Functional genomics of psoriasis

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A thesis submitted in partial
fulfilment of the requirements for the degree of
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Abstract

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This is my abstract...

Acknowledgements

Thank you, thank you, thank you.

Declarations

I declare that unless otherwise stated, all work presented in this thesis is my own. Several aspects of each project relied upon collaboration where part of the work was conducted by others.

Submitted Abstracts

Title **Year**
Authors

Associated Publications

Title
Journal
Authors

Other Publications

Title
Journal
Authors

Contents

List of Figures

List of Tables

Abbreviations

Abbreviation	Definition
Ab	Antibody
ATAC-seq	
Atopic dermatitis	AD
ChIPm	
CLE	cutaneous lupus erythematosus
DMARDs	disease-modifying antirheumatic drugs
Fast-ATAC	
GWAS	Genome-wide association studies
KC	Keratinocytes
NSAID	nonsteroidal antiinflammatory drug
Omni-ATAC	
PI	Protein inhibitor
PsA	
QC	
qPCR	quantitative polymerase chain reaction
RA	Rheumatoid arthritis
SDS	Sodium dodecyl sulfate
SF	Synovial fluid

Chapter 1

Introduction

1.1 Psoriasis and psoriatic arthritis

Psoriasis and psoriatic arthritis (PsA) have been progressively identified as two different common complex disease entities. Psoriasis is a chronic inflammatory dermatose disease with episodes of relapse and remitance (Nestle et al. 2009). On the other hand, PsA is a seronegative chronic inflammatory disease within the family of spondyloarthritis (Moll et al. 1973; Coates et al. 2016) that usually develops after the psoriasis skin manifestations(**Villanova2016**). Psoriasis and PsA have shared and distinct clinical features, which are likely a reflection of the commonalities and differences in genetic loci contributing to disease development. It is important to understand those commonalities and differences at the physiological and genetic level in order to better understand the relevance of the genetic variability in the risk to develop psoriasis and PsA.

1.1.1 Epidemiology and global impact

Psoriasis represents a serious global health problem that currently affects about 100 million people worldwide, including children and adults with no sex bias (Organization 2016). Although there is a very weak correlation with geographic latitude (Jacobson et al. 2011), it has been reported to vary upon ethnicity. For example, psoriasis prevalence in adults is lower among African,

African American and Asian (0.4-0.7%) compared to American and Canadian (4.6 and 4.7%, respectively) populations. In the UK, psoriasis prevalence ranges between 2-3% and it affects approximately 1.8 million people (Perera et al. 2012).

PsA prevalence in the general population ranges between 0.04-1.2% (Perera et al. 2012)but it dramatically increases to 10-30% within psoriasis cases (**Reich2008**; Gelfand et al. 2005) and evidences the association between the two diseases. Particularly, in the UK, 14% of the psoriasis patients develop chronic inflammatory arthritis in the form of PsA at some point of the disease course (Ibrahim et al. 2009).

Although psoriasis can be developed at any age, onset of disease seems to have a bimodal distribution strongly influenced by the Human Leukocyte Antigen (HLA) Cw*06:02 (HLA-Cw6:02), an allele for one of the genes in the Major Histocompatibility Complex (MHC), involved in antigen presentation (Henseler and Christophers 1985) and the strongest genetic association with psoriasis and PsA risk (()Ellinghaus2010, Strange2010, Stuart2010; Sun2010). The early-onset or Type I is characterised by development of disease around 16-22 and 30-39 years and a prevalence for HLA-C*06:02 (85.4% of the cases). In contrasts, the late-onset or Type II group manifests disease between 50-60 years old and presents positive HLA-C*06:02 only in 14.6% of the cases.

Psoriasis and PsA also represent an economical burden for the countries' economies due to treatment and associated morbidity. For example, in the UK treatment and management of psoriasis in 2015 ranged between 4,000 to 14,000, before and after requirements of biological therapy, respectively (Burgos-Pol and Dermo 2016) and the costs are even greater for PsA (Poole et al. 2010).

1.1.2 Psoriasis and inflammatory dermatoses

The group of inflammatory dermatoses affects up to 70% of the population, regardless age and geographic location (**ICD-10**), and it represents the 4^{th} leading

cause of nonfatal burden (**Roderick2014**). The skin is the biggest organ in the human body constituting an effective barrier between the environment and the internal organs. The most external layer, the epidermis, plays a relevant role in the innate and adaptive immunity (Proksch et al. 2008) and its alterations due to exogenous or endogenous factors can lead to development of inflammatory dermatose conditions, such as psoriasis, atopic dermatitis (AD) or cutaneous lupus erythematosus (CLE) (**Johnson-Huang**; **2009**). Lesions in psoriasis can be non-pustular and pustular which reflects the heterogeneity in the type, location and severity of the disease and impairs the clinical classification (Perera et al. 2012). As a result, several phenotypes of psoriasis including vulgaris, guttate, pustular, erythroderma and nail pitting have been defined and it is under debate whether some of those should be considered a different disease entity (**Marrakchi2011**).

1.1.3 PsA and spondyloarthropaties

PsA belong to the family known as spondylarthropaties (SpA) which also includes other subtypes such as ankylosing spondylitis (AS), reactive arthritis (ReA), idiopathic inflammatory bowel disease (IBD) and undifferentiated SpA (**Baeten2013**). All SpA subtypes are characterised by structural damage (bone formation and erosion) as well as inflammation of joints and extraarticular sites such as eyes, gut and skin. Additional SpA criteria have led to a reduced classification of SpA into axial and peripheral SpA based on the affected joint (spine/sacroilicac or peripheral) and the presence of extraarticular features (**Runwaleit2001**; **Runwaleit2001**). Studies in human families and rat models with HLA-B27 positive status have shown manifestation of different SpA forms, such as psoriasis and IBD, within a single family or individual (**Hammer1990**; **Said-Nahal2000 "parencite**). These observations support the hypothesis that SpA subtypes may be a single multifaceted condition with shared

genetic, immunophatological and structural features and dynamic phenotypes (**Baeten2013**). Conversely, some studies suggest that multiple genetic factors may be involved in the determination of the axial and peripheral arthritis and partially explain the immunopathological differences between the two (**Porcher2005**; **Appel2011**; **Noordenbos2012**).

As a phenotype, PsA can be further subdivided in five clinical groups based on Moll and Wright criteria: distal, destructive, symmetric, asymmetric and spinal (Moll et al. 1973). These subclasses mainly differed upon the location, number and distribution of the affected joints. Later studies have questioned this method of classification due overlapping of the different subsets and lack of inclusion of dactylitis (diffuse swelling of a digit) a distinctive feature of PsA (Reich et al. 2009). This phenotypic heterogeneity increases the difficulty in the design and achievement of meaningful outcomes from clinical studies.

