster (EDC).

There is a cluster of genes encoding IL-10 family cytokines IL-10, IL-19, IL-20 and IL-24 on the long arm q31-32 region of the same chromosome. Linkage to several common autoimmune diseases, such as systemic lupus erythematosus (Johanneson *et al*., 2002) and rheumatoid arthritis (Martinez *et al*., 2003; Barrera *et al*., 2001) has been detected in this region. In addition, the protective effect of the microsatellite marker IL-10.G9 allele 3 for familial psoriasis has been observed using the transmission/disequilibrium test (TDT) in families with a positive family history of psoriasis (Hensen *et al*., 2003). Moreover, the polymorphisms of IL-10 gene have the role in determining the severity for a number of immune-inflammatory diseases.

Three cytokines of IL-10 gene cluster – IL-19, IL-20 and IL-24 – have been represented as IL-19 subfamily cytokines (Gallagher *et al*., 2004). Although the exact biological functions of the IL-19 subfamily cytokines remain unclear, it is verified that these cytokines are involved in the development and maintenance of psoriasis.

Two lines of evidence suggest the role of IL-20 in the pathogenesis of psoriasis. The first line of evidence is that the over-expression of IL-20 induced lesions on transgenic mice similar to skin changes observed in psoriasis. The histological analysis of the skin of IL-20 transgenic mice showed hyperkeratosis, thickened epidermis and proliferation in the suprabasal layer resembling human psoriatic abnormalities. Immunohistochemical analysis of epidermal markers in transgenic mice demonstrated the expression of the differentiation and proliferation markers in the suprabasal layers, which are normally confined to the basal layer (Blumberg *et al*., 2001). The above-described changes in the skin appear to be caused by circulating IL-20, because even mice expressing transgene in other tissues (liver) were similarly affected with analogous skin changes.

The second line of evidence implicating the role of IL-20 receptor in the pathogenesis of psoriasis is that both the IL-20Rα and IL-20Rβ receptor

subunits are markedly upregulated in human psoriatic skin compared to normal skin (Blumberg *et al*. 2001; Romer *et al*., 2003). The upregulation of both IL-20 receptor subunit mRNAs is detected in keratinocytes as well as in endothelial cells and immune cells. These are the main cell types which interact in the pathogenesis of psoriasis.

IL-19 is the pro-inflammatory cytokine that is markedly elevated in psoriasis lesions and is strongly suppressed by administration of IL-4 during the improvement of psoriasis (Ghoreschi *et al*. 2003). Sharing the same receptor complex with IL-20 (type I IL-20R that is composed from IL-20Rα and IL-20Rβ subunits) indicates that IL-19 may have partially overlapping biological activities with IL-20.

Recently a potential role of IL-24 (MDA-7) as a cytokine and its involvement in the immune system has been described. Treatment of PBMC with IL-24 resulted in the induction of IL-6, IFN-γ, TNF-α, IL-1ß, IL-12 and GM-CSF, suggesting that IL-24 may be a member of a complex cascade of cytokines involved in inflammation (Caudell *et al*., 2002). The finding that IL-24 binds to IL-20 receptor complexes (type I IL-20R and type II IL-20R that is composed from IL-20Rβ and IL-22R subunits) indicates that similarly to IL-20 also IL-24 may be involved in the pathogenesis of psoriasis. Moreover, expression of IL-24 was detected in dermal infiltrate in psoriatic skin (Romer *et al*., 2003). Since IL-24 together with IL-19 and IL-20 are expressed by monocytes these cytokines could form innate immune pathways of psoriasis and play the part in the epidermal response to inflammation.

Descriptions of SNPs of IL-19, IL-20 and IL-24 genes are accessible in the NCBI dbSNP database. No polymorphisms of these genes have been investigated in complex diseases up to the present time. We hypothesised that association and haplotype analyses of the IL-10, IL-19, IL-20 and IL-24 genes and extended haplotype analysis across a region of the genes of IL-10 cluster might indicate supplementary markers of psoriasis susceptibility.

