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Role of TNF-α gene polymorphisms in the onset of Psoriasis and Psoriatic Arthritis using Next Generation Sequencing

MED/03 Medical Genetics

PhD candidate Federica Cannas

PhD Coordinator Prof. Amedeo Columbano

PhD Supervisor Prof. Carlo Carcassi

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Abstract

Psoriasis Vulgaris (PsV) and Psoriatic Arthritis (PsA) are a complex immune-mediated diseases resulting from the interplay between multiple genetic and environmental factors. It has been estimated that at least one-third of the genetic contribution to PsV and PsA resides in the human major histocompatibility complex region (MHC).

The tumor necrosis factor-α (TNF-α) gene, which is located in the short arm of chromosome 6 in the major histocompatibility complex class III region between the HLA-B and HLA-DR genes, has been proposed as a major candidate gene in PsV and PsA. This hypothesis is supported by studies that have found high serum, synovial fluid, and synovial membrane TNF-α levels in patients with PsA. In clinical trials for psoriatic arthritis (PsA), TNF-α blockers have been shown to have excellent clinical efficacy and to prevent further structural damage to joints.

The aim of my thesis was to clarify the mechanisms through which the genetic variability of TNFα and Lymphotoxin alpha (LTA) genes play a role in the susceptibility and progression of psoriasis and psoriatic arthritis and in therapeutic response with anti-TNFα inhibitors using Next Generation Sequencing approach.

180 patients with PsV and 130 patients with PsA were included into the study. The control cohort included 120 healthy, unrelated subject of the same ethnic origin with no family history of psoriasis and PsA. We included 70 patients treated with TNF-inhibitors, with the diagnosis of psoriasis and PsA, according to clinical criteria, starting the first TNF-blocker agent, and with at least 2 years of follow-up. DNA was extracted from peripheral blood. Genomic DNA were amplified for TNF/LTα, HLA-B and HLA-C under long range PCR using the specific forward and reverse primer pairs. Subsequently, genomic DNA was prepared for the following step.

The genetic analysis revealed no significant differences in the frequency of the variants between psoriasis patients and controls. Some of these variants, would be attributable to the linkage disequilibrium among the alleles found during the analysis of this region and the HLA loci traditionally associated with the psoriasis: HLA-C*06:02 in many populations and HLA-C*07:18- B*58 in the Sardinian population.

To evaluate if the associations found in our samples were correlated with the presence of HLA-C*06:02 and HLA-C*07:18 - B*58, a stratification analysis was carried out. Of 180 psoriatic patients, 97 (54%) were carriers of the alleles HLAC*06:02 and HLA-C*07:18 - B*58 and 83 were not carriers. The 97 alleles resulted to be in a strong linkage disequilibrium.

The analysis for the group of PsA patients compared with controls have shown no significant differences in the frequency of the variants, and stratification in the PsA group

showed a strong linkage disequilibrium of all the variants identified with the HLA alleles C*06:02 C*07:18 B*58:01.

Finally, a study relating the response to TNF-inhibitors was performed. 60 patients were analyzed, 17 non-responders and 53 responders. The *p value* for the marker rs1800750 (-376G>A) (p = 0,005445) resulted strongly associated with a frequency of 14%. The minor allele (A) is completely absent in the patients that don't responded to the anti-TNF therapy while it was present in the responders, this finding seems to be important due the fact that this allele has been found to be associated to high level of TNF.

In conclusion, the results based on the examination of 65 SNPs of tumor necrosis factor and lymphotoxin alpha genes suggest that at least in the Sardinian population there is no direct link between genotype distribution, alleles carriage and overall number of alleles and susceptibility for Psoriasis Vulgaris and Psoriatic Arthritis. A striking result was obtained in the evaluation of the anti-TNF-α biologic therapy in the cohort of patients treated with anti-TNF-α biologic therapy showing a significant association in responders compared to non-responders.

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List of Abbreviations

ADRs - Adverse drug reaction

AS - Ankylosing Spondylitis

BSA - Body Surface Area

CASPAR – Classification of Psoriatic Arthritis

CDSN - Corneodesmosin

CI – Confidence Interval

DMARDs - Disease Modifying Anti-Rheumatic Drugs

FDR - False Discovery Rate

GWAS - Genome Wide Association Study

HLA - Human Leukocyte Antigen

IBD – Inflammatory Bowel Disease

LD - Linkage Disequilibrium

LR – Likelihood Ratio

LR PCR – Long Range PCR

LTA - Lymphotoxin alpha

NGS - Next Generation Sequencing

NSAIDs - Non-Steroidal Anti-Inflammatory Drugs

OR - Odds Ratio

PASI – Psoriasis Area and Severity Index

PGBMs - PGx biomarkers

PGx - Pharmacogenomics

PsA – Psoriatic Arthritis

PsD - Psoriatic Disease

PSORS - PSORiasis Susceptibility locus

PsV – Psoriasis Vulgaris

QoL - Quality of life

ReA - Reactive arthritis

SNP - Single Nucleotide Polymorphisms

SpA – Spondyloarthropathies

SPR – Standardized Prevalence Ratio

TNF - Tumor Necrosis Factor

1. INTRODUCTION

1.1.1. Historical background of psoriasis

"Psoriasis is an antidote for dermatologists' ego"
Paul Bechet, New York (1936)

Psoriasis is a chronic, common, non-contagious, papulosquamous, skin disease of undefined aetiology. The history of psoriasis begins in ancient Greece, when "psoriasis" and "leprosy" were perceived and treated as the same disease.
For centuries, the clinical diagnosis of psoriasis remained ambiguous. It was not until the 19th century, when dermatologists attempted to establish a proper classification model to better understand and treat skin diseases, that psoriasis was distinguished from leprosy. Robert Willan, in his fundamental treatise on skin disease, "On Cutaneous Diseases", was the first to describe psoriasis as a distinct clinical entity and place it in the group of papulosquamous skin diseases (reviewed in Griffiths and Barker, 2007b).
Recent advances in the fields of histo- and immunopathology, as well as molecular genetics have substantially improved our understanding of psoriasis, however, there are still numerous questions that remain unanswered (Cowden and Voorhees, 2008).

1.1.2. Epidemiology of psoriasis

Psoriasis is a common skin inflammatory disorder affecting >125 milion peolple worldwide (J. Koo, 1996) (Fig. 1). Psoriasis is widely distributed throughout the world with a prevalence that ranges from 0.91 to 8.5% in adult patients and 0 to 2.1% in children (Parisi et al., 2012). General population-based surveys show a prevalence of approximately 2% amongst Caucasians (1.6% in the United Kingdom, 2.2% in the United states, 2.8% in the Faroe Islands, 2% in Sweden, 1.17-1.43% in Spain, 4.8% in Norway) and less than 1% in the Asian race (Hong Kong, Japan and China); (Neimann et al., 2006; Gudjonsson and Elder, 2007). Overall there is no consensus on whether gender influences the prevalence of psoriasis but it is generally believed that it affects both males and females equally (Bovenschen et al., 2005). Additionally, there is literature showing a seasonal distribution of the disease, with a higher frequency of flares or new cases reported in winter and spring (Farber and Peterson, 1961).

Despite multiple studies on the prevalence of psoriasis, relatively few have investigated its incidence (Setty et al., 2007; Icen et al., 2009a; Valdimarsson et al., 1986; Prinz, 1999; Vena et al., 2010). This is attributed to the substantial variation of the clinical phenotype of psoriasis and the absence of solid criteria for its diagnosis (Icen et al., 2009a). Although susceptible to limitations, studies on the incidence of psoriasis suggest an increase in the risk of developing the disease, in Western populations, between 1970 and 2011 (SettyAr, 2007; Icen et al., 2009b; Gudjonsson and Elder, 2007; Parisi et al., 2013; Vena et al., 2010). It is, however, unclear whether the aforementioned observations on the incidence of psoriasis illustrate a true increase or are driven from a subsequent increase in risk factors for psoriasis, including stress and obesity, as well as increased awareness of the disease, in addition to more precise diagnostic methods and improved data collection strategies (Tollefson et al., 2010). Future research is, therefore, required to determine, more precisely, the incidence rate of psoriasis, while, at the same time, identify and control for all the relevant confounding factors, described above.

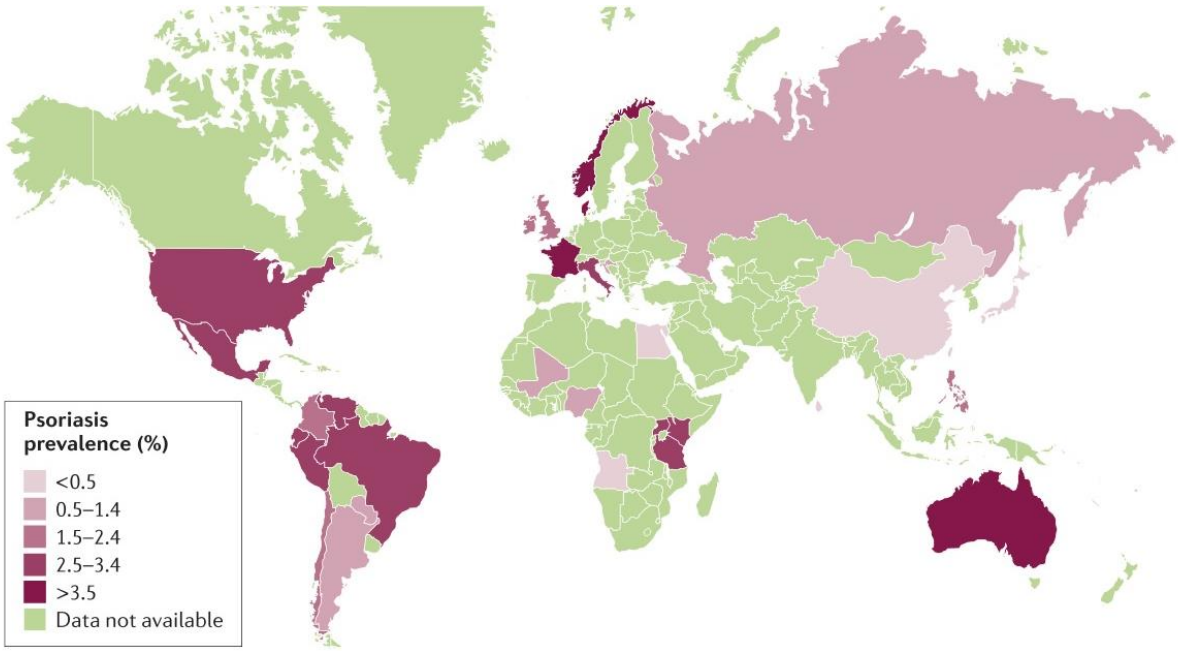

Figure 1 | **Global prevalence of psoriasis**

1.1.3. Early and Late-onset Psoriasis

Psoriasis affects men and women equally, and is seen in all races. Although psoriasis can begin at any age, there seem to be two peaks in onset: one between ages 20 and 30 and another between ages 50 and 60 (Langley RG et al., 2005). Psoriasis has been described as two types depending on the age of onset. Patients with early-onset or Type I psoriasis (before the age of 40) tend to have more severe disease with a familial history. Patients

with late-onset or Type II psoriasis (after the age of 40) tend to have a milder disease (Henseler T, et al., 1985)

1.1.4. Clinical manifestations of psoriasis

Psoriasis is usually manifested as raised, erythematous plaques with adherent silvery scales. It is usually easily recognized, but atypical or non-classic forms are more difficult to identify. There are several clinical types of psoriasis; the most common one is chronic plaque psoriasis or psoriasis vulgaris that affects 85-90% of all patients with the disease. This type usually presents in young adults with symmetrically distributed plaques involving the scalp, extensor elbows, knees, and back. Other types include flexural psoriasis, guttate psoriasis, pustular psoriasis and erythroderma (Griffiths CE, et al., 2007). Approximately 40% of patients with psoriasis have nail lesions. One of the typical nail abnormalities in psoriasis is pitting, consisting of a few to multiple tiny pits scattered over the nail plate. The pits reflect abnormal nail plate growth resulting from psoriatic involvement of the nail matrix. These changes produce friable areas of nail plate that erode away with normal friction. Another typical psoriatic nail lesion is onycholysis, that occurs as a result of a separation of the nail plate from its underlying attachment to the nail bed (Langley RG, et al., 2005). (Fig. 2)

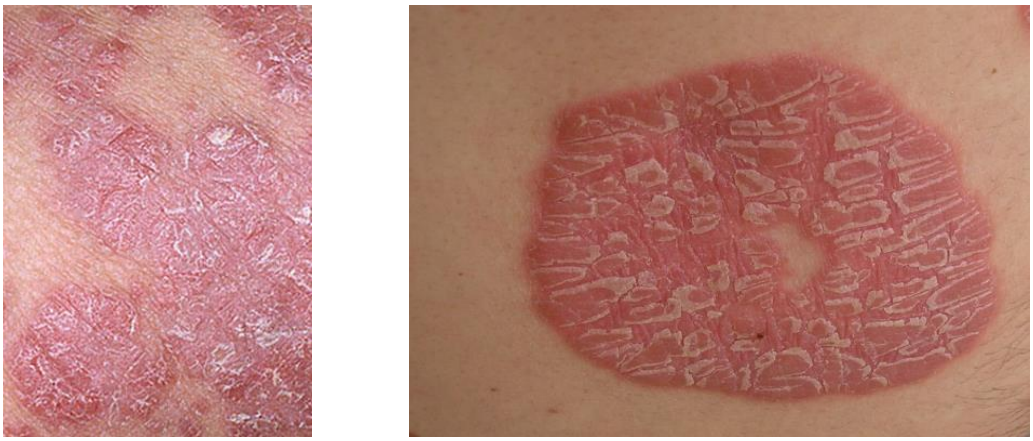

Figure 2 | **Skin manifestation of psoriasis**

1.1.5. Diagnosis of psoriasis

Although the differential diagnosis of psoriasis is broad, a skin biopsy is rarely needed. The diagnosis is usually made by history and physical examination. There are no laboratory tests that confirm or exclude the diagnosis. A detailed physical examination should focus on typical sites of involvement such as knees and elbows with a special attention to subtle findings in the scalp, umbilicus, inter gluteal cleft, and nails.

1.1.6. Measurement tools in Psoriasis: Psoriasis Area and Severity Index (PASI)

The most commonly used method for measuring psoriasis disease activity is the psoriasis area and severity index (PASI). PASI is considered the gold standard and was introduced in 1978 (Fredriksson and Pettersson 1978). It measures psoriasis in three different aspects; erythema (E; redness), induration (I; thickness) and desquamation (D; scaliness). Each aspect is graded on a scale of 0-4, 0: “no involvement”, 1: “slight”, 2: “moderate”, 3: “marked”, 4: “very marked”. These aspects are assessed in four different regions of the body, together with the area of psoriasis coverage (A) as used in the body surface area (BSA) index. However, in contrast to the body surface area index, psoriasis coverage is graded in each region on a scale of 0-6, 0: no involvement, 1: <10%, 2: 10-29%, 3: 30-49%, 4: 50-69%, 5: 70-89%, 6: ≥90%. The PASI score is calculated by the sum of all three psoriatic aspects in each region of the body, multiplied by the area coverage of that region and its respective weighting. The scores for each region of the body are then added together giving a final range of 0-72, with 0 being no disease and 72 being maximal disease (Ashcroft et al. 1999).
This is given in the following formula;

PASI = 0.1AH(EH + IH + DH) + 0.2AU(EU + IU + DU) + 0.3AT(ET + IT + DT) + 0.4AL(EL + IL + DL).

There are a number of limitations with PASI. The first is that it has poor sensitivity as a result of erythema, induration and desquamation being given equal weightings. This means that an increase in one aspect can be balanced out in the PASI score by a decrease in another. It is also poor at detecting changes in the three different aspects measured when the area of psoriasis coverage is very low. PASI does not include a number of psoriatic features such as fever, which means that some important factors in psoriasis severity are overlooked. The perception of disease severity can vary from person to person. What one patient views as mild can be viewed as severe by another patient. Therefore a major limitation of PASI is that it does not take patient assessment

into account. Another effect of psoriasis that is overlooked with PASI is the quality of life. This is also specific to each patient and can dictate what course of treatment is taken. It has been reported that the upper extremities of the scale are rarely used and are therefore redundant (Feldman and Krueger 2005). Because of these limitations, variants of PASI have been developed, but are little used. These include the simplified PASI (SPASI), which uses an average and estimated value for erythema, induration, desquamation and body coverage, and the exact PASI (PEASI), which uses exact body coverage percentages (Louden et al. 2004; Jacobson and Kimball 2004). Despite this, PASI continues to be used widely and is the scale that other psoriasis assessment tools are compared against.

1.1.7. Dermatology Life Quality Index (DLQI)

The most commonly used tool to assess patient quality of life (QoL) in psoriasis is the Dermatology Life Quality Index (DLQI). The DLQI was developed in 1994 and designed to be completed by a patient over 16 years of age, without the need for detailed explanation from a physician (Finlay and Khan 1994). It consists of ten questions spread across six categories, including two on symptoms and feelings, two on daily activities, two on leisure, one about work and school, two about personal relationships and one about treatment. For each question, the patient responds by indicating one of five responses; “very much”, “a lot”, “a little” “not at all” or “not relevant”. Each question is scored from 0-3 with “very much” assigned 3 and “not at all” or “not relevant” assigned 0. The DLQI score is calculated by the sum of the score for all ten questions, giving a range of 0-30. The DLQI score is banded into categories to give an overall assessment of the impact of psoriasis on patient quality of life. There are a number of advantages to the DLQI, including its simplicity, which allows it to be completed easily by patients, as well as its availability in multiple languages. However, it has received criticism for its focus on physical aspects of life at the expense of psychological factors (Nijsten 2012). Despite this the DLQI is the most widely used tool for measuring QoL in clinical studies.

1.1.8. Treatment of psoriasis

Treatment in psoriasis can generally be categorized into topical and systemic therapies. The treatment choice is dictated by the severity, type, and location of psoriasis. Patients with mild psoriasis can usually be managed with topical agents including: corticosteroids, tar, retinoids and vitamin D derivates. Moderate to severe psoriasis requires phototherapy or systemic therapies such as methotrexate, retinoids cyclosporine or the biologic immune

modifying agents including alefacept the anti- Tumor Necrosis Factor (TNF) agents or anti-IL12/23 monoclonal antibody (ustekinumab) (Menter A, et al., 2010).

1.1.9. Clinical course and co-morbidities of psoriasis

Psoriasis tends to be a chronic disease. However, its course is unpredictable. There may be marked variability in severity over time, and remissions at some stage are seen in up to 40% of cases (Farber EM, et al., 1974). Although generally not life threatening, psoriasis may be associated with important morbidity and disability. It can range from a very mild disease with few small hidden plaques that do not interfere with daily life to severe wide-spread skin lesions that may lead to disability and poor quality of life. Patients with psoriasis, like those with other major medical disorders, have reduced levels of employment as well as decreased quality of life (Finlay EM, et al., 1987) (Finlay EM, et al., 1995). Psoriasis is also associated with other co-morbidities including obesity and other related metabolic abnormalities such as diabetes mellitus and dyslipidemia (Christophers E, 2007) (Gottlieb AB, et al., 2008). These in turn lead to increased cardiovascular morbidity and mortality (Gelfand JM, et al., 2006). In addition, approximately 30% of the patients with psoriasis develop an inflammatory arthritis termed Psoriatic Arthritis (PsA) (Gladman DD, 2005).

1.2. Psoriatic Arthritis

Psoriatic arthritis has been defined as an inflammatory arthritis associated with psoriasis, usually seronegative for rheumatoid factor (Wright V, et al., 1976). The association between psoriasis and arthritis was first described in 1818 by the French physician, Baron Jean Louis Alibert (Alibert J, 1818). However, only in 1964 did the American Rheumatology Association recognize PsA as a unique disease entity that is separate from rheumatoid arthritis (Blumberg BS, et al., 1964). Furthermore, only in 2006, were classification criteria for PsA developed and allowed a better definition of cases for research purposes (Taylor W, et al., 2006).

Psoriatic arthritis is classified among the seronegative spondyloarthropathies (SpA). This term refers to a family of diseases that share certain clinical features. The most distinguishing features are inflammation of the axial joints, asymmetric oligoarthritis, and enthesitis (inflammation at sites of ligamentous or tendon attachment to bone). Additional features are genital and skin lesions, eye and bowel inflammation, an association with preceding or ongoing infectious disorders, and a strong association with the Human Leukocyte Antigen (HLA). The SpA group consists of the following disorders: Ankylosing

Spondylitis (AS), Reactive arthritis (ReA), PsA, Undifferentiated spondyloarthritis, SpA associated with Inflammatory Bowel Disease (IBD) and Juvenile onset spondyloarthritis (Healy PJ, et al., 2005).

1.2.1. Epidemiology of PsA

The most recent estimate of the prevalence of PsA in North America is 0.25% (95% CI: 0.18%, 0.31%) (Gelfand JM, et al., 2005). The reported incidence of PsA in the general population ranges from 3.4-8 per 100,000 (Alamanos Y, et al., 2008) (Soriano ER, et al., 2010). PsA has been reported in 7-42% of patients with psoriasis, with a recent estimate being approximately 30% (Gladman DD, 2005) (Zachariae H, 2003) (Scarpa R, et al., 1984). The marked variability in reported prevalence and incidence rates is probably related to different definitions of the disease as well as variable sources of populations. Approximately 67% of the patients develop psoriasis before arthritis and in 16% arthritis and psoriasis present within 12 month of each other (Gladman D, 2005). There is limited information about the incidence of PsA among patients with psoriasis. A retrospective study from Germany reported that the cumulative incidence of PsA among psoriasis patients reached 20.5% after 30 years from the diagnosis of psoriasis (Christophers E, et al., 2010). Another retrospective study from Rochester, Minnesota has found a lower cumulative incidence of 3.1% cases of PsA among psoriasis patients after 10 years from the onset of the skin disease (Wilson FC, et al., 2009). No study to date has prospectively assessed the incidence of PsA among psoriasis patients. The prevalence of the disease is equal among males and females. PsA usually occurs in the third or fourth decade of life (Gladman DD, 2005). There is very little information about racial and ethnic associations as most epidemiological studies has been performed in Caucasians.