1.2 Pathophysiology of psoriasis and psoriatic arthritis

1.2.1 Clinical presentation and diagnosis

Approximately 90% of all psoriasis cases are plaque psoriasis vulgaris that manifests with raising well demarcated plaques, erythema and scaling. The thickening (acanthosis) and vascularisation of the epidermis leads to the plaques formation (Perera et al. 2012) that can vary in size and distribution, being the most common the elbows, knees and scalp (Griffiths and Lancet 2007). The second most common type is psoriasis guttate (10% of all cases) characterised by acute onset of small droplike papules usually in the trunk and proximal extremities (Vence et al. 2015). Type I psoriasis commonly appears in the form of guttate lesions after bacterial infection whilst type II involves spontaneous chronic plaques (Perera et al. 2012).

In PsA the most common manifestation is the symmetric/polyarticular (more than 50%) followed by the asymmetric/oligoarticular (around 30%) PsA, that affects single or few distal interphalangeal or phalangeal joints (**McGonagle2011**; Reich et al. 2009). The psoriatic lesions precede joint inflammation in approximately 60-70% of the cases(**Gladman2005**; **McGonagle**; **2011**). Particularly, nail, scalp and intergluteal lesions constitute a predictive biomarker for development of joint inflammation (**Moll1976**; **McGonagle**; **2011**; Griffiths and Lancet 2007). This reinforces the need of appropriate coordination between dermatologists and rheumatologists for an early diagnostic and treatment that could prevent functional joint disability.

Several comorbidities have been associated with psoriasis and PsA, with comparatively greater prevalence in PsA. For example, intraocular inflammation known as uveitis affects 8% of PsA patients compared to 2% of the psoriasis ones (**Husted2011**; **Oliveira2015**). Other comorbidities include inflammatory bowel disease(IBD), cardiovascular disease (CVD) (Gelfand et al. 2006), type II diabetes (T2D) (**Saphiro2007**) and metabolic syndrome (**Cohrn20017**).

The diagnosis of psoriasis and PsA is mainly based in clinical assessment since there is a lack of appropriate biomarkers at early stages of disease (Villanova et al. 2013). Evaluating the severity of psoriasis skin lesions remains challenging and different measures have been implemented. The Psoriasis Area and Severity Index (PASI) (**Fredriksson1978**) is the most widely used in research and drug trials (**Finlay2005**). This test quantifies lesional burden weighted by body part based on the amount of affected body surface area and the degree of severity of erythema, induration and scale (Table 1.1). Disease is considered mild for PASI¡7 and it is classified as moderate-to-severe for PASI¿7-12, depending on the study (**Finlay2005**; **Schmitt2005**; **add ref from cell types**).

To evaluate PsA, analysis of performance of the previously mentioned Moll and Wright criteria together with additional ones led to the configuration of the

Classification Criteria for Psoriatic Arthritis (CASPAR) (**Taylor2006**), the most widely used. It requires the patient displaying inflammatory arthritis, enthesitis, and/or spondylitis and three points from a list of associated elements (Table 1.2) . Another composite measure commonly used to evaluate treatment efficacy for PsA is the PsA Response Criteria (PsARC) based on the number of tender joints (TJC) and swollen joints (SJC) over 68 and 66, respectively, as well as a physician global assessment based on a short questionnaire (**Philipp2011**; **Clegg1996**).

PASI	description
Body location	Head and neck, upper limbs, trunk and lower limbs
Feature	Redness, thickness and scaling
Severity scale	Absent, mild, moderate, severe or very severe
Affected area (%)	0, 1-9, 10-29, 30-49, 50-69, 70-89 or 90-100

Table 1.1: For each of the four body locations the test quantifies the percentage of affected area and the severity of three intensity features: redness, thickness and scaling.

CASPAR: a patient must have inflammatory articular disease (joint, spine, or enthesial) with three points from five categories	
Psoriasis	a. Current skin or scalp disease b. History of psoriasis c. Family history of psoriasis
Psoriatic nail involvement	Typical psoriatic nail distrophy
A negative test for RF	Using preferrably by enzyme-linked immunosorbent assay (EMSA)
Dactylitis	a. Swelling of an entire finger b. History of dactylitis
Radiologic evidence of juxtaarticular new bone formation	Ossification near joint margins

Table 1.2: xxxx

1.2.2 Aetiology of psoriasis and PsA

Psoriasis and PsA are complex chronic inflammatory diseases where a dysregulated immune response initiates as result of genetic predisposition and exposure to a particular environmental trigger (Figure**??**). One of the greater controversies has been characterising the origin of the pathologies as well as the connection between skin and joint inflammation. Particularly, for psoriasis it remains unclear whether disruption of the skin triggers activation of the immune response or viceversa.

Histopathological alterations in skin and joints

The epidermis is the most external structure of the skin and it is formed by approximately 90% keratinocytes (KC) organised in a layer structure that self-renews in a time dependent manner from the bottom to the surface (Wikramanayake et al. 2014). As the KC differentiate they undergo changes in morphology, replication ability and keratin composition of their intracellular matrix. In the context of psoriasis impaired epidermis cell renewal leads histological alterations and development of the psoriatic lesions. KC undergo upregulation in the proliferation rate (hyperplasia) that causes aberrant cell differentiation (parakeratosis) (ref) thickening of the epidermis and the subsequent scale formation (ref). Concomitantly, inflammation causes immune cell infiltration and hypervascularisation of the lesion driven by upregulation in the expression of angiogenic factors and activation of the endothelium (Perera et al. 2012).

In PsA, joint affection usually follows skin lesions and it involves a wide range of histological changes in the joints, particularly bone remodeling (**Haddad2013**). One of the most common structural changes is the arthritis caused by the swelling and inflammation of the joints (**Schett2011**). As result of this inflammation, alterations in bone remodeling leads to osteolysis with

subsequent bone resorption and erosion at the affected joints (**Mensah2017**). This phenomenon is particularly relevant in arthritis mutilans or chronic absortive arthritis, one of the most severe forms of PsA (**Haddad2013**). Bone erosion is also the main histopathological process driving dactylatis, where bone lysis resolves in shortening of the digits (**Gladman2005**). On the other hand, 35% of the PsA patients undergo inflammation of the connective tissue at the insertion of tendons or ligaments, phenomenon known as enthesitis (**McGonagle2011**; **Polachek2017**). Overtime, this causes debilitating structural changes due to formation of bony spurs along the insertion sites(**Schett2011**).

Dysregulation of the innate and adaptive immune response

The dysregulated immune response in psoriasis and PSA is the result of the interaction between innate and adaptive immune cells (ref section) resulting in feedback loops involving a complex cytokine milieu. Among the most relevant cytokines of the innate immunity involved in disease initiation are IFN-α and IFN-γ (**Leanne2009**). They are mainly produced by circulating plasmacytoid DC (pDC) and myeloid DC (mDC), respectively, upon activation by KC proinflammatory cytokines (Perera et al. 2012). Both are upregulated at the mRNA level in the lesional skin and contribute to lymphocyte recruitment and maintenance of DC activation (**Schmid1994**).