The indirect association study design for analyzing disease candidate-genes was used. Association studies are demonstrated to be more suitable in localizing susceptibility loci for common diseases than the linkage analysis (Risch *et al*., 1996). An indirect approach of an association study means to test polymorphisms under the assumption that if a risk polymorphism exists it either will be genotyped directly or by way of strong LD with one of the genotyped polymorphisms (Collins *et al*., 1997).

In the individual evaluation of SNPs we established that (1) minor alleles of the IL-10 -819 (rs1800871) and IL-10 -592 (rs1800872) had statistically significant associations with persistent eruption; (2) minor allele of the IL-19 rs2243188 revealed protective effect against psoriasis, while IL-19 rs2243169 and rs2243158 were represented only as subtype specific markers; (3) minor allele of the IL-20 -1053 (rs2981572) had susceptibility effect to psoriasis, while IL-20 3978 (rs1518108) heterozygous genotype T/C had a protective effect against psoriasis; (4) IL-24 rs1150253 minor allele was the presumable

risk allele in the subgroup of patients with eruption equal or below 10% of body involvement.

While several reports have indicated that in the detecting of associations the determination of haplotypes might offer more power than simple measuring of individual SNPs (Carlson *et al*., 2004; Crawford *et al*., 2005), we performed the LD analysis and haplotype analysis of the polymorphic loci of single genes of IL-10 cluster. The nearly complete LD was presented between the polymorphisms within the individual genes. The results of haplotype analyses of individual genes showed that (1) the IL-10 haplotype ATA has a role in determining the clinical course of plaque psoriasis (persistent eruption *vs* intermittent eruption: OR 2.445) and the haplotype ACC has a role in determining the severity of disease (PASI ≤20 *vs* controls: OR 1.463; extent ≤10% *vs* controls: OR 1.634) but does not seem to be important in susceptibility to psoriasis; (2) the IL-20 haplotype GAA has a susceptibility effect to psoriasis (OR 2.341); (3) the IL-19 haplotype TGATA has a significant protective effect in case of late onset disease (OR 0.58); (4) the IL-24 haplotype GGGT represents the potential risk haplotype in the subtype of psoriasis with limited eruption (OR 1.548).

According to recent understandings a large part of the human genome consists of the haplotype blocks (Cardon *et al*., 2003; Kauppi *et al*.; 2004). The block-like distribution of LD has been observed also in cytokine clusters (Hafler *et al*., 2005; Rioux *et al*., 2000). We have demonstrated the occurrence of three haplotype blocks within the 128498 bp fragment of human chromosome 1 locus q31-32, in the region of IL-10 gene cluster. The first haplotype block (0.5 kb) includes three SNPs from the IL-10 promoter region. The second haplotype block (30 kb) includes five SNPs across the IL-19 gene and three SNPs from the IL-20 gene. The third haplotype block (31.7 kb) includes one SNP from the IL-20 gene and four SNPs across the IL-24 gene.

Out of all the possible haplotypes three common haplotypes were considered (each frequency ≥ 1%) within block 1 and five common haplotypes within both other blocks (block 2 and block 3). Estimating the haplotype-phenotype association we identified within block 1 two haplotypes (ACC and ATA) as disease modifying haplotypes, within block 2 one haplotype (CACCGGAA) as psoriasis susceptibility haplotype and within block 3 three haplotypes (CAAAC, TGGGT and CGAGT) as protective haplotypes in psoriasis susceptibility. Our data indicate that the common haplotypes of disease-risk might produce common immune variations that have the role in autoimmune disease, like in psoriasis, therefore confirming the common variant/common disease hypothesis in complex diseases. This hypothesis presents that complex traits have underlying genetic variants that occur with a relatively high frequency (Zondervan *et al*., 2004; Crawford *et al*., 2005).

The importance of examining extended haplotypes of gene clusters have clearly been demonstrated in several studies (Smith *et al*., 2004; Undlien *et al*., 2001). Estimating the haplotype-phenotype association the protective effect of

the IL-19 gene in the context of extended haplotype analysis of the IL-19 and IL-20 genes on late onset psoriasis was not proved. However, we observed that the extended IL-19 and -20 haplotype CACCGGAA was related to increased risk (OR 2.548) for plaque-type psoriasis in the sample of unrelated patients. We suppose that the protective effect of the IL-19 gene is secondary to the susceptibility effect of the IL-20 gene. The possible role of haplotype TGATA of the IL-19 gene as the potential subtype specific marker needs to be elucidated in further studies with a larger number of patients with the late onset psoriasis.

