\chapter{Introduction}

\label{ch:Intro}

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Our growing knowledge of genetic associations with susceptibility to psoriasis and psoriatic arthritis (PsA) has not been matched by understanding of the functional basis of these associations and translation to patient benefit. To address this challenge, it is important to understand the regulatory genomic landscape within which disease associated genetic variants may act. This thesis describes functional genomic approaches to establish genome-wide epigenetic and expression profiles for disease relevant tissues and blood-isolated immune cells in psoriasis and PsA, and explore their potential significance for disease pathogenesis and genetic variation. In this chapter, I begin by reviewing current knowledge of the pathophysiology of psoriasis and psoriatic arthritis, the role of genetic variation, and the challenge of functionally characterising genome-wide association studies (GWAS) in complex traits, including the different functional genomics approaches that can be applied

\section{Psoriasis and psoriatic arthritis}

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Psoriasis and PsA have been described as two distinct common complex disease entities that nonetheless share certain clinical features and genetic architecture. Psoriasis is a chronic inflammatory skin disease characterised by episodes of relapse and remittance, most commonly manifesting as well-demarcated erythematous plaques with silver scale and associated with increased risk of joint, eye and systemic disorders \parencite{Nestle2009}. On the other hand, PsA is a seronegative chronic inflammatory disease within the spondyloarthritis (SpA) family that usually develops after psoriasis skin manifestations \parencite{Moll1973, Coates2016, Villanova2013}. Understanding similarities and differences between these conditions at the pathological level is helpful before we consider sharing and specificity at the genetic level as well as implications for the identification of new therapeutic targets.

%(Variants in RUNX3 contribute to susceptibility to PsA, exhibiting further common ground with ankylosing spondylitis, PsA Immunochip)

\subsection{Epidemiology and global impact}

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Psoriasis represents a serious global health problem that currently affects about 100 million people worldwide, including both children and adults with no sex bias \parencite{Organization2016}. Although the mean age of onset is 33 years, a bimodal distribution with psoriasis patients being classified as early-onset/ type I (with peaks between 16 and 22 years) or late-onset / type II (between 50-60 years) has also been described \parencite{Henseler1985, Perera2012}. This classification based on the age of onset also has correlates with distinctive clinical features including severity, relapse frequency and family history.

The risk of developing psoriasis shows ethnic differences with a lower prevalence among adult African, African American and Asian populations (between 0.4 and 0.7\%) compared to American and Canadian (4.6 and 4.7 \%, respectively) \parencite{Jacobson2011}. In the UK the prevalence of psoriasis ranges between 2 and 3\%, affecting approximately 1.8 million people \parencite{Perera2012}. On the other hand, cases of PsA in the general population varies between 0.04 and 1.2\% (Perera et al. 2012) occurring in 10 to 30\% of psoriasis patients, evidencing the strong association between the two diseases \parencite{Gelfand2005,Reich2015,Perera2012}. Overall, data suggest an steady increase in both psoriasis and PsA prevalence over the last thirty years \parencite{Springate2017,Organization2016}.

Several severe comorbidities have are associated with psoriasis and PsA, with comparatively greater prevalence in PsA. For example, intraocular inflammation (uveitis) affects 8\% of PsA patients compared to only 2\% of psoriasis patients \parencite{Husted2011, Oliveira2015}. Other comorbidities include inflammatory bowel disease (IBD), cardiovascular disease (CVD), type 2 diabetes (T2D) and metabolic syndrome \parencite{Gelfand2006,Shapiro2007,Cohen2008}. Psoriasis and PsA have also been associated with an increased prevalence of depression and suicidal ideation \parencite{Sampogna2012}. Overall, psoriasis and PsA represent a significant burden for the economy due to treatment costs and associated morbidity. Treatment and management-associated costs per psoriasis patient per year in 2015 in the UK accounted for \textsterling4,000 to \textsterling14,000, before and after requirements of biological therapy, respectively, and were further increased in PsA \parencite{Burgos-Pol2016, Poole2010}.

\subsection{Psoriasis and inflammatory dermatoses}

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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 an important role in innate and adaptive immunity and its alterations, due to exogenous or endogenous factors, can lead to development of inflammatory skin conditions, such as psoriasis or atopic dermatitis \parencite{Johnson-Huang2009, Proksch2008}.

Lesions in psoriasis are very heterogeneous in type (pustular and non- pustular), location and severity, which complicates its clinical classification \parencite{Perera2012}. As a result, several phenotypes including chronic plaque psoriasis (psoriasis vulgaris), guttate psoriasis, pustular psoriasis, erythrodermic psoriasis and nail psoriasis have been defined \parencite{Marrakchi2011}.

\subsection{PsA and spondyloarthropathies}

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PsA belongs to the SpA family, which includes diseases such as ankylosing spondylitis (AS), reactive arthritis (ReA), idiopathic inflammatory bowel disease (IBD) and undifferentiated SpA \parencite{Goldman2011} All these SpA subtypes are characterised by structural damage (bone formation and erosion) as well as inflammation of joints and extra-articular sites such as eyes, gut and skin. Broadly, SpA has been classified into axial and peripheral based on the affected joints (spine/sacroilicac or peripheral) and the presence of extra-articular features \parencite{Rudwaleit2009}.

Major histocompatibility (MHC) class I molecules present intracellular peptides (self or from infectious agents) to T cells, encoded by the human leukocyte antigen (HLA) A, B and C genes. HLA-B27 is the strongest genetic association for the SpA family. Studies in human families and rat models with HLA-B27 positive status have shown manifestation of different SpA, such as psoriasis and inflammatory bowel disease (IBD) , within a single family or individual \parencite{Hammer1990,Said-Nahal2001}. Based on the common pathophysiological foundations, some studies have supported the concept of SpA as a single disease that presents heterogeneous phenotypic manifestations based on current knowledge \parencite{Baeten2013}. Interestingly, the axial and peripheral classification of SpA may be supported by true immunopathological differences between the two \parencite{Porcher2005, Appel2011, Vandooren2004}. Nevertheless, the knowledge of cellular and molecular processes contributing to pathophysiology is still limited and further research will impact on the classification and understanding of SpA family.

As a phenotype, PsA can be further subdivided in five clinical groups as per the Moll and Wright criteria: distal, destructive, symmetric, asymmetric and spinal \parencite{Moll1973}. These subclasses mainly differ in the location, number and distribution of the affected joints and have been later modified to also include dactylitis (diffuse swelling of a digit), a distinctive feature of PsA \parencite{Reich2012}. Importantly, the phenotypic heterogeneity of SpA and also within PsA difficults the design and achievement of meaningful outcomes from clinical studies due to the incomplete understanding of disease classification. This may obscure findings and conclusion in pathophysiological and clinical studies and needs to be considered when interpreting the results.

\section{Pathophysiology of psoriasis and psoriatic arthritis}

\subsection{Clinical presentation and diagnosis}

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Approximately 90\% of all psoriasis cases are psoriasis vulgaris, which manifests with well demarcated plaques, erythema and scaling. Plaque formation is the result of thickening (acanthosis) and vascularisation of the epidermis and can vary in size and distribution, with the most common locations being the elbows, knees and scalp \parencite{Perera2012,Griffiths2007}. The second most common clinical presentation is guttate psoriasis (10\% of all cases) characterised by acute onset of small droplike papules usually in the trunk and proximal extremities \parencite{Vence2015}. Despite psoriasis vulgaris and guttate representing an important burden for patient wellbeing but are generally not life-threatening forms of disease.