Another key cytokine in this dysregulated inflammatory response is TNF-α which has a prominent role in bone turnover and bone remodeling in PsA (**Mensah2008**). It is produced by activated KC, mast cells but also by adaptive immune cells types, including infiltrated T helper(Th) 1 and Th17 cells infiltrated in the psoriatic lesion and PsA inflamed joints (Perera et al. 2012) and it induces activation of nuclear nactor kappa-light-chain-enhancer of activated B cells (NF-B) signaling pathways (ref). It also activates several kinase signaling pathways as well as cell death programs (ref). In the context of inflammation, NF-B represents

a master transcriptional regulator of both, the innate and adaptive immune system that induces expression of proinflammatory cytokines, antiapoptotic genes and genes involved in chronic inflammation maintenance (ref). The importance of this transcription factor (TF) in psoriasis and PsA pathogenesis is reflected by the association with disease of several genetic variants in some of the negative regulators of its proinflammatory activity, including NF-B inhibitor alpha *NFKBIA* and TNF receptor-associated factor 3 interacting protein 2 *TRAF3IP2* (ref).

Interleukin-23 (IL23) and Th17 axis represents a key loop for the maintenance of psoriasis and PsA inflammatory response and a very important link between innate and adaptive immunity. IL-23 is an innate regulatory cytokine, mainly produced by mDC and macrophages homing the inflamed skin and it binds to the IL23 receptor (IL23R), which expression is upregulated in the DC and T cells of the lesion and in circulating Th cells (ref). In psoriasis, IL23 is the mediator for the pathogenic loop between activated KC and T cells (ref). Both IL-23 and IL-23R present protective and pathogenic genetic variants associated with psoriasis and PsA risk (ref). The activation of the IL-23 pathway leads importantly to increased IL17 production through NF-activation by *TRAF3IP2* (ref). IL17 favors maintenance of the adaptive immune mediated Th17 response through recruitment and activation of neutrophils, induction of proinflammatory cytokines including $IL\text{-}1\beta and IL-6 and also perpetuation of KC activation (ref) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3580541/). More recently, interleukin 22 (IL22) has a$ cells known as Th22 (ref). It mediates some of the histological changes in skin as well as AMP production by KC (ref).

Environmental factors and disease

Several environmental triggers are known to be associated with increased risk and worsening of psoriasis and PsA development. A wide range of drugs including antidepressant, antihypertensive and anticytokine therapies have been clinically associated with initiation, exacerbation and worsening of psoriasis (**Kim2010**). Infectious agents such streptococcal throat infection have likewise been associated with development of type I psoriasis (**Gudjonsson2003**; **Valdimarsson2009**) and further supported by the presence of homologous T cell clones in tonsils and psoriatic lesions (**Diluvio2006**). Consistently with other chronic inflammatory disease such as IBD and AS, recent studies have also observed perturbation in the composition of the gut and skin microbiota in psoriasis and PsA patients. On the other hand, physical trauma, including tattoos, surgical incisions and mechanical stress, has shown to trigger the appearance of lesions in psoriatic uninvolved skin as well as joint inflammation in digits (**Weiss2002**; Nestle et al. 2009). Lastly, as for most of the complex diseases, behavioral factors including smoking, alcohol and stress have been linked to psoriasis and PsA without a clear conclusion of their involvement in triggering disease (**Meglio2014**).

1.2.3 Cell types involved in psoriasis and PsA pathogenesis

There is great debate about the most relevant cell types contributing to psoriasis and PsA pathogenesis. Progressively, both are understood as dynamic and continuous processes where different cell types became predominantly important at different stages of the pathology. Regarding levels of circulating immune cells, severe psoriasis patients (PASI¿12) showed significantly decreased numbers of PBMC compared to moderate (PASI¡12) and healthy individuals (**Langewouters2008**).

KC are one of the most relevant cell type at early stages of psoriasis pathogenesis. Several studies have shown the role of KC as immune sentinels through antigen presentation and production of antimicrobial peptides (AMP), cytokines and chemokines. There is evidence of complex formation between the cationic AMP cathelicidin or LL-37 and self-DNA/RNA released by the damaged KC in the psoriatic skin upon trigger by environmental factors (**Lande2007**). It leads to initiation of the skin inflammation through activation of skin-resident DC (**Nestle2005**) and secretion of pro-inflammatory cytokines, importantly IL-1, IL-6 and TNF-α, that reinforce activation of DC and Th lymphocytes (**Feldmeyer2007**; **Arend2008**; Nestle et al. 2009). Moreover, expression of the MHC-II allows KC to act as APC and activate memory $CD4^+$ and $CD8^+$ T cells inducing a recall immune response (**Black2007**). Studies in mouse models have shown development of psoriatic lesions in immunodeficient mice upon human xenotransplant of psoriasis skin only (**Boyman2004**). The importance of KC in psoriasis development is also reinforced by the association with psoriasis risk of genes of the late cornified envelope (LCE) family (**Tsoi2012**). Overall, these findings would support the hypothesis attributing the initiation of the chronic inflammatory response in psoriasis as the consequence of the epidermis dysfunction Proskch2008.

mDC and pDC have also been considered important innate immune cells in disease initiation (**Mahil20016**). They are professional APC that induce T cell activation and the subsequent adaptive immune response. The relevance of antigen presentation in disease has been highlighted at the cellular (**Rusell1972**; **Tiilikainen1980**) and also at the genetic level with the psoriasis and PsA GWAS association of HLA-Cw*06:02 and ERAP1 (**Strange2010**), which encodes for an aminopeptidase involved in the trimming of peptide antigens. Although pDC are circulating cells absent in healthy skin, they infiltrate into the lesional and uninvolved dermis of psoriasis lesions (**Nestle2005**) and get activated by the

aforementionedd self-DNA and LL-37 complex through Toll-like Receptor (TLR)-9 (**Lande2007**). In contrast, quiescent mDC are epidermal resident and upon secretion of IFN-α by pDC a 30-fold increase of mature mDC is observed in lesional skin but not in uninvolved or healthy tissue (ref). Different mDC subpopulation mediate the Th1 and Th17 response as well perpetuation of KC activation through IL-23 production (ref). Studies in immunodeficient psoriasis mice models have shown that blockage of downstream IFN-α signaling or its production by pDC failed to induce T cell activation and onset of psoriasis (**Nestle2005**).

Neutrophils are also though to be closely involved in disease initiation through their ability to form neutrophil extracellular traps (NET)that contain host DNA and AMP, particularly LL-37 (**Hu2016**). There is evidence of increased NET formation in peripheral blood and lesional skin of psoriasis patients and they seem to be contributing to pDC and $CD4^+$ T activation (**Hu2016**). Neutrophils have also been identified in recent studies as one of the main sources of IL-17 production in the skin lesions (**Lin2011**) and they also release a wide range of proteases which some induce KC proliferation (**Mahil2006**).

In the context of the innate immunity, the involvement of monocytes and macrophages in psoriasis and PsA has not been extensively studied. Resident macrophages in the healthy dermis undergo a 3-fold increase in lesional skin and they are involved in disease development through TNFα production (**Mahil2016**; Perera et al. 2012). Different mice models for chronic psoriasiform skin inflammation have shown a key role of macrophage migration into the affected skin and TNF-α production for maintenance of the lesions (**Stratis2006**; **Wang2006**). Some studies using psoriasis and PsA patients derived monocytes have also highlighted the systemic aspects of both pathologies. Psoriasis PBMC isolated monocytes have shown greater phagocytic and bactericidal activity compare to those from healthy individuals (**Bar-Eli1979**). Later studies have

also shown increased circulating intermediate monocytes ($CD14^+$ high $CD16^+$ high) and monocyte aggregation in psoriasis patients with subsequent enhanced platelet activation and angiogenesis (**Golden2015**).