Similarly, the susceptibility effect of the IL-24 haplotype GGGT to psoriasis with limited eruption (extent equal or below 10%) did not withstand after the combined haplotype analysis of the IL-20 and IL-24 polymorphisms. The single effect of IL-24 haplotype GGGT is probably not essential as the determination of skin involvement only by the extent of eruption seems not sufficient for discrimination for psoriasis severity. Nevertheless, we found that the extended IL-20/IL-24 haplotype CAAAC was associated with a significant protective effect against plaque-type psoriasis (OR 0.154). The protective effects against psoriasis were also observed with the extended haplotypes TGGGT (OR 0.591) and CGAGT (OR 0.457) of the IL-20 and IL-24 genes.

Our data maintained the concept of protective haplotypes in the genetics of complex diseases. The protective haplotypes of the HLA complex genes have been shown in several autoimmune diseases like diabetes, rheumatoid arthritis and systemic lupus erythematosus (Gibson *et al*., 2004; Laivoranta-Nyman *et al*., 2004; Camargo *et al*., 2004). Similarly, certain HLA haplotypes have been shown to be protective in psoriasis (Contu *et al*., 2004; Choonhakarn *et al*., 2002; Kim *et al*., 2000). The knowledge about the non-MHC protective genes in psoriasis is insufficient at the present time. However, the existence of both susceptible and protective loci in plaque-type psoriasis has been demonstrated in the chromosome 19p13 (Hensen *et al.*, 2003).

Altogether this study is the first indication that gene variants of IL-19 sub-family have the possible influencies to plaque-type psoriasis. Moreover, the results of the present study indicate that different loci within the chromosome 1q31-32 possess different effects in susceptibility to psoriasis. Further family-based studies and association studies of different populations are required to confirm the impact of the genes of IL-10 cluster in the genetic predisposition for psoriasis. The main results of genetic association studies of the genes of IL-10 cluster are demonstrated in Figure 7.

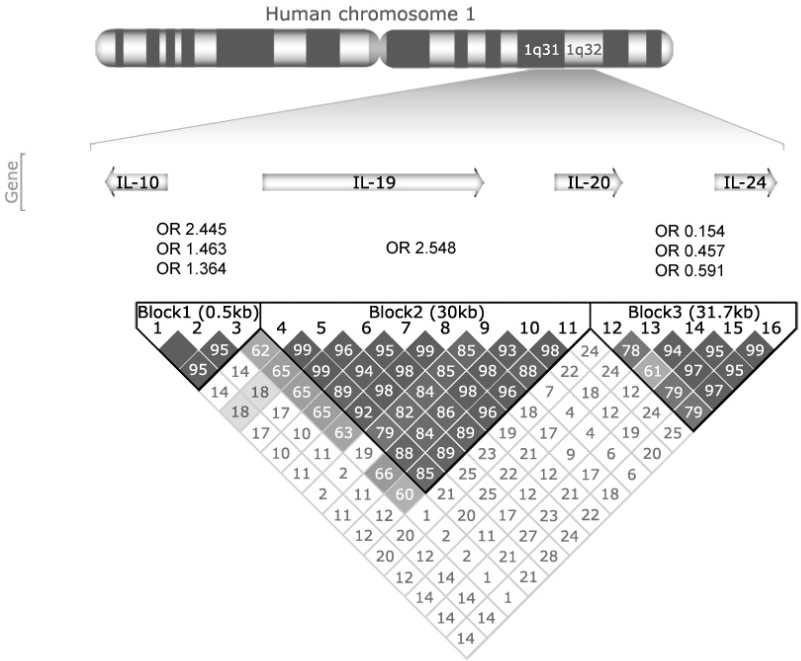



**Figure 7.** The extent of LD and formed haplotype blocks within the 128498 bp fragment of human chromosome 1q31-32, in a region of IL-10 cluster genes are demonstrated. Additionally the statistically significant haplotypic OR-s of each haplotype block in psoriasis patients compared with healthy controls are indicated.