1.2.2. Clinical manifestations

Five patterns of PsA have been described: the symmetric polyarticular pattern being the most common (Helliwell PS et al., 2007), distal arthritis that involves the distal interphalangeal joints, asymmetric oligoarthritis in which less than 5 joints are affected, arthritis mutilans that is characterized by deforming and destructive arthritis, and spondyloarthritis that includes sacroiliitis and spondylitis (Gladman DD, et al., 1987). Some of the patients present with more than one pattern or change their pattern during the course of their disease. Another common feature of PsA is enthesitis, an inflammation at the site of tendon insertion into the bone, that often affects the Achilles tendon, plantar

fascia and pelvis bones (McGonagle D, et al., 1999). Dactylitis is characterized by diffuse swelling of the entire finger or toe; it affects about half of the patients and is associated with radiographic joint damage (Brockbank JE, et al., 2005). Similarly to the other spondyloarthropathies, PsA is also associated with inflammation in other extra-articular sites including the eye (uveitis) and the gastrointestinal tract (inflammatory bowel disease). (Fig. 3)

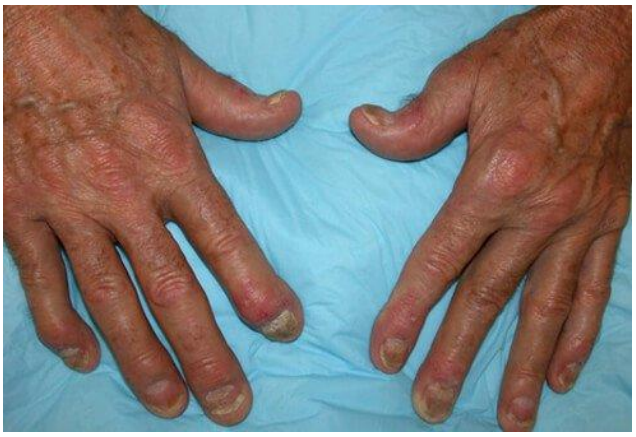

Figure 3 | **Manifestation of psoriatic arthritis**

1.2.3. Relationship between skin and joint disease

PsA may be considered as a disease within a disease as most of the patients with PsA also have psoriasis, although according to the new classification criteria, patients with PsA do not have to have established psoriasis (Taylor W, et al., 2006). Most of the patients develop PsA after or at the same time as the skin disease, however, 15% of patients with PsA present with arthritis before psoriasis (Gladman DD, et al., 1987). Since most patients develop PsA after the onset of psoriasis, the skin disease serves as a marker for the development of PsA. The paradigm that patients with severe psoriasis are the ones who develop arthritis is controversial. Several studies reported a higher prevalence of PsA among patients with severe psoriasis (Gelfand JM, et al., 2005) (Tey HL, et al., 2010). However, the fact that PsA can present before psoriasis as well as recent observations of no relationship between the severity of the skin and joint manifestations (Elkayam O, et al., 2000) (Cohen MR, et al., 1999), suggest there is no direct link between psoriasis severity and arthritis. Nail lesions are more common among PsA patients than in psoriasis and the presence of nail lesions has been suggested as marker of increased risk for PsA among psoriasis patients (Gladman DD, et al., 1986) (Griffiths CE, et al., 2007).

1.2.4. Diagnosis of PsA

The diagnosis of PsA is based on a typical combination of clinical, laboratory and radiographic findings. The Classification of Psoriatic ARthritis (CASPAR) criteria are a set classification criteria for PsA that were published in 2006. They may be used for diagnosis and allow a uniform definition of cases for research purposes. The CASPAR criteria showed high sensitivity and specificity for PsA (91.4% and 98.7%, respectively) (Taylor W, et al., 2006) (Tab. 1)

The CASPAR criteria consist of the following terms:

Required: The presence of an inflammatory arthritis, enthesitis, or spondylitis.

Plus 3 points from the following:

1. Skin psoriasis (present) (2 points), previously present by history (1 point), or a family history of psoriasis (1 point)
2. Psoriatic nail lesions (1 point)
3. Dactylitis (1 point)
4. Negative rheumatoid factor (1 point)
5. Juxta-articular bone formation on radiographs (1 point)

Table 1. Classification of Psoriatic Arthritis (CASPAR) Diagnostic Criteria.

Category	Description	Points
Current psoriasis or personal or family history of psoriasis	Current Psoriasis: Skin or plaque disease confirmed by rheumatologist or dermatologist. Personal history: obtained from patient, family physician, dermatologist, rheumatologist or other qualified health care provider Family history: presence of psoriasis in 1o or 2o relatives as reported by patient	2 (current) OR 1 (history)
Psoriatic nail dystrophy on current examination	Onycholysis, pitting, hyperkeratosis	1
Negative rheumatoid factor	Any method except latex, but preferably enzyme linked immunosorbent assay (ELISA) or nephlometry, using local laboratory referenece range	1
Dactylitis (current or on history as recorded by rheumatologist)	Swelling of an entire digit	1
Radiographic evidence of juxta-articular new-bone formation	Ill-defined ossification near joint margins but excluding osteophyte formation on plain X-Rays of the hand or foot	1

Note: A patient must have inflammatory articular disease (joint, spine, or entheseal) and ≥3 points from the above categories to be diagnosed with psoriatic arthritis. This diagnostic criteria has a sensitivity of 91.4% and specificity of 98.7% (Taylor *et al.*, 2006).

Table 1| **Classification of psoriatic arthritis (CASPAR) diagnostic criteria**

1.2.5. Clinical course of PsA

In most patients PsA runs a course of a chronic, progressive disease, although some patients can achieve a complete remission. In our cohort, 17.6% of the patients achieved a remission, however, periods of remission lasted on average 2.6 years and most patients experienced a relapse (Gladman DD, et al., 2001). PsA is more severe than previously thought (Gladman DD, 1994) (Torre Alonso JC, et al., 1991). It can lead to severe joint

damage and disability that are comparable to those that occur in rheumatoid arthritis (Rahman P, et al., 2001).Patients with PsA demonstrate clinical and radiographic progression in the course of follow-up (Siannis F, et al., 2006) (Bond SJ, et al., 2007) and have an increased mortality risk compared to the general population, although this risk has decreased over the past two decades (Wong K, et al., 1997) (Ali Y, et al., 2007). In addition to the disability that is related to the joint disease, PsA patients can suffer from the same co-morbidities as psoriasis patients (Gladman DD, et al., 2009). Thus, PsA poses a major health burden, in addition to that caused by psoriasis alone.

1.2.6. Treatment of PsA

The treatment in PsA is aimed at controlling both skin and joint inflammation in order to reduce the symptoms and to prevent joint damage. Treatment usually begins with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) that can control mild arthritis, enthesitis and spondylitis. Second line therapies are indicated when arthritis does not respond to NSAIDs. Many of the Disease Modifying Anti-Rheumatic Drugs (DMARDs), such as methotrexate, leflunomide, sulfasalazine, cyclosporine and azathioprine, were “borrowed” from rheumatoid arthritis for the treatment of peripheral arthritis in PsA. Although there are limited clinical trials that evaluated their efficacy in PsA, they are often effective in controlling articular symptoms. The new targeted biologic therapies particularly the anti-TNF agents, are the most effective treatments currently available to control all aspects of the disease and to prevent the progression of joint damage (Ritchlin CT, et al., 2009).

1.3. The Etiology of Psoriasis and PsA

1.3.1. Immunologic mechanisms in Psoriasis and PsA

Advances in immunology and skin biology provide insight into the molecular pathways that link the skin and musculoskeletal disease. Psoriatic disease is a disorder of both the innate and the adaptive immune system in which keratinocytes, dendritic cells and T cells have central roles. The first clues emerged from studies in plaque psoriasis, which pointed to Th1 and Th17 cells as the ultimate drivers of the pathobiology (Di Cesare et al., 2009; Lowes et al., 2008; 2014). In the current model, an infection or traumatic event triggers keratinocyte cell death leading to release of the cathelicidin LL37. LL37 bound to keratinocyte DNA activates toll-like receptor (TLRs) on the surface of plasmacytoid dendritic cells in the skin. TLRs in turn trigger plasmacytoid dendritic cells to release a

number of signaling proteins, including interferon α (IFNα) (Lowes et al, 2014). IFNα activates dermal myeloid dendritic cells and promotes their migration to the lymph nodes. In the lymph node, naïve T-cells are guided down one of two paths. (Fig. 4). First secretion of IL12 can induce differentiation of naïve-T-cells into Th1 cells, which secrete interferon γ (INFγ) and tumor necrosis factor (TNFα) (Zaba et al., 2009). The second cytokine, secreted by dendritic cells, is IL23, which induces the naïve T-cells to become Th17 cells (Di Meglio and Nestle, 2010). Finally, Th1 and Th17 cells exit the lymph node and re-enter circulation and return to the skin where they secrete cytokines including IL17, IL21, and IL22. Work from human and murine models indicate that IL21 and IL22 lead to proliferation of keratinocytes, hence forming the classic silvery hyperkeratosic skin in psoriasis (Nestle et al., 2009). IL17 is thought to be one of the key cytokines in the generation of psoriasis and is a central therapeutic target in psoriasis and psoriatic arthritis. Indeed, secukinumab, recently approved by the FDA, is a monoclonal antibody to IL-17 with proven efficacy in psoriasis (Thaci et al., 2015). Psoriasis is a relatively common disease, affecting about 2% of the general population; up to 30% of these patients develop musculoskeletal disease about 10 years after the development of skin disease (Ibrahim et al., 2009). What we know of the pathophysiology of PsA thus far indicates that the same cell lineages and cytokines involved in plaque psoriasis lead to development of the musculoskeletal disease. The IL23/Th17 axis has been implicated in many of the clinical features of PsA, IL23 induces the proliferation of Th17 cells, which the release proinflammatory cytokines. Both TNFα and cytokines in the IL23/Th17 pathway promote altered bone resorption and new bone formation observed in psoriatic arthritis.

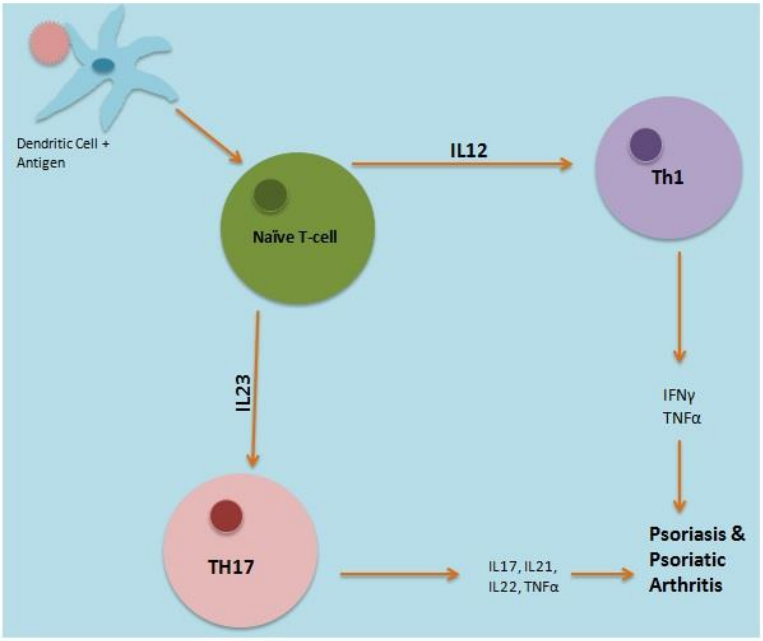

Figure 4 | **The basic pathophysiology of psoriasis and psoriatic arthritis**

Tumor necrosis factor-α (TNF-α) is a cytokine central to many aspects of the inflammatory response. Macrophages, mast cells, and activated Th cells (especially Th1 cells) secrete TNF-α. TNF-α stimulates macrophages to produce cytotoxic metabolites, thereby increasing phagocytic killing activity. Although the initial stimulus for joint inflammation is still debated, it is thought that macrophages in a diseased joint secrete TNF-α, which activates endothelial cells, other monocytes, and synovial fibroblasts. Activated endothelial cells up-regulate adhesion molecule expression, resulting in recruitment of inflammatory cells to the joint. Monocyte activation has a positive feedback effect on T-cell and synovial fibroblast activation. Activated synovial fibroblasts secrete interleukins IL6 and IL8, which recruit additional inflammatory cells. With time, the synovium hypertrophies and forms a pannus that leads to destruction of bone and cartilage in the joint, causing the characteristic deformity and pain of rheumatoid arthritis. (Fig. 5)

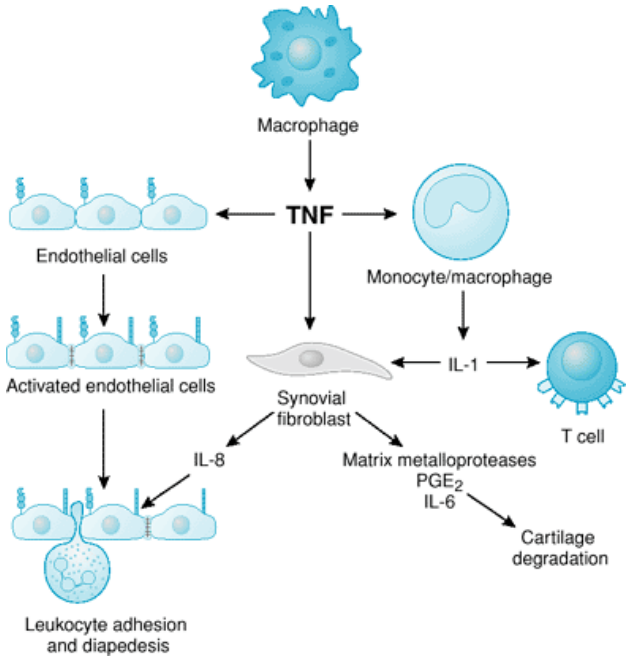

Figure 5 | **Key Actions of TNF-α in Psoriasis and PsA**

1.3.2 Environmental risk factors for Psoriasis and PsA

Psoriasis and PsA are considered complex diseases in which the interaction between genetic and environmental risk factors is thought to play a major role (Ritchlin CT, 2005). Genetic factors cannot solely account for all cases. One of the suggested pathogenic models for PsA is that psoriasis patients who carry susceptibility genes for arthritis develop PsA after being exposed to triggering environmental factors (Bruce IN, et al., 2001). Several environmental factors have been associated with psoriasis including:

infections, particularly streptococcal pharyngitis, smoking, trauma, stressful life events, alcohol, obesity, certain medications, weather changes (humidity, cold), and hormonal changes (pregnancy, pos-partum period, menopause) (Chandran V, et al., 2010). The exact molecular mechanisms by which the aforementioned exogenous factors trigger or exacerbate psoriasis are not fully understood. Human leukocyte antigen-Cw*06:02 positive patients with psoriasis are more prone in developing guttate psoriasis after streptococcal infection, while koebnarisation is common (Mallon et al., 1998).

Although the distribution of HLA-Cw*06:02, as well as other genetic susceptibility factors, covers a large number of ethnicities across the world, it is well known that there is a high incidence of psoriasis particularly in cold and humid environments, while the opposite is seen in dry places, where patients report higher improvement rates (Raychaudhuri and Farber, 2001; Riveira-Munoz et al., 2011).

Several studies discuss the role of excess smoking and alcohol consumption in the development or worsening of psoriasis. Some authors suggest that smoking is actually a triggering factor of psoriasis, while others claim that a higher numbers of cigarettes smoked per day can have a protective role for the disease (Kavli et al., 1985; Naldi et al., 1992). The theory behind smoking is that it can alter the function of PMNs, while, at the same time, induces the release of chemotactic mediators (Sonnex et al., 1988). Alcohol is another lifestyle factor that has been implicated in the pathogenesis of psoriasis. It is well documented that psoriasis patients that consume large amounts of alcohol, report a higher incidence of disease flares (Farkas and Kemény, 2010; Kirby et al., 2008; Zhu et al., 2012). It is however unclear the exact mechanism of action. One possible explanation would be that alcohol promotes central obesity, which leads to glucose intolerance and a higher risk for T2DM (Dubreuil et al., 2014). Glucose intolerance is linked to an increased production of pro-inflammatory cytokines which are also key factors in the pathogenesis of psoriasis (Gisondi et al., 2007; Gottlieb et al., 2008; Shapiro et al., 2007). In addition, alcohol can influence the immune system in many ways, including the function of both inflammatory cells and keratinocytes (Farkas and Kemény, 2010).

Another explanation is that psychological stress is directly linked to certain lifestyle habits, such as smoking, alcoholism and obesity. Emotional stress is a well-documented trigger for inflammatory skin diseases (Buske-Kirschbaum and Hellhammer, 2003; DeWeerdt, 2012; Heller et al., 2011). Stress induces the activation of the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenomedulary (SMA) axes, both of which regulate the cutaneous immune response. Normal "fight or flight" response to stress stimulates the release of stress hormones (cortisol, adrenaline and norepinephrine), which have a protective role. When there is a stress system malfunction, then the homeostasis between HPA and SMA axes is lost, which leads to upregulation of pro-inflammatory mediators in the skin (Huynh et al., 2013).

Taken together, it is likely that psoriasis patients inherit only a predisposition to the disease, that still requires an exogenous stimulus to express its phenotype (Ortonne, 1999).

1.4. Genetics of Psoriasis and PsA

Psoriasis has a strong genetic component, which was initially assessed by epidemiological studies involving twins and families (Rahman P and Elder J.T, 2005) (Lonnberg A.S, et al., 2013). Twin studies have found a substantially higher (2-3,5-fold) concordance of psoriasis in monozygotic twins than in dizygotic twins (Lonnberg A.S, et al., 2016), and estimates of heritability have ranged between 50% and 90% in populations of European descent (Lonnberg A.S, et al., 2013) (Grjibovski AM, et al., 2007). Recurrence rates range between 4% and 19% in first-degree relatives of individuals with psoriasis (Moll JM, et al., 1973) (Chandran V, et al., 2009). Even greater genetic effects have been reported for PsA, with estimates of heritability between 80% and 100% (Moll JM, et al., 1973) (Myers A, et al., 2005) and the riskof developing PsA said to be 30-49 fold greater if a first-degree relative has PsA(Moll JM, et al., 1973) (Karason A, et al., 2009). The role of genetic factors has been confirmed by linkage studies in families and genetic association studies (Gudjonsson JE, et al., 2009) (Mahil MA, et al., 2015). Although >70 genes associated with psoriasis have been identified, they only account for 30% overall psoriatic heritability, which might be explained by the cumulative effects of many genetic variations, whose individual effects are small and currently undetectable, as well as the existence of gene-gene and/or gene-environment interactions. A significant genetic component of psoriasis and PsA susceptibility was supported by the association of the disease with human leukocyte antigens (HLA), encoded by genes located within the major histocompatibility complex (MHC) on the short arm of chromosome 6 (Fig. 6). The MHC contributes approximately 40% of the detectable heritability of psoriasis, with smaller contribution for a multitude of other genetic loci (Tsoi LC, et al., 2015). Linkage-based and family-based association strategies have enabled important advances in the genetic dissection of the associations between HLA genes and psoriasis (Veal CD, et al., 2002) (Nair RP, et al., 2006), including the differential analysis of cutaneous psoriasis and PsA(Okada Y, et al., 2014). In particular HLA class I genes, have been consistently shown to contribute to the susceptibility of both psoriasis and PsA.