Historically, T lymphocytes have been considered one of the most relevant cell types in initiation and maintenance of psoriasis and PsA, and GWAS association with MHC-I also supports the role of T cells in disease. Report cases in humans have demonstrated that bone marrow transplantation can initiate or terminate psoriasis and, therefore, the role of bone marrow-derived T cells in disease pathophysiology (**Eedy1990**; **Gardembas1990**). The percentage of circulating T cells in psoriasis has been reported to be dependent of severity. Different studies have shown reduced number of T cells in moderate-to-severe and severe psoriasis patients when compared to milder phenotypes and healthy controls. Despite this reduction, increased percentage of the memory populations $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$ have been demonstrated in the same individuals(**Lecewicz-Toru2001**; **Langewouters2008**). There is still controversy regarding the total $CD4^+$ and $CD8^+$ abundance and $CD4^+/CD8^+$ ratios in PBMC, which may be due to the phenotype heterogeneity of psoriasis patients in the different studies (**Lecewicz-Toru2001**; **Cameron2003**; **Langewouters2008**). In PsA, no differences abundance of circulatings T cells have been identified compared to healthy individuals (**Costello1999**).

In healthy skin, $CD4^+$ and $CD8^+$ are found in the dermis and epidermis, respectively (**Clark2006**; Perera et al. 2012) and upon lesion development an increase in activated memory $CD4^+CD45RO^+$and $CD8^+CD45RO^+$ in the respective compartments can be detected as soon as 3 days after its appearance (**Clark2006**), highlighting the importance of the memory population. *In vivo* studies conducted in mice by Boyman and colleagues showed that development of psoriasis following engrafted human pre-lesional skin was dependent of local T cell proliferation and it did not required injection of additional factors

(**Boyle2013**). This supports the theory where recruitment of circulating T cells is restricted to the priming event and it is minimal afterward (Perera et al. 2012). The relative importance of $CD4^+$versus $CD8^+$ in psoriasis initiation has been tested immunodeficient mice with pre-lesional skin xenografts followed with injection of purified activated T cell populations (**Nickoloff1999**). These observations suggested a model where $CD4^+$ but not $CD8^+$ T cells where required for the progression of uninvolved to lesional skin in mice. Interestingly, injection of $CD4^+$ activated cells was followed by an increase in activated resident $CD8^+$ T cells expressing the acute activation marker CD69. It suggest a hypothesis where in the skin $CD4^+$ drive signaling for activation of resident T cells and the activated $CD8^+$ resident population are the main effector cells. In PsA, $CD4^+$ are significantly more abundant than $CD8^+$ cells in synovial tissues (**Diani2015**). In contrast, $CD8^+$ expressing CD45RO are prevalent in the SF and they are also significantly increased when compared to controls (**Costello1999**).

In addition to memory T cells, the contribution of regulatory T (Treg) cells have also been investigated to some extent due their role in immunosurveillance and self-tolerance in the context of autoimmune disease. Nevertheless, controversial results have been found regarding relative abundance and impaired function (Perera et al. 2012).

Based on the cytokine profile, psoriasis and PsA has been demonstrated to be a type 1 Th/Tc disease, where naive $CD4^+$ and $CD8^+$ cells get activated and proliferate in the presence of IL-12 and IFN-γ (**Austin1999**; Perera et al. 2012). Later studies also identified additional subsets including Th-17/Tc-17 and Th-22/Tc-22, which are mainly dependent on IL-23 and IL-6 for their activation, respectively (**Mahil2016**). The importance Th17 cells and their IL-17 production has been assessed in skin, joints and blood, where increased IL-17 and also IL-23 mRNA and protein levels have been found in psoriasis and PsA patients compared to controls (**Cai2012**; **reference for joints**). It has been shown that the

predominant $CD8^+$ cells in the SF are also IL-17 producers and their abundance correlates with markers of inflammation and structural changes in the joint (**Menon2014**). This finding distinguishes PsA from other forms of arthritis such as RA and is in line with findings on skin that suggest a prominent role of $CD8^+$ IL-17 producing cells in the different stages of both pathologies. There is also evidence of the synergistic interaction between Th1 and Th17 cells which overall enhances the production of AMPs by KC (**Kryczek2008**). Understanding of the importance of IL-17 has also led to the discovery of other immune cells producing this pivotal cytokine including innate immune lymphoid (ILC) cells and $\gamma\delta$ T cells which have also started to been investigated in the context of psoriasis and PsA pathophysiology and treatment (**Meglio2014**; **Leijten2015**). IL-17 producing cell have also been hypothesised to be responsible for the link between skin and joint lesions. Although the precise mechanisms for transition between psoriasis and PsA is unknown, studies using psoriasis and RA mice models have shown that skin lesions facilitate arthritis and joint inflammation It has been hypothesised that the presence of IL-17 producer cells in inflamed skin located nearby the enthesis of joint under physical stress could trigger the development of PsA.

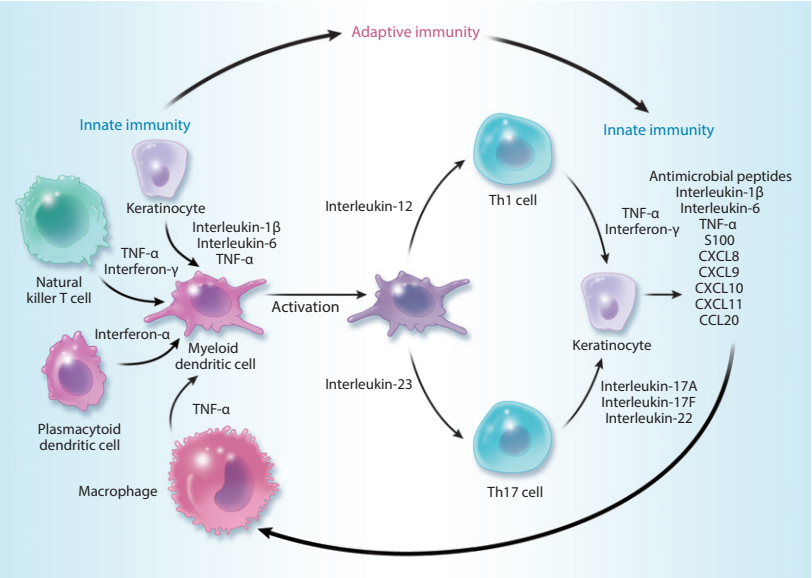

Figure 1.1: Figure adapted from (Nestle et al. 2009)

1.2.4 Therapeutic intervention and prognosis

Currently, there is no cure for either psoriasis or PsA and the different treatments available are focused in managing the disease manifestations and symptoms. The approach to treat them are usually dependent on the disease severity. Cases of mild-to-moderate psoriasis are usually managed with topical and systemic therapies (**Menter2009**). Among the topical agents, corticosteroids and emollients are the most commonly used and affordable ones. Emollients are non-medicated agents that contribute to keep the skin soft and moist minimising the symptoms of itching and tenderness and they have been accepted as an adjunctive for the treatment of psoriasis. On the other hand, cortocosteroid in creams, shampoo and spray remain as the most widely spread medical topical treatment due to their antiinflammatory, antiproliferative, and immunosuppressive actions. Corticosteroids are classified based on the potency and they all exert their effect through binding to intracellular corticosteroid receptors and regulation of gene transcription (**Tadicherla2009**). Although they are the most common way to treat mild forms of disease, some of them are approved only for short term treatments as side effects have been reported at a local and systemic level (**Menter2009**). In the case of PsA presenting swelling of two or less joints intraarticular injection of glucocorticosteroids together with joint aspiration have shown to reduce pain and inflammation as a short-time measure (Coates et al. 2016).