Early and late onset psoriasis (type I and type II) differ in clinical presentation. Type I psoriasis patients commonly present with guttate lesions followed very often by bacterial infection, particularly \textit{Streptococcus} throat infection, and have a stronger family history with a high prevalence of HLA-C\*06:02 (85.4\% of the cases) \parencite{Telfer1992}. In contrast, in type II psoriasis only in 14.6\% of the cases are positive for HLA-C\*06:02 and most commonly manifests as spontaneous chronic plaques (psoriasis vulgaris) \parencite{Perera2012}.

For PsA, symmetric/polyarticular PsA constitutes the most common manifestation (more than 50\% of the cases) followed by asymmetric/oligoarticular PsA (around 30\%), which exclusively affects single or few distal interphalangeal or phalangeal joints \parencite{Reich2009, McGonagle2011}. Skin psoriatic lesions precede joint inflammation in approximately 60 to 70\% of the cases \parencite{Gladman2005, McGonagle2011}. In particular, nail pitting and scalp and intergluteal skin lesions constitute a predictive biomarker for development of joint inflammation \parencite{Moll1973,Griffiths2007,McGonagle2011}.

The diagnosis of psoriasis and PsA is primarily based on clinical assessment of the patient`s symptoms due to the lack of molecular biomarkers at early stages of the disease \parencite{Villanova2013}. The evaluation of skin lesion severity poses an additional challenge, and different measures have been implemented for criteria unification. The Psoriasis Area and Severity Index (PASI) is the most widely quantitative rating score of skin lesion severity in research and clinical trials \parencite{Fredriksson1978,Finlay2005}. PASI quantifies the lesional burden by body part based on area of affected surface and the severity of erythema, induration and scale at each location (Table \ref{tab:PASI}). Disease is considered mild for PASI scores below 7 and is classified as moderate-to-severe for PASI scores between 7 to 12, depending on the study \parencite{Finlay2005, Schmitt2005,Langewouters2008}.

\begin{table}[htbp]

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\textbf{PASI} & \textbf{Description} \\

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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 \\

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\caption[Variables and scoring used in the Psoriasis Area and Severity Index (PASI)]{\textbf{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. The score ranges from 0 (no disease) to 72 (maximal disease).}}

\label{tab:PASI}

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To diagnose PsA, modified Moll and Wright criteria known as Classification Criteria for Psoriatic Arthritis (CASPAR) are most widely used \parencite {Taylor2006}. A positive diagnosis based on CASPAR requires the presence of inflammatory arthritis, enthesitis, and/or spondylitis and three points from a list of associated elements. In terms of disease activity and treatment efficacy, the PsA Response Criteria (PsARC) is the preferred measure \parencite{Mease2011,Clegg1996}. PsARC considers the number of tender joints (TJC) and swollen joints (SJC) over 68 and 66, respectively, as well as patient and physician global assessment of the individual's general health based on a short questionnaire (Table \ref{tab:PsARC}).

\begin{table}[htbp]

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\textbf{PsARC} & \textbf{Description} \\

\midrule

\midrule

TJC68 & Number of tender joints over 68\\

SJC66 & Number of swollen joints over 66 \\

Patient’s global health assessment & Evaluation of the patient's health\\

& by the patient (scale 0 to 5)\\

Physician global health assessment & Evaluation of the of the patient's \\

& by the physician (scale 0 to 5) \\

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\caption[Variables and scoring used in the Psoriatic Arthritis Response Criteria (PsARC)]{\textbf{Variables and scoring used in the Psoriatic Arthritis Response Criteria (PsARC).} The patient's global health assessment by the patient and the physician is scored using a 5-point Likert scale, where 0 corresponds to very good, no symptoms and 5 corresponds to very poor and severe symptoms. When used to evaluate overall improvement after 12 weeks of treatment, improvement in at least two of the four variables evaluated (one of which must be TJC or SJC score) with no worsening of any criteria is required.}

\label{tab:PsARC}

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%\multicolumn{2}{}{\textbf{CASPAR: a patient must have inflammatory articular disease (joint, spine, or enthesial) }} \\

%\multicolumn{2}{}{\textbf{ with three points from five categories}} \\

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%\multirow{3}{\*}{Psoriasis} & a. Current skin or scalp disease \\ & b. History of psoriasis \\ & c. Family history of psoriasis \\

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%\multirow{1}{\*}{Psoriatic nail involvement} & Typical psoriatic nail distrophy\\

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%\multirow{1}{\*}{A negative test for RF} & Using preferrably by enzyme-linked immunosorbent assay (EMSA)\\

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%\multirow{2}{\*}{Dactylitis} & a. Swelling of an entire finger \\ & b. History of dactylitis\\

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%\multirow{1}{\*}{Radiologic evidence of juxtaarticular new bone formation} & Ossification near joint margins\\

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%\caption[CASPAR criteria for diagnosis of PsA]{\textbf{xxxx}}

%\label{tab:CASPAR}

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%PsARC is composed of four measures,including: 1) patient global assessment of disease activity (improvement of 1 on a 5 point Likert scale is required for a response), 2) physician global assessment of disease activity (improvement of 1 on a 5 point Likert scale is required for esponse), 3) joint pain (reduction of 30% or more in total score, assessing either 68 or 78 joints, using a 4 point scale is required for a response), and 4) joint swelling (reduction of 30% or more in total score, assessing either 66 or 76 joints using a 4 point scoring scale, is required for a response).

\subsection{Aetiology of psoriasis and PsA}

Psoriasis and PsA are chronic inflammatory diseases characterised by a dysregulated immune response initiated as the result of genetic predisposition and exposure to particular environmental cues (Figure\ref{fig:PSO\_immune\_system\_diagram}). The origin of both pathologies, as well as the connection between skin and joint inflammation, still remain controversial.

%\begin{figure}[H]

%\includegraphics[width=\textwidth]{./Introduction/pdfs/PSO\_aetiology\_diagram\_Di\_Meglio\_et\_al\_2014.pdf}

%\caption[Main factors involved in psoriasis disease aetiology]{\textbf{Figure adapted from \parencite{Meglio2014}}}

%\label{fig:PSO\_aetiology\_diagram}

%\end{figure}

\subsubsection\*{Environmental factors and disease}

A variety of exposures are proposed as risk factors for the development and worsening of psoriasis and PsA. A wide range of drugs including anti-depressants, anti-hypertensives and anti-cytokine therapies have been associated with initiation, exacerbation and worsening of psoriasis \parencite{Kim2010}. Bacterial and viral infections are associated with triggering and exacerbation of psoriasis, notably guttate psoriasis after group C \textit{Streptococcus} throat infection as well as humman immunodefficiency virus (HIV) infection \parencite{Gudjonsson2003,Valdimarsson2009, Diluvio2006}. \textcolor[rgb]{1,0,0}{In PsA statistical association with antibody production against \textit{Streptococcus pyogenes}, \textit{Yersinia enterocolitica}, \textit{ Chlamydophila psittaci} and HIV have also been reported \parencite{Thrastardottir2018}. Recent studies have also observed perturbation in the composition of the gut and skin microbiota of psoriasis and PsA patients \parencite{Eppinga2014, Yan2017}.}

Physical trauma and mechanical stress can also trigger the appearance of skin lesions and digit joint inflammation \parencite {Weiss2002,Nestle2009}. Increased risk of PsA onset amongst psoriasis patients was indeed associated with lifting cumulative heavy loads as well as with several types of injuries and infections that require treatment with antibiotics \parencite{Eder2011}. Smoking has been the most confidently associated with an odds ratio (OR) of 1.78 (95\% CI 1.52-2.06) for psoriasis, in particular palmoplantar pustulosis \parencite{Armstrong2014}. Psoriasis has also shown association with obesity, alcohol dependency, vitamin D deficiency and stress, but evidence remains controversial \parencite{Meglio2014}.