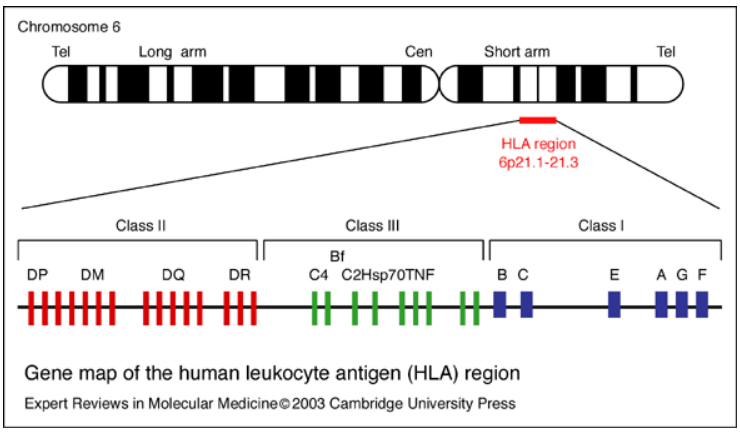

Figure 6 | **Gene map of the Human Leukocyte antigen (HLA) region**

1.5 Candidate genes within MHC region (chromosome 6p)

1.5.1. Human Leukocyte Antigen in Psoriasis

Numerous case-control association studies and later GWAS have found a strong association between psoriasis and the MHC region (Henseler T, et al., 1985) (Feng BJ, et al., 2009). The strongest association has been found with a 300kb-segment in the MHC-I region on chromosome 6p21.3 known as PSORS1 (Fan X, et al., 2008) (Elder JT, 2006). This region contains HLA genes that are associated with autoimmune diseases. Among genetic factors, the HLA loci have been the most persistently documented. Case-control studies identified the class I antigens HLA-B*13, B*17 and its split B*57, C*06, C*07 as associated with psoriasis (Tiilikainen A, et al., 1980) (Marcusson JA, et al., 1981) (Gazit E, et al., 1978). Several studies demonstrated an association with the class II antigens, HLA-DRB1*04 and HLADRB1* 07 (Russell TJ, et al., 1972) (Tiwari JL, et al., 1972). The largest and most consistently reported association is with HLAC* 06, with a relative risk of 22. The presence of HLA-C*0602 is associated with an earlier onset and more severe psoriasis (Gudjonsson JE, et al., 2002) (Enerback C, et al., 1997). The association with HLA-B and the Class II antigens, HLA-DR, was later determined to be due to extended haplotypes and LD with HLA-C (Nair RP, et al., 2006). Several studies have shown that the strongest link with psoriasis is with the 57.1 ancestral haplotype (C*06-B*57-DRB1*07-DQ*03). These findings are consistent with the association of individual components of this haplotype with psoriasis (Schmitt-Egenolf M, et al., 1996)(Jenisch S, et al., 1998). Since this region is in strong LD, the true risk allele has been difficult to determine. Candidate genes just telomeric to HLA-C, such as CDSN and HCR, seemed like good candidate genes since they are expressed in the skin (Asumalahti K, et al., 2000) (Tazi Ahnini R, et al., 1999). However, none of these candidate genes were consistently associated with psoriasis independently of HLA-C (Chia NV, et al., 2001) (Enerback C, et

al., 2000). In order to determine the psoriasis susceptibility locus within the PSORS1 region, a study that involved sequencing the putative 300-kb risk segment of PSORS1 from just telomeric to HLA-B to beyond CDSN thus including HLA-C was conducted. After sequencing this segment in 2 risk and 5 non-risk chromosomes, then examining recombinant haplotypes retaining HLA-C*06 but lacking risk alleles in CDSN, the authors concluded that HLA-C*06 is the PSORS1 risk variant that confers susceptibility to psoriasis (Nair RP, et al., 2006). Two GWAS among Caucasians and Chinese psoriasis patients confirmed previous findings. In these studies, by far, the most significant associations were of SNPs that were in tight LD with HLA-C*0602 (Fan X, et al., 2008) (Zhang XJ, et al., 2009).

1.5.2. Human Leukocyte Antigen in PsA

Case-control studies identified the HLA region as containing potential susceptibility loci for PsA. HLA-B*13, B*17 and its split B*57 and C*06 are associated with psoriasis across various population (Gladman DD, et al., 1986)(Gerber LH, et al., 1982) (McHugh NJ, et al., 1987). While HLA-C*06 is also increased in PsA patients compared to the general population, this association is stronger with psoriasis itself than with PsA (Thomson W, et al., 2008). Several studies reported an association between HLA-B*13, B*57, DRB1*07 and PsA. However, these results are most likely secondary to the presence of these alleles on the recognized ancestral haplotypes: AH.13 and AH.57 both contain HLAC* 06 (Schmitt-Egenolf M, et al., 1996) (Jenisch S, et al., 1998). HLA B*27 and B*07 have been specifically associated with PsA in case-control studies that compared patients with PsA to psoriasis. Several of the HLA antigens have been related to specific manifestations of PsA. HLA-B*27 is more common among PsA patients with axial disease while B*38 and B*39 are increased among those with peripheral polyarthritis. Within the HLA Class I region the associations are stronger with HLA-B than HLA-C alleles. It has been assumed that associations with HLA-C alleles are related to the skin disease and are not a specific marker for the joint disease. Among PsA patients, HLA-C*06 is associated only with early onset psoriasis and no association was observed with PsA patients with late onset psoriasis (Espinoza LR, et al., 1982) (McHugh NJ, et al., 1987). HLA-DRB1*04 was reported to confer a risk of PsA, but several investigators found no such associations(McKendry RJ, et al., 1984). The role of HLA genes in susceptibility to PsA was demonstrated when increased sharing of HLA haplotypes was documented among sib pairs concordant for PsA but not among those concordant for psoriasis only (Gladman DD, et al., 2003). Since almost all patients with PsA have psoriasis, it is unclear whether

the HLA associations described above are related to psoriasis, PsA, or both. The HLA alleles that may be specific to PsA are HLA-B*27 and possibly B*07, B*38 and B*39.

1.5.3. Other candidate genes within MHC region in psoriasis and PsA

The MHC is a 4 megabases region that contains more than 160 genes with alleles in strong LD. Many of the genes within this area are implicated in the pathogenesis of autoimmune diseases.

Many of the HLA alleles associated with psoriasis are in linkage disequilibrium and possible explanation of this could be, that other genes encoded within the MHC are involved in the pathogenesis of psoriasis. One such candidate is the tumor necrosis factor (TNF) gene. TNF is an important inflammatory mediator tandemly arranged in the central region of MHC with lymphotoxin alpha (LTα), and this may, therefore, be of importance for the aetiology of MHC-associated disease. TNF and LTA proteins play a central role in the initiation and regulation of the immune response. The genes for these cytokines are located within the HLA class III region of the MHC between HLA-B and HLA-DR (Fig. 7). TNF-α is a particularly strong candidate susceptibility gene because of the evidence of increased TNF production in skin and joint tissues in psoriasis and PsA (Ettehadi P, et al., 1994). TNF-α genes are known to be polymorphic. Several population studies designed to evaluate the association between some TNF gene polymorphisms, namely G to A substitutions at positions 238 and 308, and PsA lead to conflicting results. In fact, some authors found a strong association between these TNF promoter polymorphisms and the susceptibility to PsA, suggesting that both polymorphisms could be used as biomarkers to predict the risk of the disease (Hohler et al.,1997). In particular Hohler et al., (2002) reported that allele A at position -308 is associated with higher levels of TNF (constitutive and inducible) while the effect allele adenosine at position -238 has been associated with juvenile onset psoriasis and PsA in European patients. By contrast, Gonzalez et al. (2001, 2002) excluded the association between the SNPs 238 and 308 and PsA in the Jewish and Spanish populations. The discrepant results found among populations may be the consequence of ethnic admixture causing population stratification, of population-specific gene–gene or gene– environment interactions, and of statistical fluctuations (Ioannidis et al., 2001; Huizinga et al., 2004). Indeed, Gonzalez et al. (2001, 2002) suggested that the reported association between the SNPs -238 and -308 may only reflect that both are in linkage disequilibrium with major histocompatibility complex class I. TNF is known to locate quite closely to the MHC at chromosome 6p21.3, it could demonstrate that association of the promotor variant at -238 is dependent on carriage of the HLA-C risk allele. In contrast, the promoter variant at -875 was associated with PsA independently from carriers status of HLAC-*06. (Reich et al., 2007) (Giardina et al.,

2011). Giardina et al., 2011, with a collaborative work, replicated the association of TNF-857T as a susceptibility allele for PsA independent of the main PSORS1 risk allele. The authors in their large genetics study, have enrolled a total cohort of 2,224 individuals of European ancestry (Italian, German and UK cohort) and the results robustly demonstrated that TNF -857T represents a risk allele for PsA independent of the PSORS1 main locus. Although the functional role of TNF -857T remains to be determined, previous data have shown that allele T increases the transcription of TNF (Lv K, et al., 2006) . Moreover, it is well known that TNF plays a pivotal role in both the activation and the extravasation of T cells in the highly vascularized synovium, as well as in the promotion of bone erosions during subchondral osteoclastogenesis. Thus, genes encoding for TNFα as well as for the other cytokines associated with PsA (IL-11, IL-15, and IL-23 receptor) might represent candidate pharmacogenetics markers.

Multiple genes within the MHC region have been found to be associated with PsA, but the existence of high long-range LD makes it difficult to ascertain the relevance of these genes in disease susceptibility independent of HLA–C. (Giardina et al., 2011).

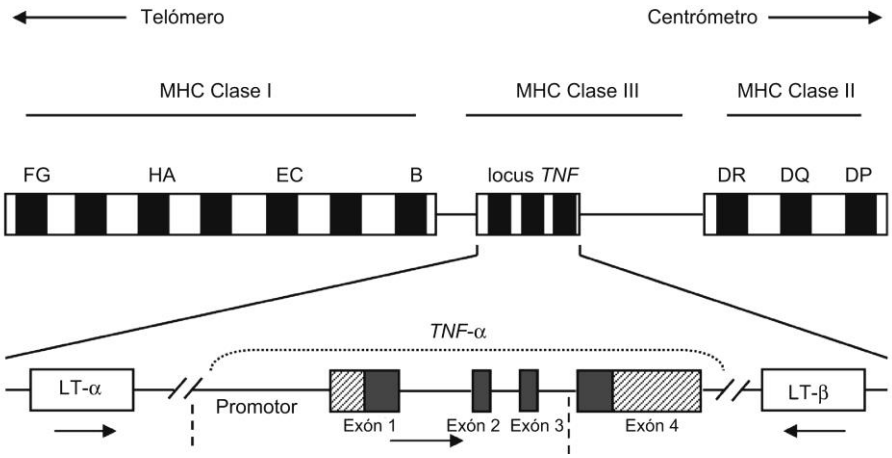

Figure 7 | **Structure of the TNF gene**

1.5.4 Candidate genes outside the MHC region

It is well recognised that the Major Histocompatibility Complex (MHC) harbours the main psoriasis susceptibility locus (PSORS1, Psoriasis Susceptibility 1). Conversely it has become apparent that the PSORS1 locus accounts for less than 50% of psoriasis familial clustering and that PSORS1 risk haplotypes are found in up to 37% of control chromosomes (Asumalahti, K, et al., 2002). The MHC region being neither necessary nor sufficient to trigger the onset of psoriasis, additional genetic factors are likely to be required. To date, at least eight putative disease susceptibility regions have been mapped outside of the MHC (PSORS2-9). Evidence supporting some non-MHC loci has been

provided by their close overlap with genomic regions conferring susceptibility to other inflammatory disorders. Parametric and non-parametric linkage analyses have mapped many susceptibility loci on different chromosomes. Evidence has been reported for additional susceptibility loci on chromosomes 17q (PSORS2), 4q (PSORS3), 1q (PSORS4), 3q (PSORS5), 19p (PSORS6), 1p (PSORS7), 16q (PSORS8) and 4q (PSORS9).

PSORS1 on chromosome 6p is the most significant psoriasis susceptibility locus and has consistently shown linkage and association with psoriasis and psoriatic arthritis.

A mixed non-parametric and parametric linkage study using a dominant inheritance model in 1999 first identified PSORS4 at four markers in the 1q21 chromosome region (Capon et al. 1999). This was a genome-wide linkage study carried out in Italy and after an initial analysis for PSORS1 and PSORS2 linkage failed. This was followed up in 2001 when the same group refined the mapped region between markers D1S2346 and 140J1D (Capon et al. 2001).

Giardina et al., 2004 mapped one of these loci, PSORS4, on human chromosome 1q21. Using the linkage disequilibrium approach, the authors refined the critical region to a specific genomic interval of about 100 Kb which contains only the loricrin (LOR) gene. LOR gene is considered, for a number of reasons, as a strongest positional and functional candidate gene to psoriasis (Giardina et al., 2004). The authors reported that LOR is the only well-defined gene mapping to the critical genomic interval, and also plays a key role in the differentiation of keratinocytes. Moreover, LOR shows a quite similar amino-acid sequence to corneodesmosin, a strong candidate for the PSORS1 locus at 6p21 (Jonca et al., 2002) (Capon et al., 2003). Finally, they showed that loricrin is an integral part of the epidermal differentiation complex (EDC), a cluster of genes at 1q21 that undergo coordinated regulation in skin lesions of psoriasis.

Interestingly, Giardina and coll. observed a down-regulation of LOR mRNA in psoriatic skin, as observed in previous studies (Takahashi et al. 1996; Elder et al. 2002). Hence LOR gene was considered to be an attractive positional and functional candidate for the PSORS4 locus. Furthermore, the authors affirmed, thus whilst these findings do not provide definitive proof of the involvement of loricrin in the pathogenesis of psoriasis, sequence analysis and association studies have allowed us to exclude variations in the LOR gene as responsible for the linkage signal at 1q, previously identified in Italian families.

1.5.5. Limitations of previous genetic studies

In summary, Ps and PsA have substantive genetic determinants. Some susceptibility genes are probably to be shared by Ps and PsA; however, it is likely that there are some distinct genes that confer an independent risk for PsA. In PsA, several candidate genes have been replicated in different studies, most of them within the MHC region. Candidate gene approaches were initially used in the genomic studies with focus on the genes known to be involved in well-defined molecular pathways for targeted human conditions through linkage and association studies. Through candidate-gene studies, certain genetic variants among many genetic loci have been successfully identified for their important attribution to specific human diseases. Subsequently, a new approach, genome-wide association study (GWAS), was widely applied to genomics. Although several early GWAS studies reported potentially promising results, the majority of GWAS studies were disappointing because of inadequate sample size and limitation of arrays for certain genetic variations. Moreover, these two kinds of studies have a fundamental limitation on the fact that both are based on the analysis of positional markers with the hope that at least one of them can identify a significant association. By their nature, none of these kinds of studies have the ability to analyze the whole genetic variability of a gene or region or even an entire genome. These obstacles may be overcome by Next Generations Sequencing (NGS), that in the last few years has emerged as a revolutionary genomic tool. The advantages of DNA sequencing in genetic studies are represented by the possibility that most variants, common and rare, can be discovered with the appropriate sequencing read coverage, appropriate algorithms, methods to identify the variants, and a sufficient careful validation to confirm true from false positive.

2. AIM OF THE STUDY

The aim of this study was to clarify the mechanisms through which the genetic variability of TNF and LTA genes play a role in the susceptibility and progression of psoriasis and psoriatic arthritis and in therapeutic response with anti-TNF inhibitors using Next Generation Sequencing approach.

3. MATERIALS AND METHODS

3.1 Patients and controls

One hundred and eighty patients with psoriasis vulgaris and one hundred and thirty patients with psoriatic arthritis originating in Sardinia were included into the study. The control cohort consisted of one hundred and twenty healthy, unrelated subject with no family history of PsV and PsA. We included 70 patients treated with TNF-inhibitors with at least 2 years of follow-up, recruited from Sardinian patients with psoriasis vulgaris and psoriatic arthritis.

3.2 Laboratory methods

Blood samples were collected from patients with psoriasis, psoriatic arthritis and healthy controls in EDTA anticoagulant. DNA was extracted from peripheral blood using QIAamp DNA Mini Kit. Genomic DNA were amplified for TNF/LTα, HLA-B and HLA-C under long range PCR using the specific forward and reverse primer pairs:
(TNF: *forward* 5' TGTGAAACCTGCCAGATGGG, *reverse* 5' CGAGAGGGTGTACGTCAACA, HLA-B: *forward* 5'AGGTGAATGGCTCTGAAAATTTGTCTC, *reverse* 5'AGAGTTTAATTGTAATGCGTT TTGACACA), (HLA-C: *forward* 5'GGCCGCCTGTACTTTTCTCAGCAG, *reverse5'* CCATGGTGAG TTTCCCTGTACAAGAG).
20 µl of reaction mixture included 1x Pstar GXL Buffer (Mg^{2+}), 50 ng of genomic DNA, 2,5 picomole of each primers, 0,2 mM dNTP, 0,04% DMSO and 0,32 unit Taq DNA polymerase (TakaraPrimeSTAR GXL) (Tab. 2). The program comprised of an initial denaturation at 98°C for 10 seconds followed by 30 cycles at 98°C for 10 sec, 60°C for 15, and 68°C for 13 min, final extension involved 1 min at 68°C (Tab. 3). The PCR products was analyzed on 1% agarose gel.

PCR COMPONENTS	Amount
DNA	50 ng
5X Pstar GXL Buffer (Mg^{2+} plus)	1x
dNTPs	0,2 mM
Mix Primers	2,5 pmol
DMSO	0,04%
Taq TakaraPrimeSTAR GXL	0,32 U
H_2O	To reach a volume
Final Volume	20 ul

Table 2 | **LR PCR components**

PCR CONDITIONS		
Numbers of cycle	Temperature	Duration
1	98°C	10''
30	98°C	10''
	60°C	15''
	68°C	13'
1	68°C	1'

Table. 3 | **PCR conditions**

After the quantify using Qubit 3.0, genomic DNA was purified and subsequently prepared for the following step. NGS library were prepared using a protocol based on Nextera XT (Illumina). This method allows to resequencing the entire gene region including introns and regulatory region. The Nextera XT DNA Sample Preparation Kit uses an engineered transposome to simultaneously fragment and tag “tagment” input DNA, adding unique adapter sequences in the process. Library preparation starts with random fragmentation of genomic DNA and adapters ligation to the DNA fragments. Than a limited-cycle PCR reaction uses these adapter sequences to amplify the insert DNA. The PCR reaction also adds index sequences on both ends of the DNA. The adapters allow covalent binding of the DNA to the flowcell. The preparation was completed by an AMpure (Beckman) purification, a subsequent quantification of the libraries using a fluorimetric Qubit (TechnoFisher) method. Finally the libreries were normalized to 4nM, pooled and denatured to be loaded onto a MiSeq flow cell Illumina V3. In a Illumina MiSeq sequencer, 300 cycles of sequence were applied in both directions for a total of 600 cycles.

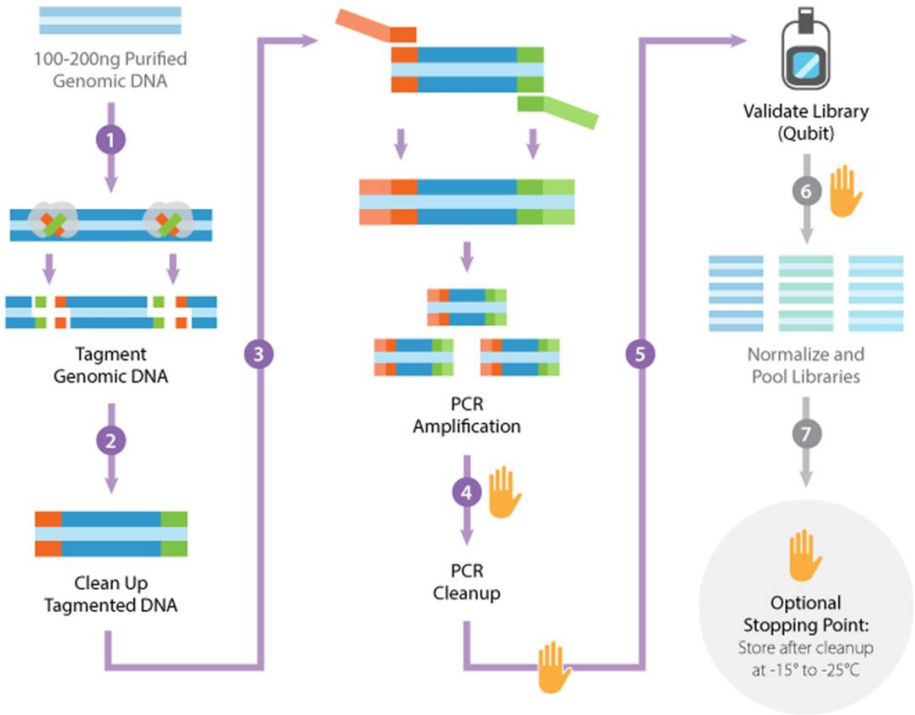

Figure 8 | **Step of Nextera XT DNA library**

3.3 NGS software analysis

Once sequencing is complete, raw sequence data must undergo several analysis steps. The analysis was carried out by the SoftwereMiSeqReporter v.2.6.1. The sequences generated were stored as a FASTQ file format (a text based format containing millions of short reads together with quality values for each base). MiSeq Reporter can be considered a pipeline performs different analytic activities can be split into three parts:

1) ALIGNMENT: is the first and arguably most crucial step of NGS analysis to map NGS reads to a reference genome (hg19). The Alignment was performed using BWA (Burrows-Wheeler Alignment tool). BWA generates BAM files. BAM files include the same information as the FASTQ format, but including genomic position(s) of the reads as well as quality information. It also includes a header where information on the reference genome. At this point the raw data can be evaluated using the Integrative Genomics Viewer (IGV).

2) VARIANT CALLING: Variant calling is the process to identifying sequence variation respect to the reference-sequence genome. Bayesian models are used by many modern variant callers, such as GATK (Genome Analysis Toolkit) and the output file is VCF (variant calling format).

3) VARIANT ANNOTATION: is the process by which the called variants are illustrated by their characteristics on the base of the available genomic information. In other words through this process it is possible to understand the nature and the effects of each identified variant. This process is crucial in identifying pathogenetic variants.

3.4 Statistical analysis

Genotype and allele frequencies were compared between the groups by the Chi2-test with Bonferroni's correction. P values less than 0.05 were considered statistically significant.

The statistical power of our samples was calculated using the GAS power calculator (http://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html).