## 6.2. The functional influence of polymorphisms of the genes of IL-10 cluster in plaque-type psoriasis

The whole blood IL-10 and IL-20 mRNA expression levels were examined to explain the functional part of the genes of the IL-10 cluster in the pathogenesis of psoriasis. We found increased levels of IL-10 mRNA transcription in psoriasis patients ($p<0.01$). Previous studies have demonstrated decreased IL-10 protein level in skin (Nickoloff *et al*., 1994; Mussi *et al.*, 1994) and blood (Asadullah *et al*., 1998). Supportive evidence favouring towards our results is that elevated mRNA expression level of IL-10 in biopsies from patients with psoriasis relative to healthy donors have been recently observed (Wolk *et al*., 2005). The inconsistency of different studies is most likely caused by our limited understanding on the post-transcriptional regulation of IL-10 gene expression.

The present study offers the additional evidence that -1082 allele G and haplotype G (+) of the IL-10 gene is associated with higher IL-10 expression levels. While the effect of -1082 allelic variants on IL-10 mRNA expression in

whole blood samples of healthy controls was weaker than in the samples of psoriasis patients, the effect is possibly amplified by other impact factors of psoriasis like smoking, body mass index, ultraviolet radiation, physical trauma or bacterial lipopolysaccharides. Altered gene expression of various cytokines, chemokines and other signalling molecules could also influence the IL-10 gene expression in psoriasis.

We could not verify that the expression of IL-10 mRNA in whole blood is significantly affected by allelic variants of -819 C/T and -592 C/A as described by Temple *et al.* (2003). However, healthy controls with genotypes -819 C/C and -592 C/C had a trend of higher IL-10 expression values than those with genotypes -819 T/T and -592 A/A ($p=0.13$). Therefore this finding supports the association of haplotypes ACC or ATA with differences in the severity and clinical course of psoriasis. Further studies are needed to clarify the effects of polymorphisms at positions IL-10 -592 and -819 on gene transcriptional activity.

The levels of IL-20 mRNA expressions in whole blood did not differ significantly between healthy controls and psoriasis patients. However, psoriasis patients with heterozygous genotype -1053 T/G and homozygous genotype 3978 C/C tended to have higher IL-20 mRNA levels. While IL-20 is primarily expressed in monocytes, the monocytic cell line should be a more relevant target to investigate genotypic effects of the IL-20 gene on the IL-20 mRNA expression in blood. Additionally, it will be necessary to identify the cellular origin of IL-20 mRNA in psoriatic plaques and in uninvolved skin of patients with certain genotypes.

## 6.3. The future prospects

With current studies we verified significant genetic influence of IL-10 family cytokines on the development of psoriasis. However, functional changes leading to higher susceptibility of psoriasis are still unclear. Our plans for further studies are following:

1. Genetic analysis of SNPs in other potential loci of psoriasis susceptibility, combining SNP and haplotype data with whole blood gene expression studies;
2. Psoriasis is a disorder, where altered function of T cells seems to be pathogenetically crucial. Therefore, genetic predisposition to psoriasis could realize by altered function of T cells. To verify this we need more detailed analysis of function of T cells isolated from blood or blister fluid. We combine gene expression analysis from blister fluid to “fine-tune” changes in gene expression and functional activity analysis (migration) of cultivated T cells after stimulation with LPS;

3. Combination of genotyping data with gene expression data should indicate whether there are any specific markers in peripheral blood predicting outcome of disease. In addition, treatment and clinical course information could give biochemical measure to predict the course of psoriasis.

In general, we are planning to expand our present studies from genotyping to more functional studies.