\subsubsection\*{Histopathological alterations in skin and joints}

The epidermis is the most external compartment of the skin, comprising approximately 90\% keratinocytes and organised in a layer-like structure that self-renews in an spatial and time-dependent manner \parencite{Wikramanayake2014}. Keratinocyte differentiation is associated with changes in morphology, replication ability and keratin composition of the intracellular matrix. In the context of psoriasis, impaired epidermis cell renewal leads to histological alterations and lesion development. Importantly, keratinocytes undergo upregulation in proliferation rate (hyperplasia) that causes aberrant cell differentiation (parakeratosis), thickening of the epidermis and subsequent scale formation \parencite{Ruchusatsawat2011}. 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 \parencite{Perera2012}.

% Check content accuracy

In PsA, the affected joint shows a wide range of histological changes \parencite{Haddad2013}. One of the most common structural changes is arthritis caused by the swelling and inflammation of the joints \parencite{Schett2011}. As a result of this inflammation, alterations in bone remodeling lead to osteolysis with subsequent bone resorption and erosion at the affected joints \parencite{Mensah2008}. Bone erosion is also the main histopathological process driving dactylatis, where bone lysis resolves in shortening of the digits \parencite{Gladman2005}. Moreover, 35\% of PsA patients also undergo inflammation of the connective tissue at the insertion of tendons or ligaments, a phenomenon known as enthesitis \parencite{McGonagle2011,Polachek2017}. The inflammatory environment at the entheses favours bony spurs formation along the insertion sites, similar to RA, causing structural debilitation of the joints \parencite{Benjamin2009,Finzel2014}.

%https://onlinelibrary.wiley.com/doi/full/10.1002/art.38794

%Schett2011

\subsubsection\*{Dysregulation of the innate and adaptive immune response}

%link to the histological changes

The dysregulated immune response in psoriasis and PsA is the result of the interaction between innate and adaptive immune cells through feedback loops and a complex cytokine milieu (Figure \ref{fig:PSO\_immune\_system\_diagram}). Interferon (IFN)-$\alpha$ and $\gamma$ are innate immune cytokines involved in disease initiation and mainly produced by circulating plasmacytoid dendritic cells (pDCs) and myeloid DC (mDCs), respectively, as well as by T cells in lesional skin \parencite{Leanne2009,Perera2012,Hijnen2013}. Increased mRNA levels for both IFNs have been detected in skin plaques and shown to contribute to lymphocyte recruitment and maintenance of DC activation \parencite{Schmid1994}. TNF-$\alpha$ is another key cytokine involved in the dysregulated innate immune response observed in psoriasis and PsA. TNF-$\alpha$ is produced by activated keratinocytes, mast cells, natural killer (NK) cells

%include NK reference

and also adaptive immune cell types, \textcolor[rgb]{1,0,0}{including T helper (Th)/CD4$^+$ cells activated Th-1 and Th-17 subsets infiltrate} skin lesions and inflamed joints \parencite{Perera2012,Lizzul2005}. TNF-$\alpha$ causes activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-$\kappa$B), a master transcriptional regulator which induces expression of pro-inflammatory cytokines, antiapoptotic genes and genes involved in maintenance of chronic inflammation \parencite{Lizzul2005, Johansen2004}. Moreover, TNF-$\alpha$ has a prominent role in bone turnover and bone remodeling, key histopathological features of PsA \parencite{Mensah2008}.

% Johansen 2010 Preferential inhibition of the mRNA expression of p38 mitogen-activated protein kinase regulated cytokines in psoriatic skin by anti-TNF-a therapy.

Interleukin-23 (IL-23) and interleukine-17 (IL-17) constitute a link between the innate and adaptive immunity as well as a key loop for the perpetuation of the psoriasis and PsA inflammatory response. IL-23 is an innate immune cytokine mainly produced by the mDCs and macrophages in inflamed skin and to a lesser degree by psoriatic keratinocytes \parencite{Lee2004, Li2018}. IL-23 exerts its function through binding to the IL-23 receptor (IL23R), highly expressed by the lesion-resident DCs and T cells and also by circulating CD4$^+$ \parencite{Tonel2010}. In psoriasis, IL-23 mediates the pathogenic loop between activated keratinocytes and T cells, where activation of the IL-23 pathway importantly leads to Th-17 cell differentiation and increased IL-17 cytokine levels as a result of NF-$\kappa$B activation \parencite{McGeachy2009}. %by \textit{TRAF3IP2} (ref).

IL-17 signalling maintains the Th-17 immune mediated response through recruitment and activation of neutrophils, induction of pro-inflammatory cytokines, including interleukine-1$\beta$ (IL-1$\beta$) and interleukine-6 (IL-6), and sustained KCs activation \parencite{Doyle2012}.

More recently, interleukin 22 (IL-22) has gained relevance as mediator of dysregulated crosstalk between the innate and adaptive immune response. IL-22 levels are increased in the skin lesions and plasma of psoriatic patients and is mainly produced by a subset of CD4$^+$ cells known as Th-22 \parencite{Wolk2006}. IL-22 contributes to some of the histological changes in skin as well as to antimicrobial peptides (AMPs) production by keratinocytes \parencite{Eyerich2009}.

% Maybe a paragraph to connect skin and joint affection Identical T cell clonality between skin and synovium https://ac.els-cdn.com/S0198885999000348/1-s2.0-S0198885999000348-main.pdf?\_tid=5efa7316-fde5-11e7-8091-00000aacb360&acdnat=1516454913\_dd20efb867f822d68d8b09873601e8ad

\subsection{Cell types involved in psoriasis and PsA pathogenesis}

%Global report on psoriasis, 2016

Psoriasis and PsA are complex dynamic pathophysiological processes, and the understanding of the relative importance of different cell types at different disease stages still remains challenging.

\textbf{\textit{T cells}}. T lymphocytes have been considered the most relevant cell types in the initiation and maintenance of psoriasis and PsA. Skin-resident memory T cells have been demonstrated to have a key role in psoriatic lesion development in mice models \parencite{Boyman2004}. In human case reports, bone marrow transplantation has shown to cause initiation or termination of psoriasis \parencite{Gardembas1990, Eedy1990}. \textit{In vivo} studies demonstrated that transition to psoriatic lesions following engrafted human pre-lesional skin in immune-deficient mice was only dependent on T cells requiring injection of autologous activated CD4$^+$ not CD8$^+$ cells \parencite{Wrone-Smith1996}. Nevertheless, preferential migration into the epidermis and clonal populations T cells have only been isolated for CD8$^+$ cells \parencite{Wrone-Smith1996, Chang1994}. %Altogether, this may suggest that CD4$^+$ are drivers of T cell activation but resident CD8$^+$ are the main effector cells in the dysregulated psoriasis immune response.