4. RESULTS

One hundred and eighty patients with psoriasis vulgaris and one hundred and thirty patients with psoriatic arthritis were included into the study. The control cohort consisted of one hundred and twenty healthy, unrelated subject with no family history of PsV or PsA. We included 70 patients treated with TNF-inhibitors, with at least 2 years of follow-up. We identified 65 SNPs of which three missense variants in LTA gene (rs2229094, rs2229092, rs1041981), and one synonymous variant in TNF gene (rs747910356). In addition in the TNF gene we found three insertions, one in the 5' UTR (rs201328097) and two in the intron region (rs3745501689, rs371135507) (Tab. 4-8). Ten new SNPs never reported before were found in our Sardinian samples. Four of these were located in the LTA region, seven in the TNFα region and one in the LTB region. The statistical power of our sample evaluated for a multifactorial model and a significance threshold of 0.0005 showed that our sample was sufficient to detect with a high probability (> 0.8) only variants with relatively high impact on the disease risk (OR> 2.5) (Figure 9). Therefore, variants with minor effects may have been lost in this study.

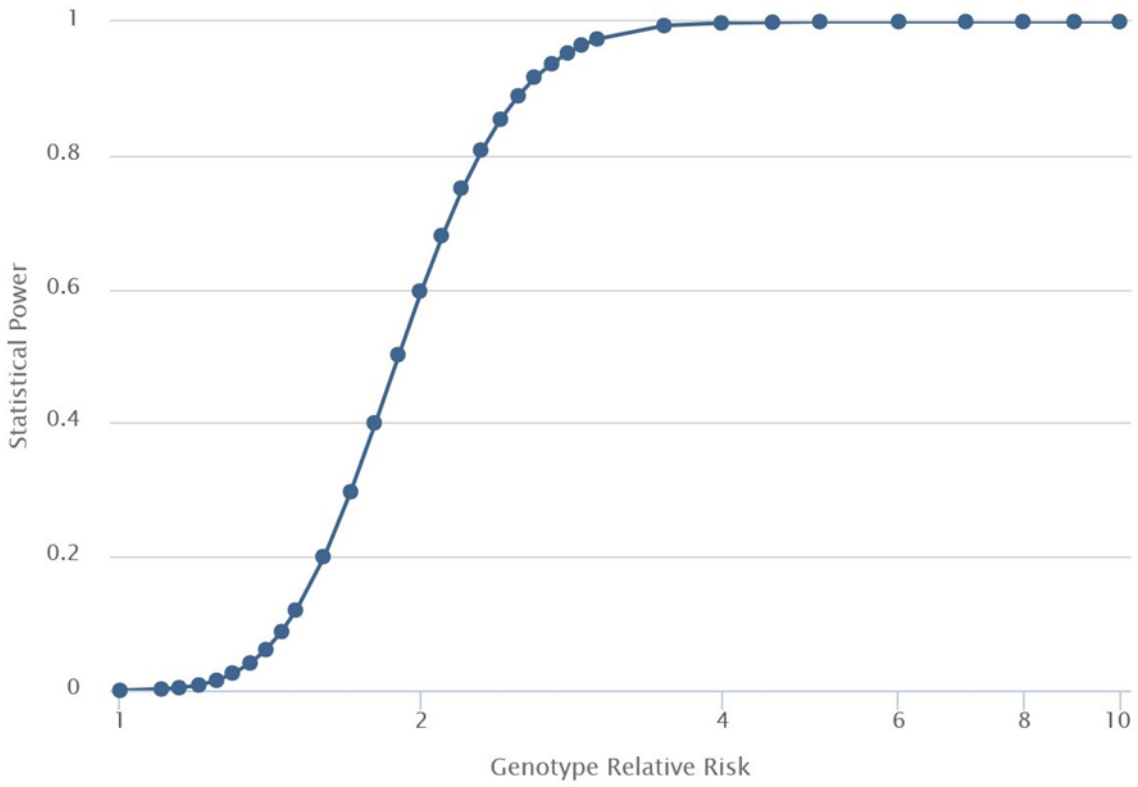

Figure 9 | **Statistical power of this study case-control**

4.1 Comparison between psoriatic patients (PsV) and control individuals

The allele frequency of the variants between psoriatic patients and controls were compared (Tab. 4). In general there was no significant differences in the frequency of the variants between psoriatic patients and controls. The analysis, leaded to the identification of nine variants SNP, three of these were located on LTA gene, rs2844482, rs18469284, rs2229092, and six were located on TNF gene, rs3093668, rs3013726, rs1799964, rs1800630, rs4248158, rs3093661 with a significant *P value* less than $p<0.05$, but after the application of Bonferroni's correction these variants did not conserved a significant *value*. In particular there was no significant differences in the frequency of the SNP -308G>A -238G>A and +489G>A. Which have been described as associated to the Psoriasis susceptibility in recently published papers (Hohler et al,.2002; Balding et al., 2003; Murdca et al., 2014). Some of these variants, as will be described later, would be attributable to the linkage disequilibrium among the alleles found during the analysis of this region and the HLA loci traditionally associated with the psoriasis: HLA-C*06:02 in many world populations and HLA-C*07:18 - B*58 in the Sardinian population. However, two variants have interesting aspects that may be susceptible to further studies: the first one, rs2229092, is a missed variant of the LTA gene (p.His51Pro) potentially capable of influencing protein functions. The second one is instead located in the TNF gene promoter (rs1799964). This variant, known as TNF-1031T>C, has been investigated in previous studies on inflammatory and autoimmune diseases (Reich K, 2007).

4.2 Psoriasis patients stratification for HLA associated alleles

To evaluate if the associations found in our samples were correlated with the presence of HLA-C*06:02 and HLA-C*07:18 - B*58, a stratification analysis was carried out. Of 180 psoriatic patients, 97 (54%) were carriers of the alleles HLAC*06:02 and HLA-C*07:18 - B*58 and 83 were not carriers (Tab. 5). As expected, most of the variants of the LTA/TNF region show alleles in linkage disequilibrium with HLA allele associated with psoriasis. However, the two variants rs2229092 (p.His51Pro) (p = 0,966) and rs1799964 (-1031T>C) (p = 0,463) do not show significant deviations from independence. Therefore, this data reinforces the hypothesis of a role of these two variants in susceptibility to psoriasis.

4.3 Psoriatic arthritis patients (PsA) compared to control individuals

Comparison of allelic frequencies of LTA / TNF variants was also conducted in the group of patients with psoriatic arthritis (Tab. 6). Seven SNPs with a significant difference

between the two groups were found (rs2229092, rs3093547, rs3093661, rs3093668, rs3013726, rs3093727). One SNP at position 31548552 was new and not present in the SNPs database. However , as with most SNPs found associated with PsV, the nature of these associations is likely to be false and probably due to LD with HLA allele. Indeed, none of these associations observed with the 0.05 threshold remains such after correction. In particular there was no significant differences in the frequency of the SNP -308G>A -238G>A and +489G>A.

4.4 Psoriatic arthritis patients (PsA) stratification for HLA associated alleles

A stratification in the psoriatic arthritis group was performed and this analysis showed a strong linkage disequilibrium of all the variants identified with the HLA alleles C*06:02 C*07:18 - B*58:01 (Tab. 7)

4.5 Associations between TNF-α gene polymorphisms and response to TNF-α inhibitors

Finally, a study relating the response to TNF-inhibitors was performed on 70 patients treated with TNF inhibitors. Of them 22 (31.4%) had a psoriasis vulgaris (PsV), while 48 had a psoriatic arthritis (PsA). Overall, seventeen (24%), did not respond to therapy while fiftythree showed a remarkable improvement after treatment with TNF inhibitors(Tab. 8). The SNPs -308, -238 and +489 were not significantly associated with the clinical outcome of PsV and PsA patients after anti-TNF-α treatment, (p = 0,881 for SNP -308) (p = 0,087 for SNP -238) (p = 0,243 for SNP +489). The *p value* for the marker rs1800750 (-376G>A) (p = 0,005445) resulted strongly associated with the response to the therapy. The minor allele (A) is completely absent in the patients that don't responded to the anti-TNF therapy while it was present in the responders, this finding seems to be important due the fact that this allele has been found to be associated to high level of TNF. It is important to note that this SNP is not significantly associated in the stratification tests for associated HLA alleles with both PsV and PsA. So the association we observe with the different drug response is independent from the alleles HLA-C*06: 02, HLA-C*07:18 - B*58:01.

4. Comparison between psoriatic patients (PsV) and control individuals

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% Psoriasis patients (n. 180)	Va% Sardinian ctrl (n. 120)	*p-value*	*p**
1	31539592	T>T/G		upstream_gene_variant	snv	,-497T>G		0,00	0,00	0	0,000
2	31539735	C>C/T		upstream_gene_variant	snv	,-354C>T	rs56161754	0,45	0,70	0,681	44,265
3	31539767	C>C/T		upstream_gene_variant	snv	.-322C>T	rs2844482	12,16	18,31	**0,037**	**2,405**
4	31539768	A>A/G		upstream_gene_variant	snv	.-321A>G	rs2071590	56,76	57,04	0,945	61,425
5	31539919	C>C/G	LTA	5_prime_UTR_variant	snv	.-170C>G		0,45	0,00	0,298	19,370
6	31539974	G>G/A	LTA	5_prime_UTR_variant	snv	.-115G>A	rs145829373	0,00	0,70	0,111	7,215
7	31540071	G>G/A	LTA	5_prime_UTR_variant	snv	.-18G>A	rs1800683	10,36	9,15	0,628	40,820
8	31540119	T>T/A	LTA	intron_variant	snv	.-10+40T>A		0,90	0,00	0,14	9,100
9	31540141	A>C/C	LTA	intron_variant	snv	.-10+62A>C	rs2239704	44,14	50,00	0,159	10,335
10	31540142	G>G/A	LTA	intron_variant	snv	.-91G>A	rs3093546	0,45	0,70	0,681	44,265
11	31540313	A>A/G	LTA	intron_variant	snv	.-9-198A>G	rs909253	10,36	9,15	0,628	40,820
12	31540429	C>G/G	LTA	intron_variant	snv	.-9-82C>G	rs746868	45,05	50,00	0,234	15,210
13	31540440	C>C/T	LTA	intron_variant	snv	-9-71C>T	rs780911663	0,00	0,00	0	0,000
14	31540457	G>G/A	LTA	intron_variant	snv	,-9-54G>A	rs184649284	0,00	2,11	**0,006**	**0,390**
15	31540556	T>T/C	LTA	missense_variant	snv	Cys13Arg	rs2229094	34,23	40,85	0,1	6,500
16	31540693	G>G/C	LTA	intron_variant	snv	100-12G>C	rs3093542	3,15	0,00	0,059	3,835
17	31540757	A>A/C	LTA	missense_variant	snv	His51Pro	rs2229092	4,95	10,56	**0,009**	**0,585**
18	31540784	C>A/A	LTA	missense_variant	snv	Thr60Asn	rs1041981	10,36	9,15	0,628	40,820
19	31540821	A>A/G	LTA	intron_variant	snv	205+11A>G	rs56285847	1,80	1,41	0,711	46,215
20	31541848	T>T/A	LTA	3_prime_UTR_variant	snv	378T>A	rs3093547	3,15	4,23	0,498	32,370
21	31541948	C>C/T	LTA	3_prime_UTR_variant	snv	478C>T	rs17207127	4,50	4,93	0,809	52,585
22	31541959	G>G/C	LTA	3_prime_UTR_variant	snv	489G>C	rs3093545	1,35	0,00	0,071	4,615
23	31542308	T>T/C		downstream_gene_variant	snv	-1031T>C	rs1799964	26,58	36,62	**0,009**	**0,585**
24	31542476	C>C/A		downstream_gene_variant	snv	-863C>A	rs1800630	12,61	19,72	**0,018**	**1,170**
25	31542482	C>C/T		downstream_gene_variant	snv	-857C>T	rs1799724	14,41	18,31	0,202	13,130
26	31542485	A>A/T		downstream_gene_variant	snv			0,45	0,00	0,298	19,370
27	31542533	C>C/T		downstream_gene_variant	snv	-986C>T	rs4248158	0,00	1,41	**0,024**	**1,560**
28	31542767	A>A/C		upstream_gene_variant	snv	-752A>C	rs4248161	2,70	0,70	0,079	5,135
29	31542963	G>G/A		upstream_gene_variant	snv	.-376G>A	rs1800750	11,71	16,90	0,07086873	4,606
30	31543031	G>G/A		upstream_gene_variant	snv	-308G>A	rs1800629	3,15	2,11	0,44412048	28,868
31	31543101	G>G/A		upstream_gene_variant	snv	-238G>A	rs361525	13,51	16,90	0,25316532	16,456
32	31543122	G>G/C		upstream_gene_variant	snv			0,45	0,00	0,29780231	19,357
33	31543261	T>T/C		upstream_gene_variant	snv	-76T>C		0,00	0,00	0	0,000

Tab. 4 | **Comparison between psoriatic patients (PsV) and control individuals**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

4. Comparison between psoriatic patients (PsV) and control individuals

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% Psoriasis patients (n. 180)	Va% Sardinian ctrl (n. 120)	*p-value*	*p**
34	31543262	A>A/T		upstream_gene_variant	snv		rs41297589	0,45	0,00	0,298	19,370
35	31543404	A>A/AC	TNF	5_prime_UTR_variant	insertion		rs201328097	0,90	0,70	0,794	51,610
36	31543758	G>G/A	TNF	5_prime_UTR_variant	snv	,186+54G>A	rs3093661	3,60	0,00	**0,033**	**2,145**
37	31543797	G>G/A	TNF	5_prime_UTR_variant	snv	.186+93G>A		0,90	1,41	0,55995397	36,397
38	31543825	A>A/T	TNF	intron_variant	snv	,+186+121A>T	rs4645839	1,80	0,00	0,13429047	8,729
39	31543827	G>G/A	TNF	intron_variant	snv	.+186+123G>A	rs1800610	14,41	18,31	0,20196659	13,128
40	31543943	.-/TGAA	TNF	intron_variant	insertion	186+240insTGAA	rs374501689	1,80	0,00	0,13429047	8,729
41	31544022	TG>TG/T	TNF	intron_variant				1,80	0,70	0,25595039	16,637
42	31544065	A>A/AAG	TNF	intron_variant	insertion		rs371135507	11,26	16,20	0,08048839	5,232
43	31544189	A>A/G	TNF	intron_variant	snv	187-122A>G	rs3093662	18,92	21,13	0,50615747	32,900
44	31544642	A>A/G	TNF	intron_variant	snv	280+51A>G	rs3093664	9,01	4,93	0,06110568	3,972
45	31545101	C>C/T	TNF	synonymous variant	snv	489C>T	rs747910356	0,00	0,70	0,11082815	7,204
46	31545366	A>A/G	TNF	3_prime_UTR_variant	snv	52A>G	rs769422678	0,00	0,70	0,11082815	7,204
47	31545391	A>A/C	TNF	3_prime_UTR_variant	snv	77A>C	rs3093665	4,95	4,23	0,67796616	44,068
48	31545432	G>G/A	TNF	3_prime_UTR_variant	snv	118G>A	rs762834115	0,45	0,00	0,29780231	19,357
49	31545691	G>G/C	TNF	3_prime_UTR_variant	snv			0,90	0,00	0,14036871	9,124
50	31545840	G>G/A		downstream_gene_variant	snv			0,00	0,00	0	0,000
51	31546495	G>G/C		downstream_gene_variant	snv	799+382G>C	rs3093668	4,05	0,00	**0,018**	**1,170**
52	31546789	T>T/C		downstream_gene_variant	snv		rs3013726	2,25	0,00	**0,019**	**1,235**
53	31546850	G>G/A		downstream_gene_variant	snv		rs1800628	1,80	0,70	0,25595039	16,637
54	31546980	A>A/G		downstream_gene_variant	snv		rs3093671	0,90	2,11	0,21350798	13,878
55	31547115	G>G/A		downstream_gene_variant	snv		rs3093727	1,80	0,00	0,13429047	8,729
56	31547174	T>T/C		downstream_gene_variant	snv		rs865848433	0,00	0,70	0,11082815	7,204
57	31547379	C>C/T		downstream_gene_variant	snv			0,00	0,00	0	0,000
58	31547420	C>C/T		downstream_gene_variant	snv		rs146233968	2,70	0,70	0,079	5,135
59	31547460	G>G/A		downstream_gene_variant	snv		rs3091258	14,86	9,86	0,073	4,745
60	31547474	G>G/A		downstream_gene_variant	snv		rs3093561	1,35	1,41	0,9530665	61,949
61	31547514	G>G/T		downstream_gene_variant	snv		rs769178	14,41	18,31	0,20196659	13,128
62	31547611	C>C/T		downstream_gene_variant	snv		rs769177	9,01	6,34	0,2358315	15,329
63	31547792	C>C/T		downstream_gene_variant	snv		rs3093559	4,95	4,23	0,67796616	44,068
64	31548379	T>T/C		downstream_gene_variant	snv		rs567175785	0,45	0,70	0,68104754	44,268
65	31548552	G>C/C		downstream_gene_variant	snv			0,45	0,00	0,29780231	19,357

Tab. 4 | **Comparison between psoriatic patients (PsV) and control individuals**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

5. Psoriatic patients stratification for HLA associated alleles

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% HLA Associated Alleles (n. 97)	Va% HLA Not Associated Alleles (n.83)	*p-value*	*p**
1	31539592	T>T/G		upstream_gene_variant	snv	,-497T>G		0,00	0,00	0,000	0,000
2	31539735	C>C/T		upstream_gene_variant	snv	,-354C>T	rs56161754	0,83	0,00	0,238	15,501
3	31539767	C>C/T		upstream_gene_variant	snv	.-322C>T	rs2844482	10,83	13,73	0,403	26,177
4	31539768	A>A/G		upstream_gene_variant	snv	.-321A>G	rs2071590	53,33	60,78	0,155	10,067
5	31539919	C>C/G	LTA	5_prime_UTR_variant	snv	.-170C>G		0,83	0,00	0,238	15,501
6	31539974	G>G/A	LTA	5_prime_UTR_variant	snv	.-115G>A	rs145829373	0,00	0,00	0,000	0,000
7	31540071	G>G/A	LTA	5_prime_UTR_variant	snv	.-18G>A	rs1800683	13,33	6,86	0,045	2,893
8	31540119	T>T/A	LTA	intron_variant	snv	.-10+40T>A		0,00	1,96	0,050	3,255
9	31540141	A>C/C	LTA	intron_variant	snv	.-10+62A>C	rs2239704	42,50	46,08	0,496	32,208
10	31540142	G>G/A	LTA	intron_variant	snv	.-91G>A	rs3093546	0,00	0,98	0,167	10,848
11	31540313	A>A/G	LTA	intron_variant	snv	.-9-198A>G	rs909253	13,33	6,86	0,045	2,893
12	31540429	C>G/G	LTA	intron_variant	snv	.-9-82C>G	rs746868	44,17	46,08	0,716	46,559
13	31540440	C>C/T	LTA	intron_variant	snv	-9-71C>T	rs780911663	0,00	0,00	0,000	0,000
14	31540457	G>G/A	LTA	intron_variant	snv	,-9-54G>A	rs184649284	0,00	0,00	0,000	0,000
15	31540556	T>T/C	LTA	missense_variant	snv	Cys13Arg	rs2229094	30,00	39,22	0,066	4,306
16	31540693	G>G/C	LTA	intron_variant	snv	100-12G>C	rs3093542	3,33	2,94	0,832	54,073
17	31540757	A>A/C	LTA	missense_variant	snv	His51Pro	rs2229092	5,00	4,90	**0,966**	**62,785**
18	31540784	C>A/A	LTA	missense_variant	snv	Thr60Asn	rs1041981	13,33	6,86	0,045	2,893
19	31540821	A>A/G	LTA	intron_variant	snv	205+11A>G	rs56285847	0,00	3,92	0,005	0,351
20	31541848	T>T/A	LTA	3_prime_UTR_variant	snv	378T>A	rs3093547	2,50	3,92	0,442	28,718
21	31541948	C>C/T	LTA	3_prime_UTR_variant	snv	478C>T	rs17207127	0,83	8,82	0,000	0,020
22	31541959	G>G/C	LTA	3_prime_UTR_variant	snv	489G>C	rs3093545	0,83	1,96	0,356	23,142
23	31542308	T>T/C		downstream_gene_variant	snv	-1031T>C	rs1799964	25,00	28,43	**0,463**	**30,066**
24	31542476	C>C/A		downstream_gene_variant	snv	-863C>A	rs1800630	10,83	14,71	0,270	17,551
25	31542482	C>C/T		downstream_gene_variant	snv	-857C>T	rs1799724	5,83	24,51	0,000	0,000
26	31542485	A>A/T		downstream_gene_variant	snv			0,00	0,98	0,167	10,848
27	31542533	C>C/T		downstream_gene_variant	snv	-986C>T	rs4248158	0,00	0,00	0,000	0,000
28	31542767	A>A/C		upstream_gene_variant	snv	-752A>C	rs4248161	4,17	0,98	0,063	4,086
29	31542963	G>G/A		upstream_gene_variant	snv	.-376G>A	rs1800750	10,00	13,73	0,273	17,763
30	31543031	G>G/A		upstream_gene_variant	snv	-308G>A	rs1800629	3,33	2,94	0,832	54,073
31	31543101	G>G/A		upstream_gene_variant	snv	-238G>A	rs361525	13,33	13,73	0,914	59,384
32	31543122	G>G/C		upstream_gene_variant	snv			0,00	0,98	0,167	10,848
33	31543261	T>T/C		upstream_gene_variant	snv	-76T>C		0,00	0,00	0,000	0,000

Tab. 5 | **Psoriatic patients stratification for HLA associated alleles**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