For psoriasis, other topical treatments are used in combination with topical corticosteroids including ultra violet (UV) light therapy and vitamin D analogues, which inhibit KC proliferation, stimulate KC differentiation and inhibits T cell proliferation and other inflammatory mediators (**Rizova2001**). Topical retinoids and calcineurin inhibitors are also used less frequently for the treatment of psoriasis and they have shown reduced systemic absorption

and better suitability for long term treatments in some studies (**Weinstein2003**; **Menter2009**).

Treatment of most forms of PsA and more moderate-to-severe psoriasis require the use of a broad range of systemic therapies. For mild cases of PsA with involvement of less than four joints and non radiological evidence of structural changes, nonsteroidal antiinflammatory drug (NSAID) are the most commonly used to help controlling the mild inflammatory symptoms and also helping to alleviate the pain and stiffness (Coates et al. 2016). However, the use of NSAID is not recommended in patients presenting CVD comorbidities due to the increased risk associated with the use of these drugs (**Bhala2013**). For those NSAID-resistant or more severe forms of PsA, disease-modifying antirheumatic drugs (DMARDs) including the an antagonist of folic acid methotrexate (MTX) and the phosphodiesterase 4 inhibitor apremilast have shown to immunosupressive effects on activated T cells and reduction of cytokine production, respectively (**Schmitt2014**; **Gossec2016**; **Keating2017**; **Polachek2017**). However, increased risk of hepatotoxicity for the MTX Menter2009 and gastrointestinal side effect of apremilast Menter2009 require requires ensuring appropriate dosing and surveillance.

The use of biologic systemic agents tend to be the most specific but also expensive treatment for severe cases of psoriasis and PsA and they are not used as the first-line of treatment. These are molecular species generated in cell-based that modulate the immune response in a physiological based manner (Perera et al. 2012). Among the biologic agents targeting cytokines, TNFα inhibitors (TNFi) have been broadly used for the past five decades to treat both, psoriasis and PsA since this is a pivotal cytokine in initiation and perpetuation of the inflammatory response. There are three TNFi approved for the treatment of psoriasis etanercept, infliximab and adalimumab (**Ahil2016**) and another two, certolizumab pegol and golimumab, also used in the management of PsA and

other rehumatoid diseases (**Coates2016b**). All the TNFi are antibody-based agents but etanercep, that is a soluble receptor, and they also show differences in the frequency and via of administration as well as the efficacy, particularly in the specific improvement of skin or joint lesions (**Mease2000**). Although TNF-α blockade is one of the most effective treatments some patients experience common side effects such as increased risk of infection, reactivation of latent infections, demyelinating disease and induced pustular psoriasis have been identified (**Nickoloff2004**). Between 20 to 50% of the patients fail to respond to the first TFNi and require switching to a second or third one (**Abramson2016**). Lately, new biologic therapies have been developed to target other key cytokines in the pathogenesis of PsA and psoriasis, such as IL-12, IL-23 (ustekinumab) or IL-17 (secukinumab and ixekizumab) (**Mahil2016**). These new biologics represent a substantial benefit for treating patients and they are routenely administered to individuals failing to respond after a switch to a second TNFi (**Coates2016b**).

indirect evidence mDC also produce interleukin 12 (IL12) that could contribute to IFN-γ production in lesional skin and inflammed synovium (ref).

1.3 Genetics of psoriasis and psoriatic arthritis

As complex diseases, the risk to develop psoriasis and PsA is not only influenced by the surrounding environmental conditions but also by the genetic background of each individual. Determining the magnitude of contribution of the genetic factors in the development of these disease and identifying the exact genes or regions involved in the predisposition to psoriasis and PsA remains challenging.

1.3.1 Heritability

Several studies have shown a trend towrads the increase of psoriasis and PsA prevalence over the last 30 years in different countries (Organization 2016).

This importantly reflects changes in life style habits and it highlights the need to better understand the genetic factors that predispose to disease upon interaction with environmental stresses.

The contribution of genetics in the development of psoriasis has been shown in several twins studies. The concordance of psoriasis has been shown to be greater in monozygotic twins(33-55%) compared to dizyogtic (13-21%) with some variation between studies and populations, estimating an 80% of heritability (**Faber1974**; **Duffy1993**; **Pendersen2008**). In the case of PsA, similar concordance has been show probably due to lack of statistical power and appropriate diagnosis (**Pendersen2008**).

Family studies where 40% of the patients have family history in first degree (**Gladman1986**). Interestingly, the genetic recurrence ratios in first-degree relatives has been shown to be greater in PsA (40) compared to psoriasis (8) in a study in Icelandic population (**Chandran2009**). although the results regarding twins concordance in PsA has failed to give conclusive results, this finding could suggest differences in the heritability between the two phenotypes and maybe an stronger genetic contribution in PsA.

1.3.2 Genetic studies

Different approaches have been undertaken to uncover the genetic variability contributing to the predisposition to psoriasis and PsA. The appearance of next generation sequencing (NGS) techniques and the progressive reduction of cost has allowed to move from candidate genes studies looking at the genetic variability at particular locus to a genome-wide approach.

Non-GWAS and linkage studies

The study of psoriasis and PsA genetics architecture started with linkage analysis in family pedigrees with autosomal dominant condition to try to .

The use of this approach yielded nine psoriasis susceptibility loci (PSORS1-9) (**Capon2017**) with the strongest association in PSORS1 (**International2003**). PSORS1 lies within chromosome 6p21.3 and its identification by linkage analysis confirmed the association previously identified in serological studies between psoriasis susceptibility and the MHC I (**Rusell1972**; **Tiilikainen1980**). Importantly, Mendelian forms of disease with rare highly penetrant mutations have been identified in family studies for two genes within PSORS2 (17q25): zinc finger protein 750 (*ZNF750*) (**Tomfohrde1994**) and caspase domain family member 14 (*CARD14*) (**Jordan2012**). Rare gain of function and textitde novo mutations and also common variants in textitCARD14 have been identified in psoriasis and PsA patients, suggesting an important role of the genetic variation in this gene for Mendelian and multifactorial forms of both (**Jordan2012**; **GWAS study**). In PsA, a region close to the psoriasis PSORS8 was also identified (**Karason2003**). Nevertheless, the lack of reproducibility by independent studies of the remaining PSORS highlighted the limitations of the linkage studies to understand the genetics of complex diseases (**Capon2017**).