In psoriasis and PsA, IL-23 together with other cytokines, including IL-1$\beta$ and IL6, induce activation and differentiation of na\"{i}ve CD4$^+$ and CD8$^+$ into pathogenic Th-17 and Tc17 cells producing IL-17 \parencite{Weaver2007}. IL-17$^+$ CD8$^+$ cells have been found in psoriatic skin and are enriched in PsA synovial fluid when compared to peripheral blood, showing correlation with markers of inflammation and structural changes in the joint \parencite{Menon2014,Ortega2009}. Likewise, Th-17 infiltrated cells have been found in the epidermis of psoriatic lesions \parencite{Lowes2008, Pene2008}. Additionally, IL-12 and IFN-$\gamma$ lead to expansion of Th-1 and Tc-1 cells, which contribute to perpetuation of the immune response through IFN$\gamma$ and IL-18 production in psoriasis and PsA \parencite{Austin1999, Perera2012}.

%Regarding the adaptive immunity, T lymphocytes have been considered the most relevant cell types in the initiation and maintenance of psoriasis and PsA. Report cases in humans have demonstrated that bone marrow transplantation can initiate or terminate psoriasis \parencite{Eedy1990, Gardembas1990}. Reduced numbers of circulating T cells but increased percentages of the memory populations CD4$^+$CD45RO$^+$ and CD8$^+$CD45RO$^+$ have been observed in moderate-to-severe and severe psoriasis patients when compared to milder phenotypes and healthy controls \parencite{Lecewicz-Torun2001,Langewouters2008}. Different studies have reported controversial results regarding the total abundance and ratios of CD4$^+$ and CD8$^{+}$ in PBMC, likely due to the phenotype heterogeneity of the psoriasis cohorts between studies \parencite{Lecewicz-Toruń2001,Cameron2003,Langewouters2008}. In PsA, no differences in abundance of circulating T cells have been identified when compared to healthy individuals \parencite{Costello1999}.In homeostasis, CD8$^+$ and CD4$^+$ lymphocytes are found in the epidermis and dermis, respectively \parencite{Clark2006}. An increase in activated memory CD4$^{+}$CD45RO$^{+}$and CD8$^{+}$CD45RO$^{+}$ cells can be detected by the third day from the lesion appearance \parencite{Clark2006,Perera2012}. \textit{In vivo} studies showed that development of psoriasis following engrafted human pre-lesional skin was only dependent on local T cell proliferation, highlighting the importance of circulating T cells recruitment during the priming event rather than at later stages of the disease \parencite{Wrone-Smith1996,Nickoloff1999,Perera2012}. The relative importance of CD4$^+$ versus CD8$^+$ cells in psoriasis initiation has been explored in pre-lesional skin mouse xenografts where CD4$^+$ but not CD8$^+$ T cells were required in the transition from uninvolved to lesional skin \parencite{Nickoloff1999}. Interestingly, the injection of activated CD4$^+$ cells in mice was followed by an acute increase in activated resident CD8$^+$ T cells. Overall, these results supported the hypothesis of skin CD4$^+$ cells being drivers of resident T-cell activation and the population of resident activated CD8$^+$ the main effector of the immune response. In synovial tissues of PsA patients, CD4$^+$ are significantly more abundant than CD8$^+$ \parencite{Diani2015}. However, amongst the CD8$^+$ populations, the memory cells are prevalent in the patients’ synovial fluid (SF) with a significant enrichment compared their counterparts in PsA PB and RA SF \parencite{Costello1999}. Based on the cytokine profile, psoriasis and PsA have been classified as a type 1 Th/Tc disease, where activation of naive CD4$^+$ and CD8$^+$ cells is driven by IL-12 and IFN-$\gamma$ \parencite{Austin1999,Perera2012}. In addition, T-cell subsets including Th-17/Tc-17 and Th-22/Tc-22, producing high levels of IL-17 and IL-22, respectively, have been identified to be relevant for the perpetuation of the inflammatory response \parencite{Mahil2016}. The importance of Th-17 cells and IL-17 production has been evaluated in skin, joints and blood, with elevated mRNA and protein levels of IL-17 and also IL-23 reported in psoriasis and PsA patients compared to controls \parencite{Cai2012, Dolcino2015}. %Mention paper Kagami about increased Th17, Th1 and Th22 in psoriasis patients bloodThe relevance of IL-17 has been further highlighted by the presence of CD8$^+$ populations in patients’ SF that are predominantly IL-17 producers and whose abundance correlates with markers of inflammation and structural changes in the joint \parencite{Menon2014}. This finding is in line with observations in skin and suggests a prominent role for CD8$^+$ IL-17-producing cells in the different stages of both pathologies. Studies directed to understand the importance of IL-17 have led to the discovery of other immune cells producing this pivotal cytokine, including innate immune lymphoid (ILC) cells and $\gamma$$\delta$ T cells, opening new research avenues in the context of psoriasis and PsA pathophysiology and treatment \parencite{Meglio2014,Leijten2015}. IL-17-producing cells have also been hypothesised to be at the link between skin and joint lesions. Although the precise mechanisms for transition between psoriasis and PsA is still poorly understood, the study of psoriasis and RA in mouse models revealed that skin lesions facilitate arthritis and joint inflammation \parencite{Yamamoto2015}. %In fact, the presence of IL-17-producing cells in the inflamed skin nearby the enthesis of joints under physical stress is likely to be a trigger for the development of PsA.

\textbf{\textit{Keratinocytes}}. In psoriasis keratinocytes have demonstrated a role as a bridge between the innate and adaptive immune response. Karatinocytes have shown ability to act as immune sentinels through MHC-class II antigen presentation to CD4$^+$ and T cytotoxic(Tc)/CD8$^+$ cells \parencite{Black2007}. Upon damage by environmental triggers keratinocytes release cationic AMP LL-37 and self-DNA/RNA that form a complex which acts as an antigen for skin-resident DCs activation and initiation of the inflammatory response \parencite{Lande2007}. Moreover, pro-inflammatory cytokines such as IL-17A or IL-22 also activate keratinocytes inducing proliferation and in turn production of cytokines, including IL-1, IL-6 and TNF-$\alpha$, and chemokines (e.g CXCL1, CXCL2, CXCL5, CXCL8 and CCL20) leading to recruitment of neutrophils and T cell to the site of inflammation \parencite{Feldmeyer2007, Arend2008, Nestle2009, Nestle2005}. Keratinocytes also release vein endothelial growth factor (VEGF), a pro-angiogenic factor that activates endothelial cells and leads to pathogenic angiogenesis \parencite{Xia2003}. The relevance of keratinocytes in the dysregulated immune response in psoriasis is further reinforced by the genetic association with genetic variants located at keratinocyte-specific genes from the late cornified envelope (LCE) family \parencite{Tsoi2012}.

\textbf{\textit{Dendritic cells}}. mDCs and pDCs are important innate immune cells in initiation of the psoriasis dysregulated immune response through antigen presentation and T cell activation \parencite{Mahil2016}. pDCs are circulating professional antigen presentation cells (APCs) that on activation through Toll-like receptor (TLR)7/9 by the keratinocytes self-DNA and LL-37 complex and infiltrate into the lesional and uninvolved dermis of psoriasis patients \parencite{Nestle2005, Lande2007}. In contrast, quiescent mDCs are epidermal resident cells that undergo maturation in presence of the IFN-$\alpha$ secreted by pDCs, expanding up to 30-fold in lesional skin \parencite{Zaba2007}. Activated mDCs mediate the Th-1 and Th-17 response as well as perpetuation of KC activation through IL-23 production \parencite{Lee2004}. %Studies in immunodeficient psoriasis mouse models have shown that blockage of downstream IFN-$\alpha$ signaling or IFN-$\alpha$ production by pDCs failed to induce T-cell activation and psoriasis onset \parencite{Nestle2005}.

\begin{figure}[htbp]