# 7. CONCLUSION

1. We have verified the occurrence of three haplotype blocks within the 128498 bp fragment of human chromosome 1 locus q31-32 in a region of IL-10 gene cluster.
2. By estimating the haplotype-phenotype association we found that IL-10 gene would be first of all the disease modifying gene, whereas the IL-19 sub-family genes appear to influence the general risk of plaque-type psoriasis.
3. We have demonstrated that the whole blood IL-10 mRNA expression of patients with plaque psoriasis is significantly higher than in healthy controls, but no significant differences between IL-20 mRNA expression levels were found.
4. The functional data of the present study indicate that IL-10 -1082 G allele and G (+) haplotype are associated with higher expression levels of IL-10 mRNA. However, there is a possibility that high expression of IL-10 mRNA in psoriasis partially results from some other causes than -1082 G allele and G (+) haplotype.
5. The trend of higher IL-20 mRNA expression among psoriasis patients with -1053 genotype T/G and 3978 genotype C/C indicates that the poly-morphisms in the promoter and 3'-UTR regions are possibly related to the level of IL-20 mRNA expression.

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# 8. SUMMARY IN ESTONIAN

## Interleukiin-10 perekonna tsütokiinide geenide polümorfismide seosed naastulise psoriaasiga

### Üldteoreetiline taust

Psoriaas on krooniline, mitteinfektsioosne, põletikuline dermatoos, mille patogeneesis on juhtiv roll T-rakkudel ning neist vabanevatel tsütokiinidel ja kemokiinidel. Interleukiin-10 (IL-10) on multifunktsionaalne tsütokiin, mida produtseeritakse erinevate T-rakkude (Th2, Tc2, Tr1), monotsüütide, makrofaagide, keratinotsüütide ja mitmete teiste rakutüüpide poolt ning mille üheks märklauaks organismis on keratinotsüüdid. Nimetatud tsütokiini ebaadekvaatset ekspressiooni on näidatud erinevate nahahaiguste (atoopilise dermatiidi, T-lümfoomi, melanoomi ja basaalrakulise kartsinoomi) korral, kusjuures psoriaasile on iseloomulik IL-10 defitsiit.

Erinevused IL-10 ekspressioonis on ~50%...75% ulatuses geneetiliselt determineeritud. IL-10 produktsiooni määrav geen paikneb 1 kromosoomi pikas õlas q31 – q32 lookuses ning tema 5' regiooni promootorala on kõrgelt polümorfne. IL-10 geeni promootorala kolm ühenukleotiidset polümorfismi (SNP-d) positsioonides -1082 G/A, -819 C/T ja -592 C/A on täielikult aheldunud ning esinevad eurooplastel enamasti kolme haplotüübina (GCC, ACC, ATA). IL-10 promootorala nimetatud geneetilised variatsioonid mõjutavad IL-10 ekspressiooni, kuid uuringute tulemused nende SNP-de efektidest tsütokiini ekspressioonile on vasturääkivad. Lisaks on kirjeldatud SNP-d seotud mitmete autoimmuunhaiguste, pahaloomuliste kasvajate ja infektsioonide avaldumise ja kliinilise kuluga. IL-10 promootoralas paikneva mikrosatelliidiga on seotud psoriaasi perekondliku vormi avaldumine.

Interleukiin-19 (IL-19), interleukiin-20 (IL-20), ja interleukiin-24 (IL-24) on suhteliselt hiljuti avastatud IL-10 perekonda kuuluvad tsütokiinid, mis moodustavad omakorda iseseisva IL-19 alamperekonna tänu oma struktuursele sarnasusele ja seondumisele IL-20 retseptor ß-alaühikuga.

IL-20 ekspresseerivad veres monotsüüdid ning tema üheks peamiseks märklauaks organismis on keratinotsüüdid, kus IL-20 mõjutab epidermises spetsiifiliste retseptorite – IL-20R1 (IL-20Rα and IL-20Rβ) ja IL-20R2 (IL-20Rβ and IL-22R) – vahendusel keratinotsüütide diferentseerumist ja proliferatsiooni. IL-20 tähtsusele psoriaasi patogeneesis viitab fakt, et tsütokiini üleekspressioon transgeensetel hiirtel põhjustab nahalööbe, mis on sarnane nimetatud kroonilisele dermatoosile. IL-19 on peamiselt monotsüütide poolt produtseeritav proinflammatoorne tsütokiin, mis omakorda stimuleerib märklaudrakkudes IL-6 ja TNF-α ekspressiooni. Nii IL-6 kui ka TNF-α olulisus psoriaasi patogeneesis on aga veenvalt tõestatud. Lisaks eeldatakse IL-19 vähemalt osaliselt sarnast bioloogilist aktiivsust IL-20-ga, kuna tsütokiin seondub IL-20R1-ga. IL-24