GWAS

-New era for genetic studies in complex diseases -Advantages versus previous approaches -Main studies in psoriasis and PsA: limitations of the phenotypes and different populations -Possibility of transethnic studies -Low frequency and rare variants -Exome GWAS -Introduction to the limitations of GWAS including INDEL and inversion that may be covering an important part of the heritability

1.3.3 MHC and non-MHC associations with disease risk

1.3.4 The role of coding and non-coding variants in disease susceptibility

1.3.5 The role of GWAS studies in highlighting disease relevant tissues

Indicate main pathways highlighted by the GWAS studies

Chapter 2

Material and Methods

2.1 Ethical approval and recruitment of study participants

Sample recruitment for the two different phenotypes and the healthy volunteers were conducted under different ethics.

2.1.1 PSO patient recruitment

Patient blood samples and normal or psoriatic skin biopsies were collected in collaboration with the Dermatology Department research nurses at the Churchill Hospital, Oxford University Hospitals NHS Trust and Professor Graham Ogg at the Weatherall Institute of Molecular Medicine, University of Oxford under approval from the Oxfordshire Research Ethics Committee (REC 09/H0606/71 and 08/H0604/129). After written informed consent, up to 60 mL of blood from eligible PSO patients were collected into 10 mL anticoagulant EDTA-containing blood tubes (Vacutainer System, Becton Dickson).

PSO patients were eligible for recruitment when meeting the following criteria:

- over 18 years old
- previously or newly diagnose, in a flare and going into biologic therapy for the first time

- fulfillment of the clinically accepted Psoriasis Area and Severity Index (PASI) classification for PSO diagnosis (**Fredriksson1978**)
- moderate to severe disease (PASI¿5)
- less than 2 weeks without antibiotics unless used for prophylaxis
- available clinical information and written consent

Detailed clinical information of the PSO cohort is included in (Chapter **??**)(Table **??**).

2.1.2 PsA patient recruitment

Sample recruitment was performed as part of the Immune Function in Inflammatory Arthritis (IFIA) study established in 2013 (REC/06/Q1606/139)in collaboration with research nurses at the Nuffield Orthopaedic Centre, Oxford University Hospitals NHS Trust and Dr Hussein Al-Mossawi at the Botnar Research Centre. Following informed written consent, blood (30 mL) and synovial fluid aspirate (variable upon disease severity) were recruited into 10 mL anticoagulant sodium heparin coated tubes (Vacutainer System, Becton Dickson).

Eligibility of the PsA patients was upon fulfillment of the following criteria:

- over 18 years old
- previously or newly diagnose, with concomitant PSO, in a flare and going into biologic therapy for the first time
- fulfillment of the clinically accepted PsA Response Criteria (PSARC) including a physician global assessment questionnaire (**Philipp2011**; **Clegg1996**)
- oligoarticular phenotype and naïve for any treatment
- less than 2 weeks without antibiotics unless used for prophylaxis

- available clinical information and written consent

Further details about the cohort and clinical information can be found in (Chapter **??**)(Table **??**).

2.1.3 Healthy volunteer recruitment

Recruitment of healthy volunteers was conducted as part of the study Genetic Diversity and Gene Expression in White Blood Cells with approval from the Oxford Research Ethics Committee (REC 06/Q1605/55). Up to 80 mL of blood were collected into 10 mL anticoagulant EDTA-containing blood tubes, similarly to the PSO sample recruitment.

The criteria for healthy individuals to participate in the study was:

- over 18 years old and preferably British or European
- no family history of PSO, PsA, RA or SpA
- matched sex and age with the PSO cohort
- less than 2 weeks since last infectious process
- available clinical information and written consent

2.2 Sample processing

Blood, synovial fluid and skin biopsies were processed straight after recruitment following the appropriate protocols.

2.2.1 PBMC and synovial fluid cells isolation

PBMC were isolated from blood samples through density gradient separation using Ficoll-Paque. Total synovial fluid (SF) cells (SFC) were isolated by centrifugation at 500g for 5 min. Both were washed twice in Hanks balanced

salt solution without calcium or magnesium (Thermo Fisher Scientific) and resuspended in phosphate saline buffer (PBS, Gibco) supplemented with 0.5% fetal calf serum (FCS, Invitrogen) and 2mM ethylenediaminetetraacetic acid (EDTA, Sigma)prior to cell types separation. Cell numbers and viability were determined by manual count using a haemocytometer and trypan blue (Sigma).

2.2.2 Primary cell isolation using magnetic-activated cell sorting

For the work related to PSO and healthy volunteers, primary cell subpopulations were separated using magnetic-activated cell sorting (MACS, Miltenyi). Positive selection was performed for consecutive isolation of $CD19^+$ B cells, $CD8^+$ T cells, $CD14^+$ monocytes and $CD4^+$ T cells with AutoMACS Pro (Miltenyi) and cells were manually counted as previously described. MACS separation was chosen over Fluorescence-associated cell sorting (FACS) due to time and logistic constrains in the sample processing and therefore cell numbers in down stream application may not be as exact.

2.2.3 Primary cell isolation using fluorescence-activated cell sorting

Primary cell subpopulations from controls to study the effect of cryopreservation in chromatin states (Chapter 3) or PsA blood and SF samples were isolated by FACS. PBMC and SFC were resuspended in PBS 1mM EDTA (FACS buffer) at $10x10^6$ cells/mL, stained with the appropriate antibody cocktail (Table 2.1) for 30 min at 4°C, washed with FACS buffer and centrifuged at 500g for 5 min at 4°C. For the samples used in Chapter 3, a modified FACS buffer supplemented with 3 mM EDTA , 2% FCS and 25mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Invitrogen) was used

to avoid cell clumping after cryopreservation and short recovery.After removing the supernatant, cells were resuspended in FACS buffer prior to separation.

In the controls samples of Chapter 3 only $CD14^+$ monocytes and $CD3^+$ $CD14^-$ $CD4^+$ T cells were isolated in the SONY SH800 cell sorter. For the PsA samples, separation of $CD19^+$ B cells, memory T cells ($CD3^+$ $CD14^-$ $CD4^+$ $CD45RA^-$ and $CD3^+$ $CD14^-$ $CD8^+$ $CD45RA^-$) ,$CD14^-$ monocytes and $CD56^-$ NK was performed using FACS Aria (BD) cell sorter from both PBMC and SFC. Bulk sorted cells were collected in 1.5mL tubes in PBS 1% FCS, whilst single cell and small bulk sorting was performed in 96-well plates in the appropriate buffer (See RNA-seq section). Different nozzle sizes were chosen for bulk and single-cell sorting and OneComp eBeads (eBioscience) were used for compensation of fluorescence spill over.