\centering

\includegraphics[width=0.7\textwidth]{./Introduction/pdfs/PSO\_adaptive\_innate\_immune\_system\_crosstalk.pdf}

\caption[Environmental triggers and genetic predisposition leading to psoriasis and PsA (adapted from Nestle \textit{et al.}, 2009).]{\textbf{Environmental triggers and genetic predisposition leading to psoriasis and PsA (adapted from Nestle \textit{et al.}, 2009).} The main cell types, cytokines and chemokines involved in the dysregulated innate and adaptive immune response found in these conditions are shown.}

\label{fig:PSO\_immune\_system\_diagram}

\end{figure}

\textbf{\textit{Monocytes and macrophages}}. Resident macrophages in the healthy dermis undergo a 3-fold increase in psoriatic skin lesions and contribute to disease development through TNF$\alpha$ production \parencite{Perera2012, Mahil2016}. Similarly, mouse models for chronic psoriasiform skin inflammation have demonstrated macrophage migration into affected skin and how production of TNF-$\alpha$ contributes to maintenance of skin lesions \parencite{Stratis2006, Wang2006}. Initial studies showed greater phagocytic and bactericidal activity in monocytes from psoriasis patients compared to those from healthy individuals \parencite{Bar-Eli1979}. Additionally, increased circulating intermediate monocytes (CD14$^{high}$ CD16$^{high}$) and monocyte aggregation was also observed in psoriasis patients, resulting in enhanced platelet activation and angiogenesis \parencite {Golden2015}. In PsA synovial membranes, the levels of monocytes/macrophage metalloproteinases responsible for bone erosion through differentiation into osteoclasts have been found to be similar to those found in RA joints \parencite{Hitchon2002}.

\textbf{\textit{Natural killer}}. NK cells are lymphoid-derived innate immune cells identified as CD3$^-$ CD56$^+$. The majority of circulating NK cells (90\%) are CD56$^{dim}$ and show strong cytotoxicity driven by high content of perforin and granzymes \parencite{Mandal2014}. In contrast, CD56$^{bright}$ commonly infiltrate into second lymph organs and other tissues, where they are activated by DCs and produce immunoregulatory cytokines such as IFN-$\gamma$, promoting Th-1 expansion and the adaptive immune response \parencite{Martin-Fontecha2004,Ferlazzo2004}. In psoriasis, significant increase of cells expressing NK markers have been found in lesional compared to uninvolved skin \parencite{Cameron2002,Ottaviani2006}. %with NK CD56$^{bright}$ cells isolated from acute plaque lesions producing abundant IFN-$\gamma$ upon activating stimuli \parencite{Cameron2002,Ottaviani2006}.

Expansion of NK CD3$^-$ CD56$^{bright}$ cells in inflamed joints was observed in a cohort including RA, PsA and AS patients \parencite{Dalbeth2002}. Moreover, NK cells in RA have shown to trigger osteoclastogenesis and bone destruction in vitro and in mice models \parencite{Soderstrom2010} . %Moreover, the cytokine IL-15, which is highly present in the the joint microenvironment can prime NK cells isolated from PsA peripheral blood to kill via activation of the receptor NKG2D and cPLA2.82 \parencite{Tang2013}.

Amongst the target cells receptors regulating NK cells function, the killer immunoglobulin-like receptor (KIR) family includes activating and inhibitory members. The inhibitory receptor KIR2DL1 and the activatory receptor KIR2DS1 recognise HLA- Cw\*06:02, strongly associated with psoriasis and PsA \parencite{Tobin2011}. Interestingly, gene based studies have shown genetic variability in \textit{KIR2DS1} gene, associated with psoriasis and PsA susceptibility and also reported for AS and RA \parencite{Luszczek 2004, Williams2005,Carter2007, Yen2001}.

\textbf{\textit{Neutrophils}}. Neutrophils are implicated in disease initiation through their ability to form neutrophil extracellular traps (NET) that contain host DNA and LL-37 \parencite{Hu2016}. Evidence of increased NET formation in peripheral blood and lesional skin of psoriasis patients has been found and seem to contribute to pDC and CD4$^+$ T cell activation \parencite{Hu2016}. Neutrophils have also been identified in recent studies as one of the main sources of IL-17 production in the skin lesions and release a wide range of proteases, some of which induce keratinocyte proliferation \parencite{Lin2011,Mahil2006}.

%Add chemokines

%HLA-Cw\*06:02 can be recognised by the inhibitory receptor KIR2DL1 and the activatory receptor KIR2DS1. Some studies have shown KIR2DS1 was present in 85\% of the patients but only in 51\% of the controls

%NK cells are important regulators of immune responses \parencite{Luszczek2004}. Their function extends beyond killing of infected or transformed cells. Interactions with dendritic cells, macrophages, and fetal trophoblast cells can regulate NK cell activity by influencing cytokine production, cytotoxicity and stimulation of T helper-1 responses.

%

%

\textbf{\textit{B cells}}. \textcolor[rgb]{1,0,0}{The role of B cells in the pathophysiology of psoriasis and PsA has remained unclear. B cells are mainly known as key players of the humoral adaptive immune response through antibody production. However, they also act as APCs, regulate CD4$^+$ activation and differentiation into Th effector cells by providing co-estimulatory signals and actively secrete cytokines \parencite{Bouaziz2007,Constant1995,Harris2000,Linton2003}. Recent studies in the imiquimod-induced psoriasis mice model have demonstrated more severe inflammation in CD19 knock-out mice a regulatory B cell subset producing IL-10 \parencite{Yanaba2013,Alrefai2016}. Furthermore, different B cell subsets have been found in PBMCs from psoriasis patients and in lesional skin and correlation with disease severity has been identified for some clinical subtypes \parencite{Lu2016}.}

\subsection{Therapeutic intervention}

Psoriasis and PsA are currently incurable diseases, with treatments available focused on alleviating symptoms. For instance, topical therapies are advocated in cases of mild-to-moderate psoriasis, including emollients and short-term corticosteroids \parencite{Menter2009}. Other treatments may be used in combination with corticosteroids, such as ultraviolet (UV) light therapy and vitamin D analogues, directed to inhibit T-cell and KC proliferation and stimulate KC differentiation \parencite{Rizova2001}. In the case of PsA, for patients presenting with swelling of two or fewer joints, nonsteroidal anti-inflammatory drugs (NSAID) to control the inflammatory symptoms and intra-articular injection of glucocorticosteroids together with joint aspiration are used to reduce pain and inflammation \parencite{Coates2016}.

Treatment of most forms of PsA and moderate-to-severe psoriasis require the use of systemic therapies. More severe forms of PsA require disease-modifying antirheumatic drugs (DMARDs) including the antagonist of folic acid methotrexate (MTX) and the phosphodiesterase 4 inhibitor Apremilast, which act as immunosuppressors of activated T cells and cytokine production, respectively \parencite{Schmitt2014, Gossec2016, Keating2017,Polachek2017}. Remarkably, biologic systemic agents represent the most specific treatment option for severe psoriasis and PsA notably TNF-alpha inhibitors (TNFi). Three TNFi have been approved for the treatment of psoriasis: etanercept, infliximab and adalimumab \parencite{Ahil2016}. In addition, certolizumab pegol and golimumab are often used in the management of PsA \parencite{Coates2016a}. However, side effects such as increased risk of infection or reactivation of latent infections have been identified \parencite{Nickoloff2004}. Moreover, between 20 to 50\% of patients fail to respond to the first TNFi administrated, requiring switching to an alternative TNFi \parencite{Abramson2016}. New biologic therapies have been developed to target other key cytokines, such as IL-12, IL-23 (ustekinumab) or IL-17 (secukinumab and ixekizumab), which represent a substantial advance in treating patients failing to respond to TNFi {Mahil2016, Coates2016a}.