5. Psoriatic patients stratification for HLA associated alleles

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% HLA Associated Alleles (n. 97)	Va% HLA Not Associated Alleles (n.83)	*p-value*	*p**
34	31543262	A>A/T		upstream_gene_variant	snv		rs41297589	0,00	0,98	0,167	10,848
35	31543404	A>A/AC	TNF	5_prime_UTR_variant	insertion		rs201328097	1,67	0,00	0,095	6,159
36	31543758	G>G/A	TNF	5_prime_UTR_variant	snv	,186+54G>A	rs3093661	5,83	0,98	0,014	0,891
37	31543797	G>G/A	TNF	5_prime_UTR_variant	snv	.186+93G>A		0,83	0,98	0,883	57,394
38	31543825	A>A/T	TNF	intron_variant	snv	,+186+121A>T	rs4645839	2,50	0,98	0,280	18,173
39	31543827	G>G/A	TNF	intron_variant	snv	.+186+123G>A	rs1800610	5,83	24,51	0,000	0,000
40	31543943	.-/TGAA	TNF	intron_variant	insertion	.86+240insTGA/	rs374501689	0,83	2,94	0,134	8,730
41	31544022	TG>TG/T	TNF	intron_variant				0,83	2,94	0,134	8,730
42	31544065	A>A/AAG	TNF	intron_variant	insertion		rs371135507	9,17	13,73	0,173	11,226
43	31544189	A>A/G	TNF	intron_variant	snv	187-122A>G	rs3093662	15,83	22,55	0,105	6,821
44	31544642	A>A/G	TNF	intron_variant	snv	280+51A>G	rs3093664	8,33	9,80	0,627	40,765
45	31545101	C>C/T	TNF	synonymous variant	snv	489C>T	rs747910356	0,00	0,00	0,000	0,000
46	31545366	A>A/G	TNF	3_prime_UTR_variant	snv	52A>G	rs769422678	0,00	0,00	0,000	0,000
47	31545391	A>A/C	TNF	3_prime_UTR_variant	snv	77A>C	rs3093665	1,67	8,82	0,002	0,117
48	31545432	G>G/A	TNF	3_prime_UTR_variant	snv	118G>A	rs762834115	0,83	0,00	0,238	15,501
49	31545691	G>G/C	TNF	3_prime_UTR_variant	snv			1,67	0,00	0,095	6,159
50	31545840	G>G/A		downstream_gene_variant	snv			0,00	0,00	0,000	0,000
51	31546495	G>G/C		downstream_gene_variant	snv	799+382G>C	rs3093668	6,67	0,98	0,006	0,412
52	31546789	T>T/C		downstream_gene_variant	snv		rs3013726	4,17	0,00	0,008	0,508
53	31546850	G>G/A		downstream_gene_variant	snv		rs1800628	1,67	1,96	0,834	54,234
54	31546980	A>A/G		downstream_gene_variant	snv		rs3093671	1,67	0,00	0,095	6,159
55	31547115	G>G/A		downstream_gene_variant	snv		rs3093727	3,33	0,00	0,018	1,145
56	31547174	T>T/C		downstream_gene_variant	snv		rs865848433	0,00	0,00	0,000	0,000
57	31547379	C>C/T		downstream_gene_variant	snv			0,00	0,00	0,000	0,000
58	31547420	C>C/T		downstream_gene_variant	snv		rs146233968	4,17	0,98	0,063	4,086
59	31547460	G>G/A		downstream_gene_variant	snv		rs3091258	20,83	7,84	0,001	0,036
60	31547474	G>G/A		downstream_gene_variant	snv		rs3093561	1,67	0,98	0,574	37,299
61	31547514	G>G/T		downstream_gene_variant	snv		rs769178	5,83	24,51	0,000	0,000
62	31547611	C>C/T		downstream_gene_variant	snv		rs769177	6,67	11,76	0,092	5,999
63	31547792	C>C/T		downstream_gene_variant	snv		rs3093559	1,67	8,82	0,002	0,119
64	31548379	T>T/C		downstream_gene_variant	snv		rs567175785	0,83	0,00	0,238	15,501
65	31548552	G>C/C		downstream_gene_variant	snv			0,83	0,00	0,238	15,501

Tab. 5 | **Psoriatic patients stratification for HLA associated alleles**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

6. Psoriatic arthritis patients (PsA) compared to control individuals

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% Psoriatic Arthritis patients (n. 130)	Va% Sardinian ctrl (n. 120)	*p-value*	*p**
1	31539592	T>T/G		upstream_gene_variant	snv	,-497T>G		1,364	0,000	0,069	4,514
2	31539735	C>C/T		upstream_gene_variant	snv	,-354C>T	rs56161754	0,000	0,704	0,175	11,393
3	31539767	C>C/T		upstream_gene_variant	snv	.-322C>T	rs2844482	15,455	18,310	0,394	25,599
4	31539768	A>A/G		upstream_gene_variant	snv	.-321A>G	rs2071590	56,364	57,042	0,878	57,096
5	31539919	C>C/G	LTA	5_prime_UTR_variant	snv	.-170C>G		0,000	0,000	0,000	0,000
6	31539974	G>G/A	LTA	5_prime_UTR_variant	snv	.-115G>A	rs145829373	0,455	0,704	0,712	46,284
7	31540071	G>G/A	LTA	5_prime_UTR_variant	snv	.-18G>A	rs1800683	10,909	9,155	0,515	33,466
8	31540119	T>T/A	LTA	intron_variant	snv	.-10+40T>A		0,000	0,000	0,000	0,000
9	31540141	A>C/C	LTA	intron_variant	snv	.-10+62A>C	rs2239704	50,000	50,000	1,000	65,000
10	31540142	G>G/A	LTA	intron_variant	snv	.-91G>A	rs3093546	2,273	0,704	0,152	9,885
11	31540313	A>A/G	LTA	intron_variant	snv	.-9-198A>G	rs909253	10,909	9,155	0,515	33,466
12	31540429	C>G/G	LTA	intron_variant	snv	.-9-82C>G	rs746868	49,545	50,000	0,919	59,742
13	31540440	C>C/T	LTA	intron_variant	snv	-9-71C>T	rs780911663	0,455	0,000	0,296	19,220
14	31540457	G>G/A	LTA	intron_variant	snv	,-9-54G>A	rs184649284	0,455	2,113	0,096	6,209
15	31540556	T>T/C	LTA	missense_variant	snv	Cys13Arg	rs2229094	38,636	40,845	0,614	39,913
16	31540693	G>G/C	LTA	intron_variant	snv	100-12G>C	rs3093542	3,182	0,000	0,064	4,185
17	31540757	A>A/C	LTA	missense_variant	snv	His51Pro	rs2229092	5,455	10,563	**0,034**	**2,238**
18	31540784	C>A/A	LTA	missense_variant	snv	Thr60Asn	rs1041981	10,909	9,155	0,515	33,466
19	31540821	A>A/G	LTA	intron_variant	snv	205+11A>G	rs56285847	1,364	1,408	0,966	62,779
20	31541848	T>T/A	LTA	3_prime_UTR_variant	snv	378T>A	rs3093547	0,909	4,225	**0,018**	**1,148**
21	31541948	C>C/T	LTA	3_prime_UTR_variant	snv	478C>T	rs17207127	3,182	4,930	0,320	20,817
22	31541959	G>G/C	LTA	3_prime_UTR_variant	snv	489G>C	rs3093545	0,455	0,000	0,296	19,220
23	31542308	T>T/C		downstream_gene_variant	snv	-1031T>C	rs1799964	33,182	36,620	0,420	27,312
24	31542476	C>C/A		downstream_gene_variant	snv	-863C>A	rs1800630	16,364	19,718	0,329	21,388
25	31542482	C>C/T		downstream_gene_variant	snv	-857C>T	rs1799724	17,273	18,310	0,762	49,518
26	31542485	A>A/T		downstream_gene_variant	snv			0,000	0,000	0,000	0,000
27	31542533	C>C/T		downstream_gene_variant	snv	-986C>T	rs4248158	1,364	1,408	0,966	62,779
28	31542767	A>A/C		upstream_gene_variant	snv	-752A>C	rs4248161	2,727	0,704	0,085	5,546
29	31542963	G>G/A		upstream_gene_variant	snv	.-376G>A	rs1800750	12,727	16,901	0,188	12,237
30	31543031	G>G/A		upstream_gene_variant	snv	-308G>A	rs1800629	5,455	2,113	0,052	3,402
31	31543101	G>G/A		upstream_gene_variant	snv	-238G>A	rs361525	16,364	16,901	0,872	56,667
32	31543122	G>G/C		upstream_gene_variant	snv			0,000	0,000	0,000	0,000
33	31543261	T>T/C		upstream_gene_variant	snv	-76T>C		0,455	0,000	0,296	19,220

Tab. 6 | **Psoriatic arthritis patients (PsA) compared to control individuals**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

6. Psoriatic arthritis patients (PsA) compared to control individuals

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% Psoriatic Arthritis patients (n. 130)	Va% Sardinian ctrl (n. 120)	*p-value*	*p**
34	31543262	A>A/T		upstream_gene_variant	snv		rs41297589	0,000	0,000	0,000	0,000
35	31543404	A>A/AC	TNF	5_prime_UTR_variant	insertion		rs201328097	0,909	0,704	0,799	51,906
36	31543758	G>G/A	TNF	5_prime_UTR_variant	snv	,186+54G>A	rs3093661	5,000	0,000	**0,006**	**0,413**
37	31543797	G>G/A	TNF	5_prime_UTR_variant	snv	.186+93G>A		1,364	1,408	0,966	62,779
38	31543825	A>A/T	TNF	intron_variant	snv	,+186+121A>T	rs4645839	1,818	0,000	0,141	9,178
39	31543827	G>G/A	TNF	intron_variant	snv	.+186+123G>A	rs1800610	17,273	18,310	0,762	49,518
40	31543943	.-/TGAA	TNF	intron_variant	insertion	186+240insTGAA	rs374501689	0,455	0,000	0,949	61,673
41	31544022	TG>TG/T	TNF	intron_variant				2,727	0,704	0,085	5,546
42	31544065	A>A/AAG	TNF	intron_variant	insertion		rs371135507	11,364	16,197	0,116	7,549
43	31544189	A>A/G	TNF	intron_variant	snv	187-122A>G	rs3093662	19,545	21,127	0,661	42,936
44	31544642	A>A/G	TNF	intron_variant	snv	280+51A>G	rs3093664	8,636	4,930	0,101	6,585
45	31545101	C>C/T	TNF	synonymous variant	snv	489C>T	rs747910356	0,000	0,704	0,175	11,393
46	31545366	A>A/G	TNF	3_prime_UTR_variant	snv	52A>G	rs769422678	0,455	0,704	0,712	46,284
47	31545391	A>A/C	TNF	3_prime_UTR_variant	snv	77A>C	rs3093665	3,182	4,225	0,536	34,835
48	31545432	G>G/A	TNF	3_prime_UTR_variant	snv	118G>A	rs762834115	0,455	0,000	0,296	19,220
49	31545691	G>G/C	TNF	3_prime_UTR_variant	snv			0,000	0,000	0,000	0,000
50	31545840	G>G/A		downstream_gene_variant	snv			0,455	0,000	0,296	19,220
51	31546495	G>G/C		downstream_gene_variant	snv	799+382G>C	rs3093668	5,455	0,000	**0,004**	**0,230**
52	31546789	T>T/C		downstream_gene_variant	snv		rs3013726	3,636	0,000	**0,003**	**0,186**
53	31546850	G>G/A		downstream_gene_variant	snv		rs1800628	1,364	0,704	0,469	30,502
54	31546980	A>A/G		downstream_gene_variant	snv		rs3093671	1,818	2,113	0,812	52,805
55	31547115	G>G/A		downstream_gene_variant	snv		rs3093727	3,636	0,000	**0,012**	**0,776**
56	31547174	T>T/C		downstream_gene_variant	snv		rs865848433	0,000	0,704	0,175	11,393
57	31547379	C>C/T		downstream_gene_variant	snv			0,455	0,000	0,296	19,220
58	31547420	C>C/T		downstream_gene_variant	snv		rs146233968	2,727	0,704	0,085	5,546
59	31547460	G>G/A		downstream_gene_variant	snv		rs3091258	10,000	9,859	0,958	62,273
60	31547474	G>G/A		downstream_gene_variant	snv		rs3093561	2,727	1,408	0,304	19,729
61	31547514	G>G/T		downstream_gene_variant	snv		rs769178	17,273	18,310	0,762	49,518
62	31547611	C>C/T		downstream_gene_variant	snv		rs769177	5,000	6,338	0,517	33,611
63	31547792	C>C/T		downstream_gene_variant	snv		rs3093559	3,182	4,225	0,536	34,835
64	31548379	T>T/C		downstream_gene_variant	snv		rs567175785	0,000	0,704	0,175	11,393
65	31548552	G>C/C		downstream_gene_variant	snv			2,273	0,000	**0,019**	**1,222**

Tab. 6 **| Psoriatic arthritis patients (PsA) compared to control individuals**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

7. Psoriatic arthritis patients (PsA) stratification for HLA associated alleles

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% HLA Associated Alleles (n. 66)	Va% HLA Not Associated Alleles (n.64)	*p-value*	*p**
1	31539592	T>T/G		upstream_gene_variant	snv	,-497T>G		1,786	0,926	0,550	35,745
2	31539735	C>C/T		upstream_gene_variant	snv	,-354C>T	rs56161754	0,000	0,000	0,000	0,000
3	31539767	C>C/T		upstream_gene_variant	snv	.-322C>T	rs2844482	24,107	6,481	0,000084	0,005
4	31539768	A>A/G		upstream_gene_variant	snv	.-321A>G	rs2071590	70,536	41,667	0,000003	0,00018
5	31539919	C>C/G	LTA	5_prime_UTR_variant	snv	.-170C>G		0,000	0,000	0,000	0,000
6	31539974	G>G/A	LTA	5_prime_UTR_variant	snv	.-115G>A	rs145829373	0,000	0,926	0,268	17,409
7	31540071	G>G/A	LTA	5_prime_UTR_variant	snv	.-18G>A	rs1800683	14,286	7,407	0,075	4,888
8	31540119	T>T/A	LTA	intron_variant	snv	.-10+40T>A		0,000	0,000	0,000	0,000
9	31540141	A>C/C	LTA	intron_variant	snv	.-10+62A>C	rs2239704	63,393	36,111	0,000	0,001
10	31540142	G>G/A	LTA	intron_variant	snv	.-91G>A	rs3093546	2,679	1,852	0,655	42,553
11	31540313	A>A/G	LTA	intron_variant	snv	.-9-198A>G	rs909253	14,286	7,407	0,075	4,888
12	31540429	C>G/G	LTA	intron_variant	snv	.-9-82C>G	rs746868	63,393	35,185	0,000005	0,00035
13	31540440	C>C/T	LTA	intron_variant	snv	-9-71C>T	rs780911663	0,893	0,000	0,284	18,457
14	31540457	G>G/A	LTA	intron_variant	snv	,-9-54G>A	rs184649284	0,893	0,000	0,284	18,457
15	31540556	T>T/C	LTA	missense_variant	snv	Cys13Arg	rs2229094	49,107	27,778	0,00041	0,027
16	31540693	G>G/C	LTA	intron_variant	snv	100-12G>C	rs3093542	1,786	4,630	0,192	12,464
17	31540757	A>A/C	LTA	missense_variant	snv	His51Pro	rs2229092	8,929	1,852	0,012	0,775
18	31540784	C>A/A	LTA	missense_variant	snv	Thr60Asn	rs1041981	14,286	7,407	0,075	4,888
19	31540821	A>A/G	LTA	intron_variant	snv	205+11A>G	rs56285847	2,679	0,000	0,062	4,048
20	31541848	T>T/A	LTA	3_prime_UTR_variant	snv	378T>A	rs3093547	0,893	0,926	0,978	63,544
21	31541948	C>C/T	LTA	3_prime_UTR_variant	snv	478C>T	rs17207127	5,357	0,926	0,042	2,706
22	31541959	G>G/C	LTA	3_prime_UTR_variant	snv	489G>C	rs3093545	0,893	0,000	0,284	18,457
23	31542308	T>T/C		downstream_gene_variant	snv	-1031T>C	rs1799964	42,857	23,148	0,001	0,048
24	31542476	C>C/A		downstream_gene_variant	snv	-863C>A	rs1800630	25,000	7,407	0,000	0,008
25	31542482	C>C/T		downstream_gene_variant	snv	-857C>T	rs1799724	16,071	18,519	0,602	39,117
26	31542485	A>A/T		downstream_gene_variant	snv			0,000	0,000	0,000	0,000
27	31542533	C>C/T		downstream_gene_variant	snv	-986C>T	rs4248158	1,786	0,926	0,550	35,745
28	31542767	A>A/C		upstream_gene_variant	snv	-752A>C	rs4248161	0,893	4,630	0,065	4,202
29	31542963	G>G/A		upstream_gene_variant	snv	.-376G>A	rs1800750	16,964	8,333	0,037	2,389
30	31543031	G>G/A		upstream_gene_variant	snv	-308G>A	rs1800629	5,357	5,556	0,944	61,350
31	31543101	G>G/A		upstream_gene_variant	snv	-238G>A	rs361525	16,964	15,741	0,790	51,334
32	31543122	G>G/C		upstream_gene_variant	snv			0,000	0,000	0,000	0,000
33	31543261	T>T/C		upstream_gene_variant	snv	-76T>C		0,000	0,926	0,268	17,409

Tab. 7 | **Psoriatic arthritis patients (PsA) stratification for HLA associated alleles**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

7. Psoriatic arthritis patients (PsA) stratification for HLA associated alleles

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% HLA Associated Alleles (n. 66)	Va% HLA Not Associated Alleles (n.64)	*p-value*	*p**
34	31543262	A>A/T		upstream_gene_variant	snv		rs41297589	0,000	0,000	0,000	0,000
35	31543404	A>A/AC	TNF	5_prime_UTR_variant	insertion		rs201328097	0,000	1,852	0,116	7,557
36	31543758	G>G/A	TNF	5_prime_UTR_variant	snv	,186+54G>A	rs3093661	0,000	10,185	0,0002	0,011
37	31543797	G>G/A	TNF	5_prime_UTR_variant	snv	.186+93G>A		1,786	0,926	0,550	35,745
38	31543825	A>A/T	TNF	intron_variant	snv	,+186+121A>T	rs4645839	0,000	3,704	0,026	1,667
39	31543827	G>G/A	TNF	intron_variant	snv	.+186+123G>A	rs1800610	16,071	18,519	0,602	39,117
40	31543943	.-/TGAA	TNF	intron_variant	insertion	186+240insTGAA	rs374501689	0,893	0,000	0,284	18,457
41	31544022	TG>TG/T	TNF	intron_variant				3,571	1,852	0,395	25,644
42	31544065	A>A/AAG	TNF	intron_variant	insertion		rs371135507	16,964	5,556	0,004	0,243
43	31544189	A>A/G	TNF	intron_variant	snv	187-122A>G	rs3093662	22,321	16,667	0,250	16,267
44	31544642	A>A/G	TNF	intron_variant	snv	280+51A>G	rs3093664	5,357	12,037	0,055	3,598
45	31545101	C>C/T	TNF	synonymous variant	snv	489C>T	rs747910356	0,000	0,000	0,000	0,000
46	31545366	A>A/G	TNF	3_prime_UTR_variant	snv	52A>G	rs769422678	0,893	0,000	0,284	18,457
47	31545391	A>A/C	TNF	3_prime_UTR_variant	snv	77A>C	rs3093665	5,357	0,926	0,042	2,706
48	31545432	G>G/A	TNF	3_prime_UTR_variant	snv	118G>A	rs762834115	0,000	0,926	0,268	17,409
49	31545691	G>G/C	TNF	3_prime_UTR_variant	snv			0,000	0,000	0,000	0,000
50	31545840	G>G/A		downstream_gene_variant	snv			0,893	0,000	0,284	18,457
51	31546495	G>G/C		downstream_gene_variant	snv	799+382G>C	rs3093668	0,000	11,111	0,00008	0,005
52	31546789	T>T/C		downstream_gene_variant	snv		rs3013726	0,000	7,407	0,001	0,094
53	31546850	G>G/A		downstream_gene_variant	snv		rs1800628	1,786	0,926	0,550	35,745
54	31546980	A>A/G		downstream_gene_variant	snv		rs3093671	2,679	0,926	0,290	18,849
55	31547115	G>G/A		downstream_gene_variant	snv		rs3093727	0,000	7,407	0,001	0,094
56	31547174	T>T/C		downstream_gene_variant	snv		rs865848433	0,000	0,000	0,000	0,000
57	31547379	C>C/T		downstream_gene_variant	snv			0,000	0,926	0,268	17,409
58	31547420	C>C/T		downstream_gene_variant	snv		rs146233968	0,893	4,630	0,065	4,202
59	31547460	G>G/A		downstream_gene_variant	snv		rs3091258	0,893	19,444	0,000001	0,000041
60	31547474	G>G/A		downstream_gene_variant	snv		rs3093561	3,571	1,852	0,395	25,644
61	31547514	G>G/T		downstream_gene_variant	snv		rs769178	16,071	18,519	0,602	39,117
62	31547611	C>C/T		downstream_gene_variant	snv		rs769177	7,143	2,778	0,106	6,903
63	31547792	C>C/T		downstream_gene_variant	snv		rs3093559	5,357	0,926	0,042	2,706
64	31548379	T>T/C		downstream_gene_variant	snv		rs567175785	0,000	0,000	0,000	0,000
65	31548552	G>C/C		downstream_gene_variant	snv			1,786	2,778	0,592	38,457

Tab. 7 | **Psoriatic arthritis patients (PsA) stratification for HLA associated alleles**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