Surface marker	Fluorochrome PsA/CTL	Manufacturer PsA/CTL	Clone PsA/CTL	Dilution PsA/CTL
Viability	eFluor780	-	eBioscience	1:250
CD3	FITC/AF700	SK7/UCHT1	BioLegend	xxx/1:50
CD4	APC	RPA-T4/RPA-T4	BioLegend	1:50/1:50
CD8a	PE	RPA-T8	BioLegend	xxx
CD45RA	BV421	HI100	BioLegend	xxx
CD19	PerCP-Cy5.5	SJ25C1	BioLegend	xxx
CD14	Pe-Cy7/FITC	M5E2/TUK4	BioLegend/Miltenyi	xxx/1:100
CD56	BV510	NCAM16.2	BD	xxx

Table 2.1: Details regarding target molecule, fluorochrome, clone, supplier and dilution used for PBMC and SFC staining are provided for each of the antibodies in the panel. In controls only CD3, CD4 and CD4 markers were used.

2.2.4 Skin biopsies processing and adherent assay

KC enrichment from skin biopsies was performed as described in Gutowska-Owsiak and colleagues (GutowskaOwsiak and Schaupp 2012). Skin biopsies (approximately 4mm) were washed with PBS, cut in 1mm width strips and incubated in 2U/mL of dispase II (Sigma) overnight at 4°C. The epidermis

was separated from the dermis and either snap-frozen in liquid nitrogen (for RNA extraction) or further digested in trypsin (Invitrogen) at 37°C for 5 min, when used for chromatin accessibility assay. After digestion the resulting cell suspension was filtered through a 70µm nylon strainer (BD) and washed with PBS. In some instances cells were manually counted and aliquoted for ATAC-seq processing. In others, cell from each of the biopsies were resuspended in KGM-2 BulletKit (Lonza) supplemented with 0.06mM Ca^2+ and cultured in a collagen IV coated 96-well plate at 4°C for 10 min or 3 hours, upon experimental requirements (see Chapter X). After culturing, cells were washed twice with 200µL of PBS and kept at 37°C for downstream processing.

2.3 Experimental protocols

2.3.1 Cryopreservation and cell culture

For the controls samples in Chapter 3, 40-50x10^6 of PBMC were freeze-thawing using a modified version of the (Kent 2009) protocol, where cells were pre-conditioned in RPMI 1640 (brand) complete medium supplemented with2 mM L-glutamine, 100U penicillin and strep 100µg/mL and 50% FCS for 30 minutes and afterwards diluted 1 in 2 in complete RPMI 1640 (supplemented as previously described) with 20% dimethyl sulfoxide (DMSO, Sigma). PBMC underwent slow cryopreservation at -80°CC in isopropanol at -1°C per minute and stored for a minimum of two weeks in liquid nitrogen. PBMC were thaw, resuspended in supplemented complete RPMI 1640 with 10% FCS at a density of 10^6 cells/mL and rested for 30 min at 37C, 5% CO2 in 25mL non-adherent polypropylene cell culture flasks followed by filtering through a 40µm to obtain an homogenous cell suspension undergoing FACS separation. Frozen Normal human epidermal keratinocytes (NHEK) in passage 3 were recovered and cultured at a cell density of 5x10^6 cells/mL in a 75 mL adherent cell

culture flask (brand) in EpiLife basal medium (Gibco) following manufacturer's instructions. After recovery NHEK were trypsinised at room temperature for 8 minutes followed by trypsin inactivation with EpiLife 10% FCS, centrifugation at 180g for 10 min at room temperature and manual counting with trypan blue. NHEK were seeded in a 96-well plate in 100uL of medium at a cell density of 160 cells/µL. NHEK were cultured for 2 days to a 90-100% confluence before being used downstream.

2.3.2 ATAC-seq, Fast-ATAC and Omni-ATAC

Improved versions of the ATAC-seq protocol were progressively used in the thesis for assessment of chromatin accessibility in different primary cells, including $CD14^+$ monocytes, $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells and $CD56^+$ NK cells. The subsequent version aimed to reduce the amount of mitochondrial DNA and improve the ratio of signal to noise for this technique.

After MACS separation, primary cells were manually counted as above specified and they were resuspended in PBS with 1% FCS. As previously stated, due to reduced accuracy of manual cell counting compared to FACS sorting, in my experiments ATAC-seq was performed using an estimated number of cells between 50,000 to 100,000. ATAC-seq was performed as described in Buenrostro *et al.*, 2013 with minor modifications. Cells were centrifuged at 500g for 5 min at 4°C. After removing the supernatant cells were lysed for 10 min, the nuclei were transposed using the Nextera Tn5 transposase (Illumina) for 40 min at 37°C and DNA was purified using the PCR MinElute kit (Qigen). Additional modifications and performance in 96-well plates were implemented for KC and they will be described in

After appropriate determination of the amount of DNA amplification using qPCR, samples were amplified and singled indexed for 11 PCR cycles using modified Nextera primers from Buenrostro *et al.*,2013 (Table2.2). The

resulting DNA libraries were purified using the MinElute kit and additional Agencourt AMPure XP Magentic Beads (Beckman Coulter), according to the manual specifications, to remove the remaining adaptors and primer dimers.

Primer name	Full sequence
Ad1.noMX	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.22	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT

Table 2.2: Name and full sequence of the PCR primers used for amplification, indexing and pooling of the ATAC-seq and ChIPm samples in this thesis. These primers were designed by Buenrostro *et al.*, 2013 and they are an modified version of the Nextera Illumina primers optimised for larger molecular weight DNA fragments from low input samples. All samples were indexed with the universal primer Ad1.noMx and one of the additional 18 primers. The indexing sequence of each of the primers is in blue text.

Following the Nature Methods publication of Corces et al., 2016, the initial ATAC-seq protocol was replaced by a modified version named Fast-ATAC. It was specifically optimised for hematopoietic cells and combined cell lysis and transposition in a single step. Fast-ATAC was performed as described by Corces et al., 2016 with minor modifications. Since 5,000 cells was considered the lower limit to generate good quality data in Fast-ATAC, in my experiments I used 20,000 MACS or FACS sorted cells, to account for inaccurate manual cell counting as well as possible cell loss over centrifugation steps. The Fast-ATAC reaction was performed for 30 min at 37°C and agitation at 400rpm. DNA was purified as in ATAC-seq and libraries were generated following 13 cycles of PCR amplification, following appropriate cell cycle determination. Purification following PCR were performed using Agencourt AMPure XP Magentic Beads only.

Omni-ATAC, a third generation of ATAC-seq was published by Corces et al., 2017. It consisted in an universal protocol with individual cell lysis and transposition reactions intercalated with a washing step, to remove mitochondrial DNA and other cell debri Omni-ATAC was performed as described by Corces and colleagues (**Corces2017**) using 50,000 cells.

Following either of the three protocols, DNA tagmentation profiles were assessed with the D1000 high sensitivity DNA tape (Agilent) as part of the quality control and quantified using the Kapa kit (Roche), following the manufacturer's instructions. Pools of 12 to 16 libraries were sequenced in one to 3 lanes of the HiSeq4000 Illumina platform by the Oxford Genomics Centre at the Wellcome Trust Centre for Human Genetics.