% Bispecific antibodies

\section{Genetics of psoriasis and psoriatic arthritis}

\subsection{Heritability}

The risk of developing psoriasis and PsA is not only influenced by environmental conditions but also by the genetic background of each individual. The concordance of psoriasis is greater in monozygotic (33-55\%) compared to dizyogtic twins (13-21\%), giving a heritability estimate of 80\%, while no difference in concordance is reported for PsA, probably due to lack of statistical power and appropriate diagnosis \parencite{Farber1974, Duffy1993, Pedersen2008}. In the general population, approximately 40\% of patients with psoriasis or PsA have a family history in first degree relatives \parencite{Gladman1986}. Interestingly, the recurrence rate in first-degree relatives has been shown to be greater in PsA (40\%) compared to psoriasis (8\%) in a study in the Icelandic population \parencite{Chandran2009}. Altogether, this may suggest differences in the heritability between the two phenotypes and a stronger genetic contribution in PsA.

\subsection{Non-GWAS and linkage studies}

Linkage analysis of psoriasis and PsA in family pedigrees presenting an autosomal dominant condition yielded nine psoriasis susceptibility loci (PSORS1-9) with PSORS1 showing the strongest genetic association \parencite{Capon2017, Consortium2003}. PSORS1 locus lies within the MHC class I region, initially associated with psoriasis susceptibility in serological studies \parencite{Russell1972, Tiilikainen1980}. Rare highly penetrant mutations have also been identified for two genes within PSORS2 (17q25): zinc finger protein 750 (\textit{ZNF750}) and caspase domain family member 14 (\textit{CARD14}), with common variants in \textit{CARD14} also reported in psoriasis and PsA patients, implicating genetic variation in this gene in Mendelian and multi-factorial forms of disease \parencite{Tomfohrde1994,Jordan2012, Jordan2012a,Tsoi2012}. Nevertheless, the inability of independent studies to reproduce these results for regions other than PSOR1, 2 and 4, highlights the limitations of linkage studies to understand the genetics of complex diseases \parencite{Capon2017}.

%Additionally, gene based studies in psoriasis and PsA disclosed the importance of genetic variability in the activating killer immunoglobulin receptors 2DS1 (\textit{KIR2DS1}) gene, also reported for AS and RA, which interestingly is mainly triggered by interaction with HLA-Cw\*06:02 \parencite{Luszczek2004, Williams2005,Carter2007, Yen2001}.

%Similarly, specific association with PsA but not psoriasis was found for microsatellites and promoter polymorphisms in TNF-$\alpha$ \parencite{H\"{o}hler2002}.

\subsection{Genome-wide association studies}

Genome-wide association studies (GWAS) have benefited from the understanding of common single base-pair changes known as single nucleotide polymorphisms (SNPs) in different populations and are focused on identifying disease-associated common SNPs (with minor allele frequency (MAF) $geq$1 to 5\% ) showing differences in allele frequency between patients and controls \parencite{Ku2010}. GWAS are thus based on the hypothesis that complex diseases are caused by the interaction of multiple common variants, which association with disease have only modest effect size with OR between 1.2 and 2 \parencite{Schork2009, Cui2010}. The genotyped SNPs in GWAS are solely used a proxy for the disease causative variant, for instance non-genotyped SNPs or other type of genetic variability such as copy number variants (CNVs) \parencite{Hirschhorn2005, Ku2010}.

%Due to the organisation of the genome into segments of strong linkage disequilibrium (LD) where genetic variants are strongly correlated with each other, the genotyped SNPs in GWAS are solely used a proxy for the disease causative variant. Therefore, disease causal variants can be non-genotyped SNPs or other type of genetic variability such as copy number variants (CNVs), also highly frequent in the genome but less widely studied by GWAS \parencite{Hirschhorn2005, Ku2010}.

The first psoriasis and PsA GWAS were published in 2007 and a total of 63 genetic associations have been identified at genome-wide significance (pval$\leq$5x10$^{-8}$) up to date (Table \ref{tab:GWAS\_studies}), explaining 28\% of the psoriasis and PsA heritability \parencite{Tsoi2017}. The majority of studies have been performed in Caucasian European or North American cohorts but increasing numbers of GWAS in large Chinese cohorts are also being published \parencite{Zhang2009, Sun2010, Yin2015}. Early GWAS with moderate power confirmed association with loci overlapping the PSOR1 and PSOR2 genomic regions identified by linkage studies \parencite{Cargill2007,Strange2010}. HLA-C has been consistently identified as the most significant locus with the greatest effect size. Additional MHC-I and MHC-II associations with disease risk have been identified for HLA-A, HLA-B and HLA-DQA1 through step-wise conditional analysis\parencite{Okada2014}.

The informativeness of GWAS was significantly enhanced with the use of the Immunochip genotyping chip, which covers 186 immune relevant loci identified in previous GWAS studies across different inflammatory diseases at a greater genotyping density \parencite{Tsoi2012}. The psoriasis Immunochip study uncovered 15 new associations, including the PSOR4 \textit{CARD14} and also included meta-analysis with the largest available psoriasis cohorts at the time \parencite{Tsoi2012}. This meta-analysis has since been further expanded yielding 16 additional associations and reinforcing the importance of NF$\kappa$B and cytotoxicity pathways in disease pathophysiology \parencite{Tsoi2015,Tsoi2017}. Meta-analysis of GWAS across Caucasian and Chinese populations revealed 4 new associations as well as population-specific effect or allelic heterogeneity in 11 loci, including MHC-I genes, demonstrating the value of this trans-ethnic approach to further understand the heterogeneous genetic susceptibility to psoriasis in different populations \parencite{Yin2015}.

%Four new non-coding loci in the vicinity of \textit{LOC144817}, \textit{COG6}, \textit{RUNX1} and \textit{TP63} were associated with psoriasis and PsA in both populations. Interestingly, genetic heterogeneity between Caucasian and Chinese cohorts was also observed for ten of the GWAS reported loci, for example \textit{ELMO1} and \textit{TYK2}.

Conducting independent GWAS for psoriasis and PsA has shown differences in HLA-C and HLA-B alleles frequencies. Interestingly comparative higher association with HLA-B has been found in PsA individuals compared to psoriasis patients not developing joint inflammation \parencite{Winchester2012, Okada2014}. GWAS association with PsA for previously identified psoriasis loci such as \textit{TRAF3IP}, \textit{IFNLR1}, \textit{IFIH1} and \textit{NFKBIA} and PsA-specific independent signals for \textit{IL23R} and \textit{TNFAIP3} have also been demonstrated \parencite{Ellinghaus2010, Stuart2015}. %Interestingly, the association for \textit{LCE3C/B}, identified in combined phenotypic studies, showed greater strength in those patients presenting psoriasis for over ten years without developing joint affection \parencite{Stuart2015}.

Furthermore, PsA GWAS using Immunochip has also revealed a specific association in chromosome 5q31 not reported previously \parencite{Bowes2015}.