ekspresseerivad veres nii monotsüütid kui ka T-rakud ning analoogselt IL-20-le seondub nimetatud tsütokiin IL20R1/IL-20R2 kompleksidega. Kirjeldatud tsütokiinide rolli psoriaasi tekkemehhanismis kinnitab fakt, et psoriaasiga haigetel on haiguskoldes võrreldes terve nahaga nii IL-20, IL-19, IL-24 kui ka IL-20 retseptorite alaühikute – IL-20Rα ja IL-20Rβ – ekspressiooni tase tõusnud.

IL-19, IL-20 ja IL-24 kodeerivad geenid paiknevad sarnaselt IL-10 geenile 1 kromosoomi pikas õlas q31-32 regioonis. IL-19 perekonna tsütokiinide SNP-de kirjeldused on küll andmebaasides olemas (www.ncbi.nlm.nih.gov/SNP/), kuid IL-19, IL-20 ja IL-24 geenide polümorfismide efekte tsütokiinide ekspressioonile ja erinevate haiguste avaldumisele varem ei ole uuritud.

## Uurimuse põhieesmärgid

1. Uurida interleukiin-10 (IL-10) ja tema perekonda kuuluvate tsütokiinide interleukiin-19 (IL-19), interleukiin-20 (IL-20) ja interleukiin-24 (IL-24) geneetiliste polümorfismide seoseid naastulise psoriaasiga.
   a) Viia läbi assotsiatsioonianalüüs leidmaks seoseid IL-10, IL-19, IL-20 ja IL-24 geenide ühenukleotiidsete polümorfismide ja psoriaasi vahel.
   b) Hinnata 1q31-32 regioonis paiknevate IL-10 perekonna (IL-10, IL-19, IL-20 ja IL-24) geenide markerpaaride vahelist ahelduse tasakaalustamatust (LD) ning määrata kindlaks LD blokkide piirid.
   c) Viia läbi haplotüübianalüüs selgitamaks kirjeldatud geenide haplotüüpide efekte haigusfenotüübi kujunemisel.

2. Uurida IL-10 ja IL-20 geneetiliste polümorfismide mõju nimetatud tsütokiinide ekspressioonile ning selgitada uuritavate geenide funktsionaalset tähtsust psoriaasi avaldumisel ja aktiivsuse määramisel.
   a) Hinnata IL-10 ja IL-20 mRNA ekspressiooni erinevusi veres tervetel kontrollidel ja psoriaasiga haigetel.
   b) Hinnata IL-10 ja IL-20 geneetiliste polümorfismide efekte vastavate geenide ekspressioonile ning selgitada IL-10 ja IL-20 geneetiliste polümorfismide funktsionaalset tähtsust haiguse avaldumisele.

## Uuringute metoodika ja tulemused

Võimalike seoste selgitamiseks 1q31-32 lookuses paiknevate IL-10 perekonna tsütokiinide geenide SNP-de ja naastulise psoriaasi fenotüübi vahel kasutasime uurimismetoodikana indirektset assotsiatsiooniuuringut ning uuritavateks olid suguluses mitteolevad naastulise psoriaasiga haiged ja terved kontrollid.

248-l psoriaasiga haigel ja 148-l tervel kontrollil analüüsisime ARMS-PCR meetodil kolme IL-10 geeni promootorala SNP-d positsioonides -1082 G/A, -819 C/T ja -592 C/A. Töö tulemused näitasid, et IL-10 promootorala haplotüübid ei mõjuta psoriaasi avaldumist, küll aga haiguse aktiivsust ning kliinilist kulgu. ACC haplotüüp esines sagedamini piirdunud lööbega (lööbe ulatus ≤10%) haigetel ($p<0,05$; OR 1,634) ja patsientidel, kellel oli PASI skoor ≤20 ($p<0,05$; OR 1,463). ATA hablotüüp määras lööbe persiteeriva kulu (persisteeriv lööve *vs* vahelduv lööve: $p<0,01$; OR 2,445).