8. Associations between TNF-α gene polymorphisms and response to TNF-α inhibitors

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% Anti-TNF No Responders (n. 17)	Va% Anti-TNF Responders (n. 53)	*p-value*	*p**
1	31539592	T>T/G		upstream_gene_variant	snv	.-497T>G		2,941	1,887	0,712	46,27
2	31539735	C>C/T		upstream_gene_variant	snv	,-354C>T	rs56161754	0,000	0,943	0,570	37,04
3	31539767	C>C/T		upstream_gene_variant	snv	.-322C>T	rs2844482	11,765	10,377	0,820	53,30
4	31539768	A>A/G		upstream_gene_variant	snv	.-321A>G	rs2071590	52,941	57,547	0,637	41,43
5	31539919	C>C/G	LTA	5_prime_UTR_variant	snv	.-170C>G		0,000	0,000	0,000	0,00
6	31539974	G>G/A	LTA	5_prime_UTR_variant	snv	.-115G>A	rs145829373	0,000	0,000	0,000	0,00
7	31540071	G>G/A	LTA	5_prime_UTR_variant	snv	.-18G>A	rs1800683	11,765	12,264	0,938	60,98
8	31540119	T>T/A	LTA	intron_variant	snv	.-10+40T>A		0,000	0,943	0,570	37,04
9	31540141	A>C/C	LTA	intron_variant	snv	.-10+62A>C	rs2239704	44,118	46,226	0,830	53,95
10	31540142	G>G/A	LTA	intron_variant	snv	.-91G>A	rs3093546	0,000	3,774	0,250	16,28
11	31540313	A>A/G	LTA	intron_variant	snv	.-9-198A>G	rs909253	11,765	12,264	0,938	60,98
12	31540429	C>G/G	LTA	intron_variant	snv	.-9-82C>G	rs746868	44,118	46,226	0,830	53,95
13	31540440	C>C/T	LTA	intron_variant	snv	-9-71C>T	rs780911663	0,000	0,943	0,570	37,04
14	31540457	G>G/A	LTA	intron_variant	snv	,-9-54G>A	rs184649284	0,000	0,943	0,570	37,04
15	31540556	T>T/C	LTA	missense_variant	snv	Cys13Arg	rs2229094	32,353	33,962	0,863	56,08
16	31540693	G>G/C	LTA	intron_variant	snv	100-12G>C	rs3093542	8,824	3,774	0,240	15,58
17	31540757	A>A/C	LTA	missense_variant	snv	His51Pro	rs2229092	5,882	0,943	0,084	5,43
18	31540784	C>A/A	LTA	missense_variant	snv	Thr60Asn	rs1041981	11,765	12,264	0,938	60,98
19	31540821	A>A/G	LTA	intron_variant	snv	205+11A>G	rs56285847	0,000	3,774	0,250	16,28
20	31541848	T>T/A	LTA	3_prime_UTR_variant	snv	378T>A	rs3093547	0,000	0,943	0,570	37,04
21	31541948	C>C/T	LTA	3_prime_UTR_variant	snv	478C>T	rs17207127	5,882	1,887	0,224	14,54
22	31541959	G>G/C	LTA	3_prime_UTR_variant	snv	489G>C	rs3093545	2,941	0,000	0,076	4,97
23	31542308	T>T/C		downstream_gene_variant	snv	-1031T>C	rs1799964	20,588	29,245	0,323	21,01
24	31542476	C>C/A		downstream_gene_variant	snv	-863C>A	rs1800630	14,706	11,321	0,599	38,93
25	31542482	C>C/T		downstream_gene_variant	snv	-857C>T	rs1799724	5,882	13,208	0,243	15,78
26	31542485	A>A/T		downstream_gene_variant	snv			0,000	0,000	0,000	0,00
27	31542533	C>C/T		downstream_gene_variant	snv	-986C>T	rs4248158	0,000	0,000	0,000	0,00
28	31542767	A>A/C		upstream_gene_variant	snv	-752A>C	rs4248161	0,000	2,830	0,321	20,89
29	31542963	G>G/A		upstream_gene_variant	snv	**.-376G>A**	**rs1800750**	**0,000**	**14,15**	**0,020**	**0,00545**
30	31543031	G>G/A		upstream_gene_variant	snv	-308G>A	rs1800629	5,882	6,604	0,881	57,29
31	31543101	G>G/A		upstream_gene_variant	snv	-238G>A	rs361525	5,882	17,925	0,087	5,66
32	31543122	G>G/C		upstream_gene_variant	snv			0,000	0,943	0,570	37,04
33	31543261	T>T/C		upstream_gene_variant	snv	-76T>C		0,000	0,000	0,000	0,00

Tab. 8 | **Associations between TNF-α gene polymorphisms and response to TNF-α inhibitors**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

8. Associations between TNF-α gene polymorphisms and response to TNF-α inhibitors

N	Coordinate	Variant	Gene	Consequence	Type	Alternative name	dbSNP ID	Va% Anti-TNF No Responders (n. 17)	Va% Anti-TNF Responders (n. 53)	*p-value*	*p**
34	31543262	A>A/T		upstream_gene_variant	snv		rs41297589	0,000	0,000	0,000	0,00
35	31543404	A>A/AC	TNF	5_prime_UTR_variant	insertion		rs201328097	2,941	0,000	0,076	4,97
36	31543758	G>G/A	TNF	5_prime_UTR_variant	snv	,186+54G>A	rs3093661	5,882	4,717	0,786	51,10
37	31543797	G>G/A	TNF	5_prime_UTR_variant	snv	.186+93G>A		0,000	0,943	0,570	37,04
38	31543825	A>A/T	TNF	intron_variant	snv	,+186+121A>T	rs4645839	0,000	1,887	0,420	27,29
39	31543827	G>G/A	TNF	intron_variant	snv	.+186+123G>A	rs1800610	5,882	13,208	0,243	15,78
40	31543943	.-/TGAA	TNF	intron_variant	insertion	.86+240insTGAA	rs374501689	2,941	0,000	0,076	4,97
41	31544022	TG>TG/T	TNF	intron_variant				2,941	3,774	0,820	53,30
42	31544065	A>A/AAG	TNF	intron_variant	insertion		rs371135507	2,941	12,264	0,115	7,47
43	31544189	A>A/G	TNF	intron_variant	snv	187-122A>G	rs3093662	11,765	19,811	0,286	18,62
44	31544642	A>A/G	TNF	intron_variant	snv	280+51A>G	rs3093664	11,765	7,547	0,445	28,90
45	31545101	C>C/T	TNF	synonymous variant	snv	489C>T	rs747910356	0,000	0,000	0,000	0,00
46	31545366	A>A/G	TNF	3_prime_UTR_variant	snv	52A>G	rs769422678	2,941	0,000	0,076	4,97
47	31545391	A>A/C	TNF	3_prime_UTR_variant	snv	77A>C	rs3093665	5,882	1,887	0,224	14,54
48	31545432	G>G/A	TNF	3_prime_UTR_variant	snv	118G>A	rs762834115	0,000	0,943	0,570	37,04
49	31545691	G>G/C	TNF	3_prime_UTR_variant	snv			0,000	0,000	0,000	0,00
50	31545840	G>G/A		downstream_gene_variant	snv			0,000	0,000	0,000	0,00
51	31546495	G>G/C		downstream_gene_variant	snv	799+382G>C	rs3093668	5,882	5,660	0,961	62,48
52	31546789	T>T/C		downstream_gene_variant	snv		rs3013726	5,882	3,774	0,597	38,83
53	31546850	G>G/A		downstream_gene_variant	snv		rs1800628	2,941	1,887	0,712	46,27
54	31546980	A>A/G		downstream_gene_variant	snv		rs3093671	0,000	0,943	0,570	37,04
55	31547115	G>G/A		downstream_gene_variant	snv		rs3093727	5,882	3,774	0,597	38,83
56	31547174	T>T/C		downstream_gene_variant	snv		rs865848433	0,000	0,000	0,000	0,00
57	31547379	C>C/T		downstream_gene_variant	snv			0,000	0,000	0,000	0,00
58	31547420	C>C/T		downstream_gene_variant	snv		rs146233968	0,000	2,830	0,321	20,89
59	31547460	G>G/A		downstream_gene_variant	snv		rs3091258	8,824	13,208	0,496	32,23
60	31547474	G>G/A		downstream_gene_variant	snv		rs3093561	2,941	0,943	0,393	25,55
61	31547514	G>G/T		downstream_gene_variant	snv		rs769178	5,882	13,208	0,243	15,78
62	31547611	C>C/T		downstream_gene_variant	snv		rs769177	5,882	10,377	0,432	28,08
63	31547792	C>C/T		downstream_gene_variant	snv		rs3093559	5,882	1,887	0,224	14,54
64	31548379	T>T/C		downstream_gene_variant	snv		rs567175785	0,000	0,000	0,000	0,00
65	31548552	G>C/C		downstream_gene_variant	snv			5,882	2,830	0,404	26,26

Tab. 8 | **Associations between TNF-α gene polymorphisms and response to TNF-α inhibitors**

p= p values after Bonferroni's correction*

yellow boxes represent new SNPs not present in the database

5. DISCUSSION

From the genetic point of view, psoriasis vulgaris and psoriatic arthritis belongs to the category of ‘‘complex’’ diseases: an individual carrying several genetic risk factors develops the disease under the additional influence of environmental risk factors. It has been estimated that at least one-third of the genetic contribution to psoriasis and PsA resides in the major histocompatibility complex region (Bowcock and Cookson, 2004). Characterization of the exact MHC gene or genes involved in susceptibility to psoriasis and psoriatic arthritis has been controversial. This is due to the high density of polymorphic genes located in this region, the extensive ranges of polymorphism, and the preservation of HLA haplotypes (Traherne J.A, 2008). Although extensive LD makes identification of the true disease susceptibility locus/loci, it is believed that the HLA region, especially HLA-B and C, are the most important loci associated with psoriatic disease. The TNFα gene, which is located 250 kb centromeric from HLA-B, has been proposed as high priority candidate gene in psoriasis and psoriatic arthritis. Several studies have investigated the possible association between the two most widely studied TNF promoter polymorphisms (-308G/A and -238G/A) in psoriatic disease. In particular, Hohler et al. (2002) reported a highly significant association of the SNP 238A with PsA in the German population and Balding et al. (2003) observed the association of the SNP 308 with PsA in Irish patients. Finally, Rahman et al. (2006) carried out a meta-analysis of all TNF-α association studies in White PsA patients and confirmed that the SNP 238A showed a significant risk factor for this disease. By contrast, Gonzalez et al. (2001, 2002) excluded the association between the two, SNPs 238 and 308 and PsA in the Jewish and Spanish populations. The discrepant results found among populations may be the consequence of ethnic admixture causing population stratification, of population-specific gene–gene or gene - environment interactions, and of statistical fluctuations (Ioannidis et al., 2001; Huizinga et al., 2004).

The present analysis showed no significant association between TNF and the susceptibility to psoriasis vulgaris and psoriatic arthritis and in particular the common promoter single nucleotide polymorphism (SNPs) -238G/A (rs361525) and -308G/A (rs1800629) have not shown a significant difference in the frequency between PsV and PsA patients compared with controls (PsV -308G/A p = 0,44, -238G/A p = 0,253), (PsA -308G/A p = 0,0523, 238G/A p = 0,8718) in the Sardinian population, although there was a clear trend for the association with the SNP -238 G allele that was previously found to be associated with an older age of onset and the disease (Balding et al., 2003).

Murdaca et al., 2014 report the strong associations between the SNP +489 variant allele A in the first intron of the TNF-α gene, whose frequency of 14% in the Italian population (D’Alfonso and Momigliano Richiardi, 1996) overlaps the distribution found in the Dutch

(12%) (van Krugten et al., 1999) and the British (15%) (Mullighan et al., 1997) populations, and both PsA susceptibility and severity. Indeed, for the authors, the presence and progression of joint erosions of the hands and feet as well as the presence of periostitis, sacroiliitis, and spondylitis were associated with the SNP +489 A allele. Mullighan et al. (1997) reported that the SNP +489 A allele may be associated with increased TNF-a levels, suggesting that a genetic predisposition to produce higher levels of TNF-a is an important factor in the development of erosions. We did not find significant association between the SNP +489 variant allele A in the first intron of the TNF-α gene and the susceptibility to psoriasis and PsA. The SNP +489G/A (rs1800610) have not shown a significant difference in the frequency between Ps and PsA patients compared with controls (Ps +489G/A p = 0,202), (PsA +489G/A p = 0,762) in the Sardinian population.

PsA main genetic risk factor is considered an HLA-C allele (HLA-C*06), explaining a considerable portion of heritability in PsA and even more in PsV. According to the current state of knowledge, 144 variants are disease-causing candidates, and, because of the location of a subset in an enhancer element, an influence on the expression of the HLA-C risk allele is suspected (Clop et al., 2013). However, the exact mechanism by which HLA-C*06 or other highly significantly associated HLA-C/HLA-B risk alleles and/or haplotypes (Chandran et al., 2013) contribute to psoriatic arthritis and psoriasis vulgaris remains to be elucidated.

In the past era of candidate gene studies, psoriasis vulgaris, but also psoriatic arthritis, has been independently associated with variants in the promoter region of the TNF gene coding for TNF-α (Reich et al., 2007; Giardina et al., 2011). As TNF is known to locate quite closely to the MHC, it could be shown that association of the promotor variant at -238 is dependent on carriage of the HLA-C risk allele; the latter was more significantly associated with psoriasis and PsA and therefore the more probable disease-causing risk factor (Reich et al., 2007). This dependence is referred to as linkage disequilibrium. In contrast, the promotor variant at -857 was associated with PsA independently from carrier status of HLA-Cw*06 (Reich et al., 2007). Giardina et al., 2011 replicated the association of TNF -857T as a susceptibility allele for PsA independent of the main PSORS1 risk allele. The authors in their large genetics study, have enrolled three independent cohorts, comprising independent case-control samples from Germany, Italy and UK for a total cohort of 2,224 individuals. The same cohort was typed for TNF-875 and the analysis revealed an higher significance in the frequency of allele in individuals with PsA (27%) than in control subjects (20%). To verify the existence of an association independent of the PSORS1 risk allele, Giardina and coll. carried out a stratification of the subjects according to the presence or absence of the PSORS1 susceptibility allele and the results

robustly demonstrated that TNF -857T represents a risk allele for PsA independent of the PSORS1 main locus.

Interestingly, current data provide evidence that the promotor variant at -857 is in strong linkage disequilibrium to variant +489. Therefore, both variants at the TNF locus, or perhaps their combination, are candidate variants (Huffmeier et al., 2014).

The current stratification analysis conducted in the PsV and PsA patients, evaluating the association with HLA alleles and in particular HLA-C*06:02 in general population and HLA-C*07:18 - HLA-B*58:01 in Sardinian population, revealed a strong association between marker analyzed and HLA alleles considered. In particular the analysis for the psoriasis patients shown a strong associations for two markers rs1799724 (-857C>T) ($p = 5{,}04x10^{7}$), rs1800610 (+489G>A) ($p = 5{,}04x10^{7}$). This may only reflect that the SNPs analyzed are in linkage disequilibrium with major histocompatibility complex class I genes. There is a high linkage disequilibrium within the densely packed genomic region at chromosome 6p21.3. Therefore, an isolated candidate approach at the TNF locus, without considering linked risk alleles, might lead to false-positive associations (Daly and Day, 2001).

The hypothesis that TNF gene has been proposed as a major candidate gene in psoriatic disease, is supported by studies that have found high serum, synovial fluid, and synovial membrane TNF-α levels in patients with PsA (Partsch et al., 1997; Danning et al., 2000). The TNF functions are mediated by two distinct TNF receptors, TNFRSF1A and TNFRSF1B, which exist as monomers on cell surfaces and in soluble forms. Intensive studies on TNF-driven inflammation processes have led to the development of TNF blockers for PsA treatment. They are derived from a recombinant TNF receptor, TNFRSF1B (for etanercept), or an anti-TNFA monoclonal antibody (for infliximab and adalimumab). The molecular mechanisms of these TNF blockers are similar: They inhibit binding of TNFs to cell-surface TNF receptors and thereby block signal transduction pathways that are induced or regulated by TNFs. However, etanercept binds to both TNFA and LTA, whereas infliximab and adalimumab bind to TNFA only. Indeed, TNF-α is presently a therapeutic target for patients responding poorly to conventional disease-modifying antirheumatic drugs (DMARDs). The observation that anti-TNF-α biologic therapy retards or even stops the progression of the anatomic damage has changed our views on the inevitable relentless progression that was thought to occur with DMARDs treatment.

Murdaca et al., 2014 report preliminary evidence for an association between the single nucleotide polymorphism (SNP) +489 but not the association with -308 and -238 at the TNF locus and both susceptibility to the development of PsA and to treatment responses to TNF-α blockers. The author describes a trend for a higher frequency of the TNF SNP +489G allele in responders to etanercept therapy compared with non-responders, but not

in patients treated with adalimumab, in whom, by contrast, the genotype SNP +489AA was more frequent. It might be expected that the genetic bases of treatment responses are similar for all of the TNF-a blockers; however, molecular differences may explain some of the differences in therapeutic responses, and possibly genetic predisposition.

The study of Murdaca and coll. prompted me to perform an analysis of associations between TNF-α gene polymorphisms and response to TNF-α inhibitors in 70 patients, 53 responders and 17 non-responders. This analysis shown no significant difference in the frequency of the SNP +489 between responders and no-responders. Previous studies examined whether the SNP 308 and 238 could influence the therapeutic response to TNF-α inhibitors in patients with rheumatoid arthritis. In particular, the SNP 308G/G genotype seems to favor a better response to TNF-α inhibitors (Seitz et al., 2007), whereas the SNP 308A allele predicts poor response to these biological drugs in this disease (O'Rielly et al., 2009). By contrast, it has been reported that the SNP 238A/G genotype seems to be associated with the response to infliximab (Lee et al., 2010). Interesting, Kang CP et al., 2005, report evidence that in the treatment of rheumatoid arthritis, the presence of TNF-857T was associated with a good therapeutic response to etanercept, These results are in disagreement with our present study, as we found that the SNPs -308 and -238 and -857 do not seem to influence the clinical outcome of PsA patients treated with TNF-α inhibitors. Of note, a significant association between the marker rs1800750 (-376G>A) ($p = 0,005445$) with a frequency of 14% was found in responders compared to non-responder. The minor allele (A) is completely absent in the patients that don't responded to the anti-TNF therapy while it was present in the responders, this finding seems to be important due the fact that this allele has been found to be associated to high level of TNF. Our present knowledge on the association of -376G>A polymorphism with Ps and PsA response to TNF inhibitors are not reported in the literature.

Summarising, clinical studies showed that TNF-α −308 G/G, +489 GG and the +489 GA, TNF-α −857C SNPs favor the response to etanercept. 30–60% of patients do not respond sufficiently to treatment with TNF-α, but the reasons are largely unknown.

However, most of these studies are often small and not sufficiently powered to detect an effect and markers tend to be more prognostic than predictive of therapeutic response. Furthermore, studies often examines only the effects of a single SNP, while it would be more useful to analyze more haplotypes in contemporary in the same patients. Appropriately designed clinical trials are needed before a pharmacogenetic approach may be applicable in daily clinical therapeutic practice. Pharmacogenetics represents the new frontier for the discovery of potential genetic markers of biological response to TNF-α inhibitors (Murdaca et al., 2017). For Cascella et al., 2016 pharmacogenomics (PGx) plays a crucial role in the application of personalized medicine to the clinical care. The technology and scientific progress achieved in the postgenomic era provided the basis for

the development of personal ‘omics’ profiles, conceived as the combination of genomic, transcriptomic, proteomic, metabolomic and autoantibody panels specific for each individual (Benson M, 2016). In particular, the interactions among these ‘omics’ information are able to provide a personal profile either in health or disease conditions. Personal ‘omics’ profiles may be successfully applied to provide the optimal personalized treatment to patients with specific genomic signatures affecting the drug response. This approach combines the ‘omics’ and the environment information with the purpose of implementing new strategies able to prevent and improve the disease management as well as to enhance the patients quality of life (QoL).

The ‘omics’ information is provided by specific biomarkers that can be detected in different biological sources (blood, saliva, buccal swabs and tissue biopsies). Given the variability of patient response to psoriatic arthritis (PsA) therapies, pharmacogenomic biomarkers may be utilized to predict the response and the risk for adverse drug reaction (ADRs) to specific disease treatments. The author reviewed the current knowledge about the PGx of psoriatic arthritis, putting in evidence the perspectives and the challenges for its implementation in the clinical practice. Concerning the treatment of PsA, the available therapeutic approaches are mainly oriented to relief the inflammation and/or the associated-symptoms and to ‘slow-down’ the progression to more advanced and severe stages of the disease. The poor scientific knowledge concerning the main pathogenetic and therapeutic response mechanisms obstructs the creation of a detailed pharmacogenomics (PGx) picture of PsA. To date, PsA-specific PGx information are only available for some drugs. However, these findings often refer to unpowered studies, which have been performed in small sample cohorts, including different inflammatory artropathies (mainly rheumatoid arthritis and osteoarthritis) other than PsA. To date, very few techniques have been applied for the detection of such PGx biomarkers (PGBMs). Over the limits presented by the availability of adequate techniques, the introduction of precision/personalized medicine approaches specific for PsA has to overcome several difficulties. The current molecular technologies may be additionally improved by massive and parallel genotyping on next generation sequencing (NGS) platforms. In fact, the NGS approach may be employed to effectively scan the whole genome with the purpose of selecting the optimal PsA treatment according to the patient ‘omics’ profile. Furthermore, the NGS may contribute to reveal the gene × environment connections that are responsible for the onset of PsA. In particular, the ‘gene’ component should be considered not only as the analysis of the DNA sequence per se but also as the study of the chemical changes that affect the gene expression. The development of personalized PsA therapies based on PGx testing will allow the application of a ‘stratified medicine’ approach to the disease. In fact, according to the results of the predictive PGx test, the patients will be assigned to different treatment classes: high/intermediate/poor responders and subjects at

high/intermediate/poor risk for adverse drug reactions (ADRs). The precision/personalized medicine and the web-based platform will be finalized to a new-generation medicine approach: the ‘welfare medicine’.