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\begin{landscape}

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\caption[Main GWAS studies in psoriasis and PsA]{\textbf{Main GWAS studies in psoriasis and PsA.} Summary table describing the most relevant psoriasis and PsA GWAS studies. Information regarding sample size, patients phenotypes and the main reported associations in each study is included. The Ellinghaus \textit{et al.}, 2010 and the Stuart \textit{et al.}, 2015 studies included stratified association analysis of psoriasis and PsA independently. WA=white American; Eur=European; $^\star$ Meta-analysis performed.}

\label{tab:GWAS\_summary} \\

\toprule

\textbf{Study} & \textbf{Etnicity} & \textbf{Sample size} & \textbf{Phenotype} & \textbf{Main associations} \\

& & \textbf{(Cases/Controls)} & & \textbf{(putative genes)} \\

\midrule

\midrule

\parencite{Cargill2007} & WA & 1,446/1,432 & Psoriasis, PsA & HLA-C (PSOR1) and \textit{IL-12B} \\

\parencite{Nair2009} & Eur & 1,409/1,436 & Psoriasis, PsA & \textit{IL-23A}, \textit{IL23R}, \textit{IL-12B}, \textit{TNIP1}, \textit{TNFIP3}, \textit{IL-4} and \textit{IL-13} \\

\parencite{Stuart2010} & WA, Eur & 1,831/2,546 & Psoriasis, PsA & \textit{NOS2}, \textit{FBXL19},\textit{PSMA6-NFKBIA} \\

\parencite{Ellinghaus2010} & German & 472/1,146 & Psoriasis & \textit{TRAF3IP2} \\

\parencite{Strange2010} & Eur & 2,622/5,667 & Psoriasis, PsA & \textit{LCE3D} (PSOR2), \textit{IL28RA}, \textit{REL}, \textit{IFIH1}, \textit{ERAP1}, \textit{TYK2} and \textit{HLA-C/ERAP1} epistasia \\

\parencite{Zhang2008} & Chinese & 1,139/1,132 & Psoriasis (type I) & \textit{LCE} gene family and \textit{IL-12B} \\

\parencite{Sun2010} & Chinese & 8,312/12,919 & Psoriasis, PsA & \textit{ERAP1}, \textit{PTTG1}, \textit{CSMD1}, \textit{GJB2}, \textit{SERPINB8} , \textit{ZNF816A} \\

\parencite{Tsoi2012}$^\star$ & WA, Eur & 10,588/22,806 & Psoriasis, PsA & \textit{CARD14} (PSOR4), \textit{RUNX3}, \textit{B3GNT2}, \textit{ELMO1}, \textit{STAT3} \\

\parencite{Tsoi2015}$^\star$ & WA, Eur & 15,000/27,000 & Psoriasis, PsA & 1q31.1, 5p13.1, \textit{PLCL2}, \textit{NFKBIZ}, \textit{CAMK2G} \\

\parencite{Bowes2015} & British, Irish, Australians & 1,962/8,923 & PsA & 5q31 PsA-specific \\

\parencite{Stuart2015} & WA and Eur & 1,430/1,417 & Psoriasis, PsA & PsA-specific secondary signals (main text), 1p36.23 psoriasis-specific, stronger psoriasis \textit{LCE} association\\

\parencite{Yin2015} & WA, Eur, Asian & 15,369/19,517 & Psoriasis, PsA & \textit{LOC144817}, \textit{COG6}, \textit{RUNX1} and \textit{TP63}; signals with ethnic heterogeneity \\

\parencite{Tsoi2017}$^\star$ & WA, Eur & 19,032/39,498 & Psoriasis, PsA & \textit{CHUK}, \textit{IKBKE}, \textit{FASLG}, \textit{KLRK1}, \textit{PTEN} \\

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Overall, GWAS studies have demonstrated shared and distinct genetic architectures for psoriasis and PsA. It is important to take into account that these results are affected by imprecise phenotyping of cases, which is one of the many challenges in the systematic comparison between the two diseases.

\subsection{Relevance of non-coding variants in disease susceptibility}

Approximately 88\% of all GWAS associations map within non-coding regions \parencite{Welter2013}. Psoriasis exome association studies in Chinese and Caucasian populations have increased the number of coding variants with putative effects on the protein structure \parencite{Tang2014, Zuo2015, Dand2017}. These studies have confirmed some previously identified missense associations in \textit{CARD14} and \textit{ERAP1}, revealed new common coding variants at these previously associated loci and identified rare protective missense changes, for example in the \textit{TYK2} gene \parencite{Tang2014,Dand2017}. Nevertheless, results from extensive exome studies suggest that non-synonymous SNPs have a limited contribution to the overall genetic risk of psoriasis compared to non-coding variants \parencite{Tang2014}.

The association of non-coding variants with disease can be explained by their ability to regulate gene expression in a cell and context specific manner \parencite{Fairfax2012}. These variants can be located in different regulatory elements, including enhancer, silencers, promoters and the 5' and 3' untranslated region (UTR) of genes \parencite{Ward2012}. Non-coding GWAS variants can alter the expression of target genes through different mechanisms including changes in chromatin accessibility, histone modifications, protein binding such as transcription factors (TFs), DNA methylation and binding of non-coding RNA molecules \parencite{Knight2014} (\ref{subsec:Epigenetics}).

Identification of the target gene regulated by non-coding variants represents a challenge in the field of functional genetics. This limitation can be partially addressed by conducting expression quantitative trait loci (eQTL) analysis, which identifies genome-wide statistical associations between gene transcript levels and SNPs in \textit{cis} ($<$1Mb) or \textit{trans} to the gene. For instance, in T2D an such approach revealed a \textit{cis}-eQTL involving the TF \textit{KLF4} and a haplotype of non-coding GWAS SNPs located 14kb up-stream \parencite{Small2011}. Moreover, this haplotype also showed association with genes in \textit{trans}, highlighting downstream targets regulated by KLF4. Nonetheless, eQTL mapping alone only provides statistical suggestion of transcriptomic regulation, and additional functional assays, such as chromatin conformation and genome editing, are required to demonstrate causality \parencite{Edwards2013}.

\subsection{The role of GWAS in highlighting immune-relevant cell types and pathways}

\textcolor[rgb]{1,0,0}{GWAS represent a biologically unbiased approach to shed some light on pathophysiological relevant cell types and molecular pathways associated with disease. GWAS have underlined some of the most important cell types for which genetic variation may be functionally relevant by overlapping them with epigenetic features mapped in cell lines or primary cells isolated from healthy control \parencite{Farh2015}. In psoriasis and PsA, enrichment of associated variants has been found for regulatory elements in several cell types (e.g Th-1 and Th-17 cells) and the majority of GWAS risk loci have been linked to genes that belong to a limited number of pathways, as detailed below \parencite{Tsoi2017,Capon2017}. A number of these candidate genes are selected by proximity to the associated variant and genes unknown to be regulated at a distance fail to be include, a limitation when interpreting GWAS results. Additional criteria has also been used to link non-coding GWAS variants to a target gene, including LD with a deleterious variant, direct functional characterisation of the regulatory element and/or genetic variant or association of gene expression with the genotype of the GWAS lead SNP \parencite{Capon2008,Tsoi2012,Tsoi2017,Meglio2011}.

Systematic comparison of the genetic architecture across different conditions has revealed associated psoriasis and PsA risk loci shared, in the same or opposite directions, with AS, Crohn’s disease (CD), multiple sclerosis (MS), RA or type 1 diabetes (T1D) (\url{https://www.immunobase.org}). This has also supported the use of therapeutic interventions such as anti- IL-23 and anti- IL-17 antibodies across a number of immune-related phenotypes including psoriasis, PsA, AS and IBD, amongst others \parencite{Visscher2017}.}

%GWAS represent a biologically unbiased approach to shed some light on pathophysiological relevant cell types and molecular pathways associated with disease. In the field of common immune-mediated diseases, GWAS have underlined some of the most important cell types for which genetic variation is functionally relevant. Better understanding of immune-related diseases has likewise led to identification of shared susceptibility loci and the use of therapeutic interventions across diseases, such as anti- IL-23 and anti- IL-17 antibodies to treat psoriasis, PsA, AS and IBD \parencite{Visscher2017}.