2.3.3 Chromatin Immunoprecipitation with sequencing library preparation by Tn5 transposase

Assessment of histone marks modification in the chromatin of PSO patients from Cohort 1B and four age matched healthy volunteers was performed

using a low cell input Chromatin Immunoprecipitation (ChIP) method know as ChIPmentation (ChIPm). For each individual and cell type three histone marks, including H3K27ac, H3K4me1 and X were tested in 100,000 cells and compared to an input control processed in parallel. Samples were processed following the protocol published by Schmidl and colleagues (**Schmidl2015**) with some modifications. Aliquots of 600,000 cells of MACS sorted cell types, as described in 2.2, were fixed with 1% formaldehyde (Sigma) and snap frozen in dry ice and ethanol prior to storage at -80°C. Chromatin sonications of the different individuals and cell types were performed in one batch using Covaris M220(Covaris). Each of the aliquots was resuspended in 130µL of SDS lysis buffer (Table2.3), sonicated for 8 min using a duty factor of 5% and aliquoted for single ChIPm reactions prior to long term storage at -80°C.

Sonicated chromatin aliquots were thawed and resuspended in appropriate volume of ChIP equilibration buffer (Table2.3), in order to achieve the appropriate concentration of NaCl and Triton-X100. For the immunoprecipitation step, samples were incubated with the appropriate amount of antibody (Table 2.4) overnight in rotation at 4°C. Protein-A Dynabeads (Invitrogen) were also washed three times with Beads wash buffer (Table2.3) and blocked with yeast tRNA (supplier) overnight in rotation at 4°C. Beads were washed twice with the aforementioned washing buffer and resuspended in SDS lysis buffer before being added to the sample-antibody mix. One of the advantages of this protocol is the tagmentation of the chromatin when still bound to the beads prior to protein decross-linking, which allows preventing overtagmentation of the DNA.

qPCR was performed in each of the purified ChIPm DNA samples to identify the number of full cycles required to reach one-third of the final fluorescence. Libraries were amplified for the number of cycles minus one determined with this strategy, allowing to minimise the total number of PCR replicates. The primers used for amplification and indexing are the ones

optimised by Buensotro and colleagues (Table2.2). The number of amplification cycles for each of the samples is recorded in

2.3.4 Nucleic acids extraction and RNA-seq

Bulk RNA-seq

Following MACS isolation of the different cell types from the PSO and matched healthy controls, between 2-3x10^6 $cells were re suspended in 350 \mu L of RNAProtect (Qiag$ $mercaptoethanol (BM, Sigma) and snap frozen in dry ice before storage at$ – 80°C. Cells isolated from PSO and control Cohort 1A (Chapter **??**) were preserved in RNAProtect, which stops any biochemical reaction and transcriptional activity maintaining cell integrity. At early stages of the project, when I was uncertain of time frames to process the different material from the acquires samples, I decided to use RNAProtect to preserve cells for future RNA extraction to guarantee high quality in case storage exceeded 6 months. In the PSO and control samples from Cohort 1B

DNA and total RNA were extracted using the AllPrep DNA/mRNA/microRNA Universal kit (Qiagen) following the manufacturer's instructions. RNA extractions were performed in batches of 12 samples, including all cell types from each individual and a balanced numbers of PSO and control samples, to minimise batch effect correlation with phenotype groups. Basic quantification was performed with NanoDrop (Thermo Scientific) before storage of DNA and RNA at -80°C.

RNA-seq quality control, quantification, library preparation and sequencing were carried out by Oxford Genomics Centre at the Wellcome Trust Centre for Human Genetics in two independent batches of samples, each including Cohort 1A or Cohort 1B, respectively. Processing of samples in two batches was due to logistics of patients recruitment in the project. Quality control

Reagent	Final concentration	Supplier
SDS lysis buffer		
SDS	0.25%	Sigma
EDTA	1mM	X
Tris-HCl pH 8	10mM	Sigma
PI	1X	Roche
Water	-	Ambion
ChIP equilibration buffer		
Triton-X100	1.66%	Sigma
EDTA	1mM	X
NaCl	233mM	X
Tris-HCl pH 8	10mM	Sigma
PI	1X	Roche
Water	-	Ambion
Beads washing buffer		
SDS	0.1%	Sigma
EDTA	50mM	X
NaCl	150mM	X
NP-40	1%	Sigma
Tris-HCl pH 8	10mM	Sigma
PI	1X	Roche
Water	-	Ambion

Table 2.3: Composition of the three modified buffers in house for the ChIPm protocol: SDS lysis buffer, ChIP equilibration buffer and beads washing buffer. For each of the buffers the reagents, composition and supplier are indicated.The final volume prepared for each buffer was adjusted depending on the number of samples processed at the time. Sodium dodecyl sulfate (SDS), PI (proteinase inhibitor).

Histone mark	Feature	µL per sample	Manufacturer and catalo
H3K427ac	Promoter	X	
H3K4me1	Active enhancer	X	
X	X	X	

Table 2.4: Details regarding the histone marks, the the most likely chromatin state delineated, the amount of antibody required per reaction and the supplier and catalog num of the antibodies.

and quantification were assessed with the Bioanalyzer (Agilent). Preparation of RNA-seq libraries was performed using Ribo-Zero rRNA Removal kit (Illumina), based on ribosomal RNA depletion. Unlike strategies using polyadenylated transcripts selection, this method allowed to preserve non-polyadenylated transcripts including nascent pre-mRNA (unspliced) and functionally relevant lncRNAs. For each of the cohorts, all libraries were pooled together and sequenced over several lanes of HiSeq4000 to a depth of 50 million total reads per sample in order to maintain an appropriate level of sensitivity for subsequent expression analysis given the greater complexity of these libraries.

PsA memory $CD4^+$ and $CD8^+$ single-cell RNA-seq

Single $CD4^+$ or $CD8^+$ memory T cells were sorted in 2µL of cell lysis buffer into 96-well plates. Four and three plates were prepared for $CD4^+$ and $CD8^+$, respectively, including wells containing 50 cells in each of the plates for control purposes. Libraries were prepared,indexed and sequenced at the Sanger Institute (Cambridge) using the Smart-seq2 methodology as described by Picelli and colleagues (**Picelli2014**). Samples were sequenced using 50-bp single-end Illumina HiSeq2500 at a depth of approximately 4 million reads per cell.

scRNA-seq was also generated in FACS sorted $CD4^+$ or $CD8^+$ memory T cells and in PBMC using 3'and 5' (kit catalogue) RNA library prep 10X Genomics technology ref by the Oxford Genomics Centre at the Wellcome Trust Centre

for Human Genetics. Libraries were sequenced with Illumina HiSeq4000 xxxbp single-end at a depth of 50,000 reads per cell.

Small-bulk RNA-seq

Between 100 to 500 cells of the five populations isolated from PsA patients were FACS sorted into 2µL of cell lysis buffer and processed for library prep as in Picelli *et al.*, 2014 by the Oxford Genomics Centre at the Wellcome Trust Centre for Human Genetics. Libraries were sequenced with Illumina HiSeq4000 at a depth of XXX reads per cell.

2.3.5 CyTOF

Look at my notes

Very brief

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