6. CONCLUSIONS

In conclusion, as regards Psoriasis, this study led to the identification of two candidate SNPs (rs2229092 and rs1799964) for further studies on larger samples of psoriatic patients and controls. As regards the sample of Psoriatic Arthritis patients, the results of our study suggest that at least in the Sardinian population there is no direct link between allele distribution and susceptibility for Psoriatic Arthritis.

An interesting result was obtained in the evaluation of the anti-TNF-α biologic therapy were the marker rs1800750 (-376G>A) (p = 0,005445) show a significant association with the response to TNF inhibitors therapy. Finally, the deep sequencing through the NGS sequencing is confirmed to be a useful method for fine mapping and the identification of genetic variants involved in the susceptibility to complex diseases.

References

- Alamanos Y, Voulgari PV, Drosos AA: Incidence and prevalence of psoriatic arthritis: a systematic review. J Rheumatol 2008, 35(7):1354-1358.
- Ali Y, Tom BD, Schentag CT, Farewell VT, Gladman DD: Improved survival in psoriatic arthritis with calendar time. Arthritis Rheum 2007, 56(8):2708-2714.
- Alibert JL (ed.): Précis théorique et pratique sur les maladies de la peau. Paris: Caille et Ravier; 1818.
- Ashcroft, D. M., Li Wan Po, A., Williams, H. C., and Griffiths, C. E. M. (1999). Clinical measures of disease severity and outcome in psoriasis: a critical appraisal of their quality. Br J Dermatol, 141, (2), 185-191.
- Asumalahti K, Laitinen T, Itkonen-Vatjus R, Lokki ML, Suomela S, Snellman E, Saarialho-Kere U, Kere J: A candidate gene for psoriasis near HLA-C, HCR (Pg8), is highly polymorphic with a disease-associated susceptibility allele. Hum Mol Genet 2000, 9(10):1533-1542.
- Asumalahti, K., Veal, C., Laitinen, T., Suomela, S., Allen, M., Elomaa, O., Moser, M., de Cid, R., Ripatti, S., Vorechovsky, I.,. Marcusson, J.A., Nakagawa, H., Lazaro, C., Estivill, X., Capon, F., Novelli, G., Saarialho-Kere, U., Barker, J., Trembath, R., Kere, J. and Psoriasis Consortium. Coding haplotype analysis supports HCR as the putative susceptibility gene for psoriasis at the MHC PSORS1 locus. Hum. Mol. Genet., 2002, 11: 589-97.
- Balding J, Kane D, Livingstone W et al. (2003) Cytokine gene polymorphisms: association with psoriatic arthritis susceptibility and severity. Arthritis Rheum 48:1408–13
- Benson M. Clinical implications of omics and systems medicine: focus on predictive and individualized treatment. J. Intern. Med. 279, 229–240 (2016).
- Blumberg BS, Bunim JJ, Calkins E, Pirani CL, Zvaifler NJ: ARA Nomenclature and Classification of Arthritis and Rheumatism (Tentative). Arthritis Rheum 1964, 7:93-97.
- Bond SJ, Farewell VT, Schentag CT, Gladman DD: Predictors for radiological damage in psoriatic arthritis: results from a single centre. Ann Rheum Dis 2007, 66(3):370-376.
- Bovenschen, H. J., Seyger, M. M. B. & Van De Kerkhof, P. C. M. (2005) Plaque psoriasis vs. atopic dermatitis and lichen planus: a comparison for lesional T-cell subsets, epidermal proliferation and differentiation. British Journal of Dermatology, 153(1), 72-78.

- Bowcock AM, Cookson WO (2004) The genetics of psoriasis, psoriatic arthritis, and atopic dermatitis. Hum Mol Genet 13:R43–55
- Brockbank JE, Stein M, Schentag CT, Gladman DD: Dactylitis in psoriatic arthritis: a marker for disease severity?Ann Rheum Dis 2005, 64(2):188-190.
- Bruce IN, Silman AJ: The aetiology of psoriatic arthritis. Rheumatology (Oxford) 2001, 40(4):363-366.
- Buske-Kirschbaum, A. & Hellhammer, D. H. (2003) Endocrine and Immune Responses to Stress in Chronic Inflammatory Skin Disorders. Annals of the New York Academy of Sciences, 992(1), 231-240.
- Capon, F., Novelli, G., Semprini, S., Clementi, M., Nudo, M., Vultaggio, P. et al. (1999). Searching for psoriasis susceptibility genes in Italy: genome scan and evidence for a new locus on chromosome 1. J Invest Dermatol, 112, (1), 32-35.
- Capon, F., Semprini, S., Chimenti, S., Fabrizi, G., Zambruno, G., Murgia, S. et al. (2001). Fine mapping of the PSORS4 psoriasis susceptibility region on chromosome 1q21. J Invest Dermatol, 116, (5), 728-730.
- Cascella R, Claudia Strafella, Giuliana Longo, Mara Maccarone, Paola Borgiani, Federica Sangiuolo, Giuseppe Novelli, Emiliano Giardina. Pharmacogenomics of multifactorial diseases: a focus on psoriatic arthritis. (2016) Review
- Chandran V, Bull SB, Pellett FJ et al. (2013) Human leukocyte antigen alleles and susceptibility to psoriatic arthritis. Hum Immunol 74: 1333–8
- Chandran V, Raychaudhuri SP: Geoepidemiology and environmental factors of psoriasis and psoriatic arthritis. J Autoimmun 2010, 34(3):J314-321.
- Chandran V, Schentag CT, Brockbank JE, Pellett FJ, Shanmugarajah S, Toloza SM, Rahman P, Gladman DD. Familial aggregation of psoriatic arthritis. Ann Rheum Dis. 2009 May; 68(5):664-7. doi: 10.1136/ard.2008.089367. Epub 2008 Jun 4.
- Chia NV, Stuart P, Nair RP, Henseler T, Jenisch S, Lim HW, Christophers E, Voorhees JJ, Elder JT: Variations in the HCR (Pg8) gene are unlikely to be causal for familial psoriasis. J Invest Dermatol 2001, 116(5):823-824.
- Christophers E, Barker JN, Griffiths CE, Dauden E, Milligan G, Molta C, Sato R, Boggs R: The risk of psoriatic arthritis remains constant following initial diagnosis of psoriasis among patients seen in European dermatology clinics. J Eur Acad Dermatol Venereol 2010, 24(5):548-554.
- Christophers E: Comorbidities in psoriasis. Clin Dermatol 2007, 25:529-534.
- Clop A, Bertoni A, Spain SL et al. (2013) An in-depth characterization of the major psoriasis susceptibility locus identifies candidate susceptibility alleles within an HLA-C enhancer element. PLoS One 8:e71690

- Clop A, Bertoni A, Spain SL et al. (2013) An in-depth characterization of the major psoriasis susceptibility locus identifies candidate susceptibility alleles within an HLA-C enhancer element. PLoS One 8:e71690
- Cohen MR, Reda DJ, Clegg DO: Baseline relationships between psoriasis and psoriatic arthritis: analysis of 221 patients with active psoriatic arthritis. Department of Veterans Affairs Cooperative Study Group on Seronegative Spondyloarthropathies. J Rheumatol 1999, 26(8):1752-1756.
- Cowden, A. & Voorhees, A. S. (2008) Introduction: History of psoriasis and psoriasis therapy. In:
- D'Alfonso S, Momigliano Richiardi P (1996) An intragenic polymorphism in the human tumor necrosis factor alpha (TNFA) chain encoding gene. Immunogenetics 44:321–2
- Daly AK, Day CP (2001) Candidate gene case-control association studies: advantages and potential pitfalls. Br J Clin Pharmacol 52:489–99
- Danning CL, Illei GG, Hitchon C et al. (2000) Macrophage-derived cytokine and nuclear factor kappaB p65 expression in synovial membrane and skin of patients with psoriatic arthritis. Arthritis Rheum 43:1244–56
- DeWeerdt, S. (2012) Psychodermatology: An emotional response. Nature, 492(7429), S62-S63.
- Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. J Invest Dermatol 129(6):1339-1350,2009.
- Di Meglio P, Nestle FO. The role of IL-23 in the Immunopathogenesis of psoriasis. F1000 Biol Rep2, 2010.
- Dubreuil, M., Rho, Y. H., Man, A., Zhu, Y., Zhang, Y., Love, T. J., Ogdie, A., Gelfand, J. M. & Choi, H. K. (2014) Diabetes incidence in psoriatic arthritis, psoriasis and rheumatoid arthritis: a UK population-based cohort study. Rheumatology, 53(2), 346-352.
- Elder JT, Nair RP, Guo SW, Henseler T, Christophers E, Voorhees JJ. The genetics of psoriasis. Arch Dermatol. 1994; 130:216–24
- Elder JT: PSORS1: linking genetics and immunology. J Invest Dermatol 2006, 126(6):1205-1206.
- Elkayam O, Ophir J, Yaron M, Caspi D: Psoriatic arthritis: interrelationships between skin and joint manifestations related to onset, course and distribution. Clin Rheumatol 2000, 19(4):301-305.
- Enerback C, Martinsson T, Inerot A, Wahlstrom J, Enlund F, Yhr M, Swanbeck G: Evidence that HLA-Cw6 determines early onset of psoriasis, obtained using sequence-specific primers (PCR-SSP). Acta Derm Venereol 1997, 77(4):273-276.

- Enerback C, Nilsson S, Enlund F, Inerot A, Samuelsson L, Wahlstrom J, Swanbeck G, Martinsson T: Stronger association with HLA-Cw6 than with corneodesmosin (S-gene) polymorphisms in Swedish psoriasis patients. Arch Dermatol Res 2000, 292(11):525-530.
- Espinoza LR, Vasey FB, Gaylord SW, Dietz C, Bergen L, Bridgeford P, Germain BF: Histocompatibility typing in the seronegative spondyloarthropathies: a survey. Semin Arthritis Rheum 1982, 11(3):375-381.
- Ettehadi P, Greaves MW, Wallach D et al. Elevated tumour necrosis factor-alpha (TNF-alpha) biological activity in psoriatic skin lesions. Clin Exp Immunol 1994; 96: 145-51
- Fan X, Yang S, Huang W, Wang ZM, Sun LD, Liang YH, Gao M, Ren YQ, Zhang KY, Du WH et al: Fine mapping of the psoriasis susceptibility locus PSORS1 supports HLA-C as the susceptibility gene in the Han Chinese population. PLoS Genet 2008, 4(3):e1000038.
- Farber EM, Nall LM: The natural history of psoriasis in 5600 patients.Dermatologica 1974, 148:1–18.
- Farber, E. & Peterson, J. (1961) Variations in the natural history of psoriasis. Calif Med, 95, 6-11.
- Farkas, Á. & Kemény, L. (2010) Psoriasis and alcohol: is cutaneous ethanol one of the missing links? British Journal of Dermatology, 162(4), 711-716
- Feldman, S. R. and Krueger, G. G. (2005). Psoriasis assessment tools in clinical trials. Ann Rheum Dis, 64, (suppl 2), ii65-ii68.
- Feng BJ, Sun LD, Soltani-Arabshahi R, Bowcock AM, Nair RP, Stuart P, Elder JT, Schrodi SJ, Begovich AB, Abecasis GR et al: Multiple Loci within the major histocompatibility complex confer risk of psoriasis. PLoS Genet 2009, 5(8):e1000606.
- Finlay AY, Coles EC: The effect of severe psoriasis on the quality of life of 369 patients.Br J Dermatol 1995, 132:236–244.
- Finlay AY, Kelly SE: Psoriasis—an index of disability. Clin Exp Dermatol 1987, 12:8–11.
- Finlay, A. Y. and Khan, G. K. (1994). Dermatology Life Quality Index (DLQI) - a simple practical measure for routine clinical use. Clin Exp Dermatol, 19, (3), 210-216.
- Fredriksson, T. and Pettersson, U. (1978). Severe psoriasis - oral therapy with a new retinoid. Dermatologica, 157, (4), 238-244.
- Gazit E, Brenner S, Efter T, Orgad S, Mizrachi Y, Krakowski A: HLA antigens in patients with psoriasis. Tissue Antigens 1978, 12(3):195-199.

- Gelfand JM, Gladman DD, Mease PJ, Smith N, Margolis DJ, Nijsten T, Stern RS, Feldman SR, Rolstad T: Epidemiology of psoriatic arthritis in the population of the United States. J Am Acad Dermatol 2005, 53(4):573.
- Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB: Risk of myocardial infarction in patients with psoriasis. . JAMA 2006, 296:1735-1741.
- Gerber LH, Murray CL, Perlman SG, Barth WF, Decker JL, Nigra TA, Mann DL: Human lymphocyte antigens characterizing psoriatic arthritis and its subtypes. J Rheumatol 1982, 9(5):703-707.
- Giardina E, F. Capon, MC. De Rosa, R. Mango, G. Zambruno, A. Orecchia, S. Chimenti, B. Giardina and G. Novelli. Characterization of the loricrin (LOR) gene as a positional candidate for the PSORS4 psoriasis susceptibility locus. Annals of Human Genetics (2004) 68,639–645.
- Giardina E, Ulrike Hu¨ffmeier, Jaya Ravindran, Frank Behrens, Tiziana Lepre, Neil J. McHugh, Eleanor Korendowych, Harald Burkhardt, Giuseppe Novelli, and Andre´ Reis. Tumor Necrosis Factor Promoter Polymorphism TNF*-857 Is a Risk Allele for Psoriatic Arthritis Independent of the PSORS1 Locus. ARTHRITIS & RHEUMATISM Vol. 63, No. 12, December 2011, pp 3801–3806.
- Gisondi, P., Tessari, G., Conti, A., Piaserico, S., Schianchi, S., Peserico, A., Giannetti, A. & Girolomoni, G. (2007) Prevalence of metabolic syndrome in patients with psoriasis: a hospital-based case–control study. British Journal of Dermatology, 157(1), 68-73.
- Gladman D (ed.): Psoriatic arthritis. Berlin: Springer-Verlag; 2005
- Gladman DD, Ang M, Su L, Tom BD, Schentag CT, Farewell VT: Cardiovascular morbidity in psoriatic arthritis. Ann Rheum Dis 2009, 68(7):1131-1135.
- Gladman DD, Anhorn KA, Schachter RK, Mervart H: HLA antigens in psoriatic arthritis. J Rheumatol 1986, 13(3):586-592.
- Gladman DD, Farewell VT, Pellett F, Schentag C, Rahman P: HLA is a candidate region for psoriatic arthritis. evidence for excessive HLA sharing in sibling pairs. Hum Immunol 2003, 64(9):887-889.
- Gladman DD, Hing EN, Schentag CT, Cook RJ: Remission in psoriatic arthritis. J Rheumatol 2001, 28(5):1045-1048
- Gladman DD, Shuckett R, Russell ML, Thorne JC, Schachter RK: Psoriatic arthritis (PSA)--an analysis of 220 patients. Q J Med 1987, 62(238):127-141.
- Gladman DD: Natural history of psoriatic arthritis. Baillieres Clin Rheumatol 1994, 8(2):379-394.
- Gladman DD: Psoriatic arthritis: epidemiology, clinical features, course, and outcome. Annals of the Rheumatic Diseases 2005, 64(suppl_2):ii14-ii17.

- Gonzalez S, Brautbar C, Martinez-Borra J et al. (2001) Polymorphism in MICA rather than HLA-B/C genes is associated with psoriatic arthritis in the Jewish population. Hum Immunol 62:632–8

- Gonzalez S, Brautbar C, Martinez-Borra J et al. (2001) Polymorphism in MICA rather than HLA-B/C genes is associated with psoriatic arthritis in the Jewish population. Hum Immunol 62:632–8

- Gonzalez S, Martinez-Borra J, Lopez-Vazquez A et al. (2002) MICA rather than MICB, TNFa, or HLADRB1 is associated with susceptibility to psoriatic arthritis. J Rheumatol 29:973–8

- Gonzalez S, Martinez-Borra J, Lopez-Vazquez A et al. (2002) MICA rather than MICB, TNFa, or HLADRB1 is associated with susceptibility to psoriatic arthritis. J Rheumatol 29:973–8

- Gottlieb AB, Dann F, Menter A: Psoriasis and the metabolic syndrome.J DrugsDermatol 2008, 7:563-572.
- Gottlieb, A. B., Chao, C. & Dann, F. (2008) Psoriasis comorbidities. Journal of Dermatological Treatment, 19(1), 5-21.
- Griffiths CE, Barker JN: Pathogenesis and clinical features of psoriasis.Lancet 2007, 370:263-271.
- Griffiths CE, Christophers E, Barker JN, Chalmers RJ, Chimenti S, Krueger GG, Leonardi C, Menter A, Ortonne JP, Fry L: A classification of psoriasis vulgaris according to phenotype. Br J Dermatol 2007, 156(2):258-262.
- Griffiths, C. E. M., Christophers, E., Barker, J. N. W. N., Chalmers, R. J. G., Chimenti, S.
- Grjibovski AM, Olsen AO, Magnus P, Harris JR. Psoriasis in Norwegian twins: contribution of genetic and environmental effects. J Eur Acad Dermatol Venereol. 2007 Nov; 21(10):1337-43
- Gudjonsson JE, Elder JT. Psoriasis: epidemiology. Clin Dermatol. 2007 Nov-Dec;25(6):535-46.
- Gudjonsson JE, Karason A, Antonsdottir AA, Runarsdottir EH, Gulcher JR, Stefansson K, Valdimarsson H: HLA-Cw6-positive and HLA-Cw6-negative patients with Psoriasis vulgaris have distinct clinical features. J Invest Dermatol 2002, 118(2):362-365.
- Gudjonsson, J. E. & Elder, J. T. (2007) Psoriasis: epidemiology. Clinics in Dermatology, 25(6), 535-546.
- Healy PJ, Helliwell PS: Classification of the spondyloarthropathies. Curr Opin Rheumatol 2005, 17(4):395-399.

- Heller, M. M., Lee, E. S. & Koo, J. Y. (2011) Stress as an influencing factor in psoriasis. Skin Therapy Lett., 16(5), 1-4.
- Helliwell PS, Porter G, Taylor WJ: Polyarticular psoriatic arthritis is more like oligoarticular psoriatic arthritis, than rheumatoid arthritis. Ann Rheum Dis 2007, 66(1):113-117.
- Henseler T, Christophers E: Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris.J Am Acad Dermatol 1985, 13:450-456.
- Ho PY, Barton A, Worthington J, Plant D, Griffiths CE, Young HS, Bradburn P,
- Hohler T, Grossmann S, Stradmann-Bellinghausen B, et al.: Differential association of polymorphisms in the TNFalpha region with psoriatic arthritis but not psoriasis. Ann Rheum Dis 2002, 61:213–218.
- Hohler T, Kruger A, Schneider PM, et al.: A TNF-alpha promoter polymorphism is associated with juvenile onset psoriasis and psoriatic arthritis. J Invest Dermatol 1997, 109:562–565.
- Huffmeier U and Mossner R (2014) Complex Role of TNF Variants in Psoriatic arthritis and Treatment Response to Anti-TNF Therapy: Evidence and Concepts. The Society for Inv. Dermat.
- Huizinga TW, Pisetsky DS, Kimberly RP (2004) Associations, populations, and the truth: recommendations for genetic association studies in arthritis and rheumatism. Arthritis Rheum 50:2066–71 Ioannidis JP, Ntzani EE, Trikalinos TA et al. (2001) Replication validity of genetic association studies. Nat Genet 29:306–9
- Huizinga TW, Pisetsky DS, Kimberly RP (2004) Associations, populations, and the truth: recommendations for genetic association studies in arthritis and rheumatism. Arthritis Rheum 50:2066–71
- Huynh, M., Gupta, R. & Koo, J. Y. (2013) Emotional stress as a trigger for inflammatory skin disorders. Seminars in cutaneous medicine and surgery, 32(2), 68-72.
- Ibrahim G, Waxman R, Helliwell PS. The prevalence of psoriatic arthritis in people with psoriasis. Arthritis Rheum 61(10):1373-1378, 2009.
- Icen, M., Crowson, C. S., McEvoy, M. T., Dann, F. J., Gabriel, S. E. & Maradit Kremers, H. (2009a) Trends in incidence of adult-onset psoriasis over three decades: A population-based study. Journal of the American Academy of Dermatology, 60(3), 394-401.
- Icen, M., Crowson, C. S., T. McEvoy, M., J. Dan, F., E. Gabriel, S. & Kremers, H. M. (2009b) Trends in incidence of adult-onset psoriasis over three decades: A population-based study. Journal of the American Academy of Dermatology, 60(3), 394-401.