IL-19 perekonna tsütokiinide geenide ja psoriaasi vahelise assotsiatsiooniuuringu viisime läbi 254-l naastulise psoriaasiga haigel ja 148 tervel kontrollil. IL-19 perekonna tsütokiinide SNP-de tuvastamiseks kasutasime tetra-ARMS-PCR meetodit.

Töö tulemusena leidsime, et IL-20 geeni promootorala -1053G alleel oli riskialleeliks ($p<0,05$) ja IL-20 geeni SNP-de -1053, 1380 ja 1462 alleelsetest variantidest kombineerunud GAA haplotüüp riskihaplotüübiks ($p<0,01$; OR 2,341) naastulise psoriaasi avaldumisel.

IL-19 geeni SNP rs2243188 minoorne alleel oli seotud kaitsva efektiga psoriaasi suhtes ($p<0,05$), rs2243158 ja rs2243168 olid aga alatüübi-spetsiifilised markerid II tüübi naastulise psoriaasi ($p<0,05$) ning rs2243168 täiendavalt ka hilise haigusvormi avaldumisel ($p<0,02$). IL-19 geeni haplotüübianalüüs näitas, et kuigi IL-19 TGATA haplotüübiga isikuid oli psoriaasiga haigetegrupis harvem kui kontrollgrupis, esines statistiliselt oluline erinevus vaid kontrollgrupi võrdluses hilise psoriaasiga patsientidega ($p=0,05$; OR 0,58).

IL-19 ja IL-20 geenide LD analüüs näitas, et IL-19 geeni SNP-d ja IL-20 geeni SNP-d positsioonides -1053, 1380 ja 1462 on peaaegu täielikult aheldunud (|D'| 0.78 kuni 0.99) ning IL-19 ning IL-20 geenide laiendatud haplotüübianalüüsil leidsime, et haplotüüp CACCGGAA on riskihaplotüübiks naastulise psoriaasi avaldumisel ($p<0,01$; OR 2,548), kusjuures nimetatud haplotüübi riskiefektis oli määravaks IL-20 -1053 G alleeli efekt ($p<0,01$). IL-19 TGATA haplotüübi protektiivne efekt hilise haigusvormi avaldumisel ei püsinud kombineeritud IL-19 ja IL-20 haplotüübianalüüsis (TGATAGGG: $p=0,34$; OR 0,722)

IL-24 geeni SNP-de assotsiatsiooni- ja haplotüübianalüüsil leidsime, et rs1150253 ($p<0.05$) ja IL-24 haplotüüp GGGT ($P=0,05$; OR 1,548) on alatüübi spetsiifilisteks markeriteks psoriaasi piirdunud vormi (nahalööbe ulatus ≤10%) avaldumisel. Lisaks esines IL-24 geeni SNP-de ja IL-20 geeni 3'-UTR regiooni SNP vahel märkimisväärne aheldatus (|D'| between 0.61 and 0.79) ning IL-20/IL-24 laiendatud haplotüübianalüüs näitas, et haplotüübid CAAAC ($P<0,001$; OR 0,154), TGGGT ($P<0,05$; OR 0,591) ja CGAGT ($P<0,05$; OR 0,457) vähendavad haiguse avaldumise riski, kusjuures CAAAC haplotüübi protektiivses efektis oli oluline IL-20 geeni 3'-UTR regiooni SNP 3978 C alleeli efekt ($p<0,001$). IL-24 GGGT haplotüübi riskiefekt ei püsinud laiendatud haplotüübianalüüsis (CGGGT: $P=0,06$; OR 1,618; TGGGT: $P=0,84$; OR 0,930).