- Ioannidis JP, Ntzani EE, Trikalinos TA et al. (2001) Replication validity of genetic association studies. Nat Genet 29:306–9
- J.A.Traherne, “HumanMHCarchitecture and evolution: implications for disease association studies,” International Journal of Immunogenetics, vol. 35, no. 3, pp. 179–192, 2008.
- Jacobson, C. C. and Kimball, A. B. (2004). Rethinking the Psoriasis Area and Severity Index: the impact of area should be increased. Br J Dermatol, 151, (2), 381-387.
- Jenisch S, Henseler T, Nair RP, Guo SW, Westphal E, Stuart P, Kronke M, Voorhees JJ, Christophers E, Elder JT: Linkage analysis of human leukocyte antigen (HLA) markers in familial psoriasis: strong disequilibrium effects provide evidence for a major determinant in the HLA-B/-C region. Am J Hum Genet 1998, 63(1):191-199.
- Kang CP, Lee KW, Yoo DH, Kang C, Bae SC. The influence of a polymorphism at position 7 of the tumour necrosis factor gene on clinical response to etanercept therapy in rheumatoid arthritis. Rheumatology (Oxford) 2005;44:547–52.
- Karason A, Love TJ, Gudbjornsson B. A strong heritability of psoriatic arthritis over four generations--the Reykjavik Psoriatic Arthritis Study. Rheumatology (Oxford). 2009 Nov;48(11):1424-8. doi: 10.1093/rheumatology/kep243. Epub 2009 Sep 9.
- Karimi M, Goldie LC, Cruickshank MN, Moses EK, Abraham LJ. A critical assessment of the factors affecting reporter gene assays for promoter SNP function: a reassessment of -308 TNF polymorphism function using a novel integrated reporter system, Eur J Hum Genet, 2009, vol. 17 (pg. 1454-62)
- Kavli, G., Førde, O. H., Arnesen, E. & Stenvold, S. E. (1985) Psoriasis: familial predisposition and environmental factors. British Medical Journal (Clinical research ed.), 291(6501), 999-1000.
- Kimball, A. B., Robinson, J., Wu, Y., Guzzo, C., Yeilding, N., Paramore, C. et al. (2008). Cardiovascular disease and risk factors among psoriasis patients in two US healthcare databases, 2001-2002. Dermatology, 217, (1), 27-37.
- Kirby, B., Richards, H. L., Mason, D. L., Fortune, D. G., Main, C. J. & Griffiths, C. E. M. (2008) Alcohol consumption and psychological distress in patients with psoriasis. British Journal of Dermatology, 158(1), 138-140.
- Koo J, Population-based epidemiologic study of psoriasis with emphasis on quality of life assessment. Dermatol Clin, 14 (1996), pp. 485-496
- Krueger, G. G., Leonardi, C., Menter, A., Ortonne, J. P. & Fry, L. (2007b) A classification of psoriasis vulgaris according to phenotype. British Journal of Dermatology, 156(2), 258-262.

- Langley RG, Krueger GG, Griffiths CE: Psoriasis: epidemiology, clinical features, and quality of life. Ann Rheum Dis 2005, 64 Suppl 2:ii18-23; discussion ii24-15.
- Lee YH, Ji JD, Bae SC et al. (2010) Associations between tumor necrosis factor alpha (TNF-alpha) -308 and -238 G/A polymorphisms and shared epitope status and responsiveness to TNF-alpha blockers in rheumatoid arthritis: a metaanalysis update. J Rheumatol 37:740–6
- Lonnberg A.S, et al. Heritability of psoriasis in a large twin sample Br J Dermatol. 2013 Aug;169(2):412-6. doi: 10.1111/bjd.12375.
- Lønnberg AS, Skov L, Duffy DL, Skytthe A, Kyvik KO, Pedersen OB, Thomsen SF. Genetic Factors Explain Variation in the Age at Onset of Psoriasis: A Population-based Twin Study. Acta Derm Venereol. 2016 Jan; 96(1):35-8. doi: 10.2340/00015555-2171.
- Louden, B. A., Pearce, D. J., Lang, W., and Feldman, S. R. (2004). A Simplified Psoriasis Area Severity Index (SPASI) for rating psoriasis severity in clinic patients. Dermatol Online J, 10, (2), 7.
- Louis E, Franchimont D, Piron A, et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans, Clin Exp Immunol , 1998, vol. 113 (pg. 401-6)
- Lowels MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, Bowman EP, Krueger JG. Psoriasi vulgaris lesions contain discrete populations of Th1 and Th17 cells. J Invest Dermatol 128(5):1207-1211,2008
- Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasi. Annu Rev Immunol 32:227-255, 2014
- Lv K, Chen R, Cai Q, Fang M, Sun S. Effects of a single nucleotide polymorphism on the expression of human tumour necrosis factor-α. Scand J Immunol 2006;64:164–9.
- Mahil MA, Capon Francesca, Barker J.N. Genetics of Psoriasis. Dermatol Clin. 2015 Jan;33(1):1-11. doi: 10.1016/j.det.2014.09.001.
- Mallon, E., Young, D., Bunce, M., Gotch, F. M., Easterbrook, P. J., Newson, R. & Bunker, C. B. (1998) HLA-Cw*0602 and HIV-associated psoriasis. Br J Dermatol, 139, 527-533.
- Marcusson JA, Johannesson A, Moller E: HLA-A,B,C and DR antigens in psoriasis. Tissue Antigens 1981, 17(5):525-529.
- McGonagle D, Khan MA, Marzo-Ortega H, O'Connor P, Gibbon W, Emery P: Enthesitis in spondyloarthropathy. Curr Opin Rheumatol 1999, 11(4):244-250.

- McHugh NJ, Laurent MR, Treadwell BL, Tweed JM, Dagger J: Psoriatic arthritis: clinical subgroups and histocompatibility antigens. Ann Rheum Dis 1987, 46(3):184-188.
- McKendry RJ, Sengar DP, DesGroseilliers JP, Dunne JV: Frequency of HLA antigens in patients with psoriasis or psoriatic arthritis. Can Med Assoc J 1984, 130(4):411-415.
- Menter A, Korman NJ, Elmets CA, Feldman SR, Gelfand JM, Gordon KB, Gottlieb A, Koo JY, Lebwohl M, Lim HW et al: Guidelines of care for the management of psoriasis and psoriatic arthritis: Section 5. Guidelines of care for the treatment of psoriasis with phototherapy and photochemotherapy. J Am Acad Dermatol 2010, 62(1):114-135.
- Moll JM, Wright V. Familial occurrence of psoriatic arthritis. Ann Rheum Dis. 1973 May;32(3):181-201.
- Mullighan CG, Fanning GC, Chapel HM et al. (1997) TNF and lymphotoxinalpha polymorphisms associated with common variable immunodeficiency: role in the pathogenesis of granulomatous disease. J Immunol 159:6236–41
- Murdaca G, Gulli R, Spano F et al. (2014) TNF-a gene polymorphisms: association with disease susceptibility and response to anti-TNF-a treatment in psoriatic arthritis. J Invest Dermatol 134:2503–9
- Murdaca G, Negrini, Magnani, Penza, Pellecchio, Puppo. Impact of pharmacogenomics upon the therapeutic response to etanercept in psoriasis and psoriatic arthritis. Expert Opin Drug Saf. 2017 Oct;16(10):1173-1179. doi: 10.1080/14740338.2017.1361404. Epub 2017 Aug 4.
- Myers A, Kay LJ, Lynch SA, Walker DJ. Recurrence risk for psoriasis and psoriatic arthritis within sibships. Rheumatology (Oxford). 2005 Jun;44(6):773-6. Epub 2005 Mar 9.
- Nair RP, Stuart PE, Nistor I, Hiremagalore R, Chia NV, Jenisch S, Weichenthal M, Abecasis GR, Lim HW, Christophers E, Voorhees JJ, Elder JT. Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. Am J Hum Genet. 2006 May;78(5):827-51. Epub 2006 Mar 31
- Naldi, L., Parazzini, F., Brevi, A., Peserico, A., Fornasa, C. V., Grosso, G., Rossi, E., Marinaro, P., Polenghi, M. M., Finzi, A., Galbiati, G., Recchia, G., Cristofolini, M., Schena, D. & Cainelli, T. (1992) Family history, smoking habits, alcohol consumption and risk of psoriasis. British Journal of Dermatology, 127(3), 212-217.
- Neimann, A. L., Porter, S. B. & Gelfand, J. M. (2006) Epidemiology of psoriasis. Expert Review of Dermatology, 1(1), 63-75.

- Nestle FO, Kaplan DH, Barker J, Psoriasis. Nengl J Med 361(5):496-509, 2009.
- Nijsten, T. (2012). Dermatology life quality index: time to move forward. J Invest Dermatol, 132, (1), 11-13.
- Okada Y, Han B, Tsoi LC, Stuart PE, Ellinghaus E, Tejasvi T, Chandran V, Pellett F, Pollock R, Bowcock AM, Krueger GG, Weichenthal M, Voorhees JJ, Rahman P, Gregersen PK, Franke A, Nair RP, Abecasis GR, Gladman DD, Elder JT, de Bakker PI, Raychaudhuri S. Fine mapping major histocompatibility complex associations in psoriasis and its clinical subtypes. Am J Hum Genet. 2014 Aug 7;95(2):162-72. doi: 10.1016/j.ajhg.2014.07.002. Epub 2014 Jul 31.
- Ortonne, J. P. (1999) Recent developments in the understanding of the pathogenesis of psoriasis. British Journal of Dermatology, 140(S54), 1-7.
- Parisi, R., Symmons, D. P. M., Griffiths, C. E. M. & Ashcroft, D. M. (2012) Global Epidemiology of Psoriasis: A Systematic Review of Incidence and Prevalence. J Invest Dermatol.
- Parisi, R., Symmons, D. P. M., Griffiths, C. E. M. & Ashcroft, D. M. (2013) Global Epidemiology of Psoriasis: A Systematic Review of Incidence and Prevalence. J Invest Dermatol, 133(2), 377-385.
- Partsch G, Steiner G, Leeb BF et al. (1997) Highly increased levels of tumor necrosis factor-alpha and other proinflammatory cytokines in psoriatic arthritis synovial fluid. J Rheumatol 24:518–23
- Prinz. (1999) Which T cells cause psoriasis? Clinical and Experimental Dermatology, 24(4), 291-295.
- Rahman P and Elder J.T. Genetic epidemiology of psoriasis and psoriatic arthritis. Ann Rheum Dis 2005; 64 (Suppl II):ii37–ii39. doi: 10.1136/ard.2004.030775
- Rahman P, Gladman DD, Schentag C, Petronis A. Excessive paternal transmission in psoriatic arthritis. Arthritis Rheum. 1999; 42:1228–31.
- Rahman P, Nguyen E, Cheung C, Schentag CT, Gladman DD: Comparison of radiological severity in psoriatic arthritis and rheumatoid arthritis. J Rheumatol 2001, 28(5):1041-1044.
- Rahman P, Siannis F, Butt C et al. (2006) TNFa polymorphisms and risk of psoriatic arthritis. Ann Rheum Dis 65:919–23
- Raychaudhuri, S. P. & Farber, E. M. (2001) The prevalence of psoriasis in the world. Journal of the European Academy of Dermatology and Venereology, 15(1), 16-17.
- Reich K, Huffmeier U, Konig IR, Lascorz J, Lohmann J, et al. (2007) TNF polymorphisms in psoriasis: association of psoriatic arthritis with the promoter

polymorphism TNF*-857 independent of the PSORS1 risk allele. Arthritis Rheum 56: 2056–2064.

- Ritchlin CT, Kavanaugh A, Gladman DD, Mease PJ, Helliwell P, Boehncke WH, de Vlam K, Fiorentino D, Fitzgerald O, Gottlieb AB et al: Treatment recommendations for psoriatic arthritis. Ann Rheum Dis 2009, 68(9):1387-1394.
- Ritchlin CT: Pathogenesis of psoriatic arthritis. Curr Opin Rheumatol 2005, 17(4):406-412.
- Riveira-Munoz, E., He, S.-M., Escaramis, G., Stuart, P. E., Huffmeier, U., Lee, C., Kirby, B., Oka, A., Giardina, E., Liao, W., Bergboer, J., Kainu, K., de Cid, R., Munkhbat, B., Zeeuwen, P. L. J. M., Armour, J. A. L., Poon, A., Mabuchi, T., Ozawa, A., Zawirska, A., Burden, A. D., Barker, J. N., Capon, F., Traupe, H., Sun, L.-D., Cui, Y., Yin, X.-Y., Chen, G., Lim, H. W., Nair, R. P., Voorhees, J. J., Tejasvi, T., Pujol, R., Munkhtuvshin, N., Fischer, J., Kere, J., Schalkwijk, J., Bowcock, A., Kwok, P.-Y., Novelli, G., Inoko, H., Ryan, A. W., Trembath, R. C., Reis, A., Zhang, X.-J., Elder, J. T. & Estivill, X. (2011) Meta-Analysis Confirms the LCE3C_LCE3B Deletion as a Risk Factor for Psoriasis in Several Ethnic Groups and Finds Interaction with HLA-Cw6. J Invest Dermatol, 131(5), 1105-1109.
- Russell TJ, Schultes LM, Kuban DJ: Histocompatibility (HL-A) antigens associated with psoriasis. N Engl J Med 1972, 287(15):738-740.
- Scarpa R, Oriente P, Pucino A, Torella M, Vignone L, Riccio A, Biondi Oriente C: Psoriatic arthritis in psoriatic patients. Br J Rheumatol 1984, 23(4):246-250.
- Schmitt-Egenolf M, Eiermann TH, Boehncke WH, Stander M, Sterry W: Familial juvenile onset psoriasis is associated with the human leukocyte antigen (HLA) class I side of the extended haplotype Cw6-B57-DRB1*0701-DQA1*0201-DQB1*0303: a population- and family-based study. J Invest Dermatol 1996,106(4):711-714.
- Schmitt-Egenolf M, Eiermann TH, Boehncke WH, Stander M, Sterry W: Familial juvenile onset psoriasis is associated with the human leukocyte antigen (HLA) class I side of the extended haplotype Cw6-B57-DRB1*0701-DQA1*0201-DQB1*0303: a population- and family-based study. J Invest Dermatol 1996, 106(4):711-714.
- Seitz M, Wirthmu¨ ller U, Mo¨ ller B et al. (2007) The -308 tumour necrosis factor alpha gene polymorphism predicts therapeutic response to TNF alphablockers in rheumatoid arthritis and spondyloarthritis patients. Rheumatology (Oxford) 46:93–6
- Setty Ar, C. G. C. H. K. (2007) Obesity, waist circumference, weight change, and the risk of psoriasis in women: Nurses's health study II. Archives of Internal Medicine, 167(15), 1670-1675.

- Setty, A., Curhan, G. & Choi, H. (2007) Obesity, waist circumference, weight change, and the risk of psoriasis in women: Nurses' health study II. Archives of Internal Medicine, 167(15), 1670-1675.
- Shapiro, J., Cohen, A. D., David, M., Hodak, E., Chodik, G., Viner, A., Kremer, E. & Heymann, A. (2007) The association between psoriasis, diabetes mellitus, and atherosclerosis in Israel: A case-control study. Journal of the American Academy of Dermatology, 56(4), 629-634.
- Siannis F, Farewell VT, Cook RJ, Schentag CT, Gladman DD: Clinical and radiological damage in psoriatic arthritis. Ann Rheum Dis 2006, 65(4):478-481.
- Sonnex, T. S., Carrington, P., Norris, P. & Greaves, M. W. (1988) Polymorphonuclear leukocyte random migration and chemotaxis in psoriatic and healthy adult smokers and non-smokers. British Journal of Dermatology, 119(5), 653-659.
- Soriano ER, Rosa J, Velozo E, Schpilberg M, Imamura PM, Diaz J, Catoggio LJ: Incidence and prevalence of psoriatic arthritis in Buenos Aires, Argentina: a 6-year health management organization-based study. Rheumatology (Oxford) 2010.
- Swanbeck G, Inerot A, Martinsson T, Wahlstrom JA. Population genetic study of of psoriasis. Br JDermatol. 1994; 131:32–39.
- Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H: Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 2006, 54(8):2665-2673
- Tazi Ahnini R, Camp NJ, Cork MJ, Mee JB, Keohane SG, Duff GW, di Giovine FS: Novel genetic association between the corneodesmosin (MHC S) gene and susceptibility to psoriasis. Hum Mol Genet 1999, 8(6):1135-1140.
- Tey HL, Ee HL, Tan AS, Theng TS, Wong SN, Khoo SW: Risk factors associated with having psoriatic arthritis in patients with cutaneous psoriasis. J Dermatol 2010, 37(5):426-430.
- Thaci D, Humeniuk J, Frambach Y, Bissonnette R, Goodman JJ, Shevade S, Gong Y, Papavassilis C; STATURE Study Group. Secukinumab in psoriasis: randomized, controlled phase 3 trial results assessing the potential to improve treatment response in partial responders (STATURE). Br J Dermatol 173(3):777-787, 2015
- The International Psoriasis Genetics Consortium. The International Psoriasis Genetics Study: assessing linkage to 14 candidate susceptibility loci in a cohort of 942 affected sib pairs. Am. J. Hum. Genet., 2003, 73: 430-437.

- Thomson W, Silman AJ, Bruce IN: Investigating the role of the HLA-Cw*06 and HLA-DRB1 genes in susceptibility to psoriatic arthritis: comparison with psoriasis and undifferentiated inflammatory arthritis. Ann Rheum Dis 2008, 67(5):677-682.
- Tiilikainen A, Lassus A, Karvonen J, Vartiainen P, Julin M: Psoriasis and HLACw6. Br J Dermatol 1980, 102(2):179-184.
- Tiwari JL, Lowe NJ, Abramovits W, Hawkins BR, Park MS: Association of psoriasis with HLA-DR7. Br J Dermatol 1982, 106(2):227-230.
- Tollefson, M. M., Crowson, C. S., McEvoy, M. T. & Maradit Kremers, H. (2010) Incidence of psoriasis in children: A population-based study. Journal of the American Academy of Dermatology, 62(6), 979-987.
- Torre Alonso JC, Rodriguez Perez A, Arribas Castrillo JM, Ballina Garcia J, Riestra Noriega JL, Lopez Larrea C: Psoriatic arthritis (PA): a clinical, immunological and radiological study of 180 patients. Br J Rheumatol 1991, 30(4):245-250.
- Traherne JA (2008) Human MHC architecture and evolution: implications for disease association studies. Int J Immunogenet 35: 179–192.JA Traherne2008Human MHC architecture and evolution: implications for disease association studies.Int J Immunogenet35179192
- Tsoi LC, Spain SL, Ellinghaus E, Stuart PE, Capon F, Knight J, Tejasvi T, Kang HM, Allen MH, Lambert S, Stoll SW, Weidinger S, Gudjonsson JE, Koks S, Kingo K, Esko T, Das S, Metspalu A, Weichenthal M, Enerback C, Krueger GG, Voorhees JJ, Chandran V, Rosen CF, Rahman P, Gladman DD, Reis A, Nair RP, Franke A, Barker JN, Abecasis GR, Trembath RC, Elder JT. Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. Nat Commun. 2015 May 5; 6:7001. doi: 10.1038/ncomms8001.
- Valdimarsson, H., Bake, B. S., Jónsdótdr, I. & Fry, L. (1986) Psoriasis: a disease of abnormal Keratinocyte proliferation induced by T lymphocytes. Immunology Today, 7(9), 256-259.
- van Krugten MV, Huizinga TW, Kaijzel EL et al. (1999) Association of the TNF +489 polymorphism with susceptibility and radiographic damage in rheumatoid arthritis. Genes Immun 1:91–6
- Veal CD, Capon F, Allen MH, Heath EK, Evans JC, Jones A, Patel S, Burden D, Tillman D, Barker JN, Trembath RC. Family-based analysis using a dense single-nucleotide polymorphism-based map defines genetic variation at PSORS1, the major psoriasis-susceptibility locus. Am J Hum Genet. 2002 Sep;71(3):554-64. Epub 2002 Jul 29.

- Vena, G., Altomare, G., Ayala, F. & al., e. (2010) Incidence of psoriasis and association with comorbidities in Italy: a 5-year observational study from a national primary care database. Eur J Dermatol 20, 593-598.
- Weinberg, J. M. (ed.) Treatment of Psoriasis. Birkhäuser Basel
- Wilson FC, Icen M, Crowson CS, McEvoy MT, Gabriel SE, Kremers HM: Incidence and clinical predictors of psoriatic arthritis in patients with psoriasis: a population-based study. Arthritis Rheum 2009, 61(2):233-239.
- Wong K, Gladman DD, Husted J, Long JA, Farewell VT: Mortality studies in psoriatic arthritis: results from a single outpatient clinic. I. Causes and risk of death. Arthritis Rheum 1997, 40(10):1868-1872.
- Wright V, Moll JMH (eds.): Psoriatic arthritis. Amsterdam: North Holland Publishing Co; 1976.
- Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, Abello MV, Novitskaya I, Pierson KC, Gonzalez J, Krueger JG, Lowes MA. Psoriasis in Characterized by accumulation of immunostimolatory and Th1/Th17 cell-polarizing myeloid dendritic cells. J Invest Dermatol 129(1):79-88,2009.
- Zachariae H: Prevalence of joint disease in patients with psoriasis: implications for therapy. Am J Clin Dermatol 2003, 4(7):441-447
- Zhang XJ, Huang W, Yang S, Sun LD, Zhang FY, Zhu QX, Zhang FR, Zhang C, Du WH, Pu XM et al: Psoriasis genome-wide association study identifies susceptibility variants within LCE gene cluster at 1q21. Nat Genet 2009, 41(2):205-210.
- Zhu, K.-J., Zhu, C.-Y. & Fan, Y.-M. (2012) Alcohol consumption and psoriatic risk: A meta-analysis of case–control studies. The Journal of Dermatology, 39(9), 770-773.