IL-10 ja IL-20 tsütokiinide mRNA ekspressiooni tasemete analüüsi viisime läbi TaqMan® QRT-PCR meetodil. Erinevate proovide võrdlemiseks olid IL-10 ja IL-20 ekspressioonide tasemed normaliseeritud sama proovi koduhoidjageeni HPRT-1 ekspressiooni taseme suhtes. Psoriaasiga patsientidel oli IL-10 geeni ekspressiooni tase kõrgem võrreldes tervete kontrollidega ($p<0.01$) ja IL-10 geeni -1082 positsiooni alleelsed variandid olid seotud statistiliselt oluliste erinevustega IL-10 ekspressioonis. IL-20 mRNA ekspressioonianalüüsil ei leidnud me statistiliselt olulisi erinevusi haigete võrdlusel kontrollgrupiga ning analüüsitud IL-20 promootorala ja 3'-UTR regiooni SNP-de alleelsed variandid ei mõjutanud statistiliselt oluliselt tsütokiini ekspressiooni taset, kuigi IL-20 -1053 T/G genotüübiga ja IL-20 3978 C/C genotüübiga haigetel esines tendents IL-20 kõrgemale ekspressioonile.

## Järeldused

1. IL-10 geeni klastris esimese kromosoomi q31-32 regioonis on kolm haplotüübi blokki, mis hõlmavad 128498 bp pikkuse segmendi.
2. Hinnates hablotüübi-fenotüübi vahelisi seoseid leidsime, et IL-10 geen on pigem haigust modifitseeriv kui psoriaasi avaldumist mõjutav geen. See-vastu IL-19 perekonna tsütokiinide geenid (IL-19, IL-20 ja IL-24) mõjutavad aga naastulise psoriaasi avaldumise üldist riski.
3. Meie töö tulemused näitasid, et IL-10 mRNA ekspressioon vererakkudes on psoriaasiga haigetel statistiliselt oluliselt kõrgem kui tervetel. IL-20 mRNA ekspressioonianalüüsil täisverest ei leidnud me statistiliselt olulisi erinevusi patsientide võrdlusel kontrollgrupiga.
4. Käesoleva töö funktsionaalsed andmed osutavad, et IL-10 -1082 G alleel ja G (+) haplotüüp on seotud IL-10 mRNA kõrgema ekspressiooni tasemega. Samas võib haigetel leitud kõrgem IL-10 ekspressioonitase vererakkudes olla osaliselt tingitud ka muudest faktoritest.
5. Psoriaasiga haigetel, kellel oli IL-20 -1053 T/G genotüüp ja IL-20 3978 C/C genotüüp, leidsime tendentsi kõrgemale IL-20 mRNA ekspressioonile. See tulemus viitab asjaolule, et IL-20 promootorala ja 3'-UTR regiooni SNP-de alleelsed variandid võiksid mõjutada IL-20 mRNA ekspressiooni taset.

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Journal of Dermatological Science (2005) 37, 111–113.

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Member of the Society for Estonian Dermatologists and Venereologists, member of the Estonian Society of STD, member of the Baltic Association of Dermatologists and Venereologists, member of the Central and East European Dermatovenereologists Association.

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### Teadustöö

Teadustöö eesmärgiks on uurida interleukiin-10 (IL-10) ja tema perekonda kuuluvate tsütokiinide interleukiin-19 (IL-19), interleukiin-20 (IL-20) ja interleukiin-24 (IL-24) geneetilisi polümorfisme ja nende seoseid erinevate psoriaasi vormidega. Lisaks geneetilise variatsiooni ja psoriaasi vahelise seose analüüsile üritame leida ka IL-10, IL-19, IL-20 ja IL-24 põhjuslikku või patogeneetilist rolli psoriaasi kujunemisel, määrates nimetatud tsütokiinide mRNA ekspressioone täisveres eelnevalt genotüpeeritud haigetel ja kontrollidel.

## Kuuluvus erialaühingutesse

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## Tunnustused

1. I preemia Eesti üliõpilaste teadustööde 2004. a riiklikul konkursil arsti-teaduste valdkonnas teadustöö "Interleukiin-10 ja Interleukiin-20 geenide ühenukleotiidsete polümorfismide seosed psoriaasiga" eest, 10.12.2004.
2. Tartu Ülikooli Kliinikumi teadustööpreemia uurimustöö "Interleukiin-20 geneetiliste polümorfismide seos psoriaasiga" eest, 11.10.2003.
3. Molekulaarse ja Kliinilise Meditsiini Keskuse 2003 a. doktorandipreemia uurimustöö "Interleukiin-20 geneetiliste polümorfismide seos psoriaasiga" eest.

## Publikatsioonid

Teaduslike publiktsioonide üldarv – 30