%Systematic comparison of the genetic architecture across different conditions has revealed psoriasis and PsA risk loci to be shared, in the same or opposite directions, with AS, Crohn's disease (CD), multiple sclerosis (MS), RA and type 1 diabetes (T1D) (\url{https://www.immunobase.org}). Interestingly, cross-disease association studies performed for AS, UC, primary sclerosing cholangilitis (PSC), CD and psoriasis has revealed significant overlap of the multi-trait associated loci in regulatory elements in bone marrow, NK and T cells as well as immune response pathways \parencite{Ellinghaus2016}. %This study also identified genetic pleiotropy of psoriasis with AS and CD, illustrating how the same alleles can predispose individuals for different diseases, and demonstrating the contribution of GWAS to the biological understanding of disease.

%In the case of psoriasis and PsA, the majority of GWAS risk loci have been linked to genes that belong to a limited number of pathways and show enrichment for regulatory elements in several cell types \parencite{Capon2017}.

\subsubsection\*{Antigen presentation}

In psoriasis \textit{HLA-Cw\*0602} represents the strongest GWAS association, also shared with other diseases such as hepatitis C, primary sclerosing cholangitis and Grave´s disease \parencite{Blais2011}. No differences at the transcript level have been identified for HLA-Cw\*0602 when comparing psoriasis patients versus controls, suggesting alterations in antigen presentation as the mechanism explaining disease association \parencite{Hundhausen2012}. The relevance of antigen presentation in psoriasis and PsA has been reinforced by the GWAS association of the endoplasmic reticulum aminopeptidase 1 \textit{ERAP1} gene, involved in the trimming of peptide antigens. Moreover, GWAS studies identified that \textit{ERAP1} was associated with psoriasis and PsA only in individuals carrying one copy of the rs10484554 \textit{HLA-C} risk allele \parencite{Strange2010}. %Similarly, the same study identified a dependent association between \textit{HLA-Cw\*0602} and SNPs in the vicinity of the zeta chain of T cell receptor associated protein kinase 70 (\textit{ZAP70}) gene \parencite{Picard2009}.% involved in the regulation of CD8$^+$ cells auto-reactivity

These epistatic phenomena, whereby association of one gene is dependent on the presence of another, have also been reported between \textit{HLA-B\*27} and \textit{ERAP1} in AS \parencite{Evans2011, Cortes2015b}. Interestingly, the \textit{ERAP1} haplotype associated with increased risk of SpA increases \textit{ERAP1} expression and also alters splicing, resulting in an ERAP1 protein isoform with increased activity in monocyte-derived DCs and lymphoblastoid B cell lines \parencite{Constatino2015, Hanson2018}.%which shares signal and direction with psoriasis

\subsubsection\*{Skin barrier}

\textcolor[rgb]{1,0,0}{GWAS have highlighted keratinocyte-specific genes such the previously mentioned \textit{LCE} gene cluster and genes with a key role in skin biology such as \textit{CARD-14}. Further studies in the \textit{PSORS2} region have revealed that association with disease is driven by a deletion in two of the genes within this family, \textit{LCE3B} and \textit{LCE3C} (\textit{LCE3C$\\_ $LCE3B$\\_ $del})\parencite{Cid2009}. Lack of \textit{LCE3B} and \textit{LCE3C} expression in psoriasis patients has been hypothesised to impair the repair following skin disruption, potentially facilitating microorganism infection and triggering a dysregulated immune response \parencite{Bergboer2011}. Similarly to the \textit{LCE} gene cluster, common and rare pathogenic mutations of the PSOR4 \textit{CARD-14}gene lead to increased activation of NF-$\kappa$B as well as overexpression of psoriasis pathophisiocal relevant genes (including \textit{IL-6} and \textit{TNFA}) in keratinocyte cell lines \parencite{Jordan2012}.}

%https://www.sciencedirect.com/science/article/pii/S0002929712001577?via%3Dihub

%GWAS have highlighted KC specific genes such the previously mentioned \textit{LCE} gene cluster and genes with a key role in skin biology such as \textit{CARD-14}. Further studies in the \textit{PSORS4} region have revealed that association with disease is driven by a deletion in two of the genes within this family, \textit{LCE3B} and \textit{LCE3C} (\textit{LCE3C$\\_ $LCE3B$\\_ $del})\parencite{Cid2009}. Expression of \textit{LCE3B} and \textit{LCE3C} is induced upon barrier disruption, where these proteins participate in the formation of the cornified envelope at the most external layer of the epidermis and are likely involved in KC terminal differentiation \parencite{Bergboer2011}.

%The lack of \textit{LCE3B} and \textit{LCE3C} expression in psoriasis patients has been hypothesised to impair the repair following skin disruption, potentially facilitating microorganism infection and triggering a dysregulated immune response \parencite{Bergboer2011}. In fact, the use of UVB radiation has been shown to upregulate \textit{LCE3E} expression 48 hours after treatment, contributing to amelioration of the skin lesions\parencite{Jackson2005}. % Interestingly, epistasia between this deletion and \textit{HLA-Cw\*0602} has also been identified in Dutch and American populations amongst others \parencite{Cid2009, Riveira-Munoz2011}.

%Similarly to the \textit{LCE} gene cluster, \textit{CARD14} is primarily expressed in epithelial tissues mediating the recruitment and activation of the NF-$\kappa$B pathway in this tissue \parencite{Blonska2011}. Common and rare pathogenic mutations of \textit{CARD14} in KC cell lines lead to increased activation of NF-kB as well as overexpression of psoriasis-associated genes including \textit{IL6}, \textit{TNFA} and \textit{TNFAIP2}, among others \parencite{Jordan2012b}.

%%https://www.sciencedirect.com/science/article/pii/S0002929712001577?via%3Dihub

\subsubsection\*{NF-$\kappa$B and TNF pathways}

The NF-$\kappa$B pathway is involved in the regulation of the innate and adaptive immune response and NF-$\kappa$B contributes to the development of many chronic inflammatory diseases \parencite{Liu2017}. In fact, elevated levels of NF-$\kappa$B are present in psoriatic lesional compared to uninvolved and normal skin \parencite{Lizzul2005}.

Several psoriasis and PsA GWAS loci have been mapped to gene members of the NF-$\kappa$B and TNF signalling pathways including \textit{TNIP1}, \textit{TNFAIP3}, \textit{NFKBIA}, \textit{REL}, \textit{TRAF3IP2}, \textit{CHUK}, \textit{IKBKE} and \textit{FASLG} \parencite{Nair2008, Ellinghaus2010, Huffmeier2010, Wang2008, Idel2003, Bowes2012, Tsoi2017}.

%\textit{NF-$\kappa$B} is a dimeric TF that translocates into the nuclei upon cytokine stimuli, including TNF-$\alpha$ itself.

For example, a haplotype including missense mutations and intronic variants in \textit{TRAF3IP2} has been reported to drive psoriasis and PsA association by reducing its affinity for TRAF interacting proteins and concomitantly altering NF-$\kappa$B activation and the IL-17/IL-23 axis\parencite{Huffmeier2010, Ellinghaus2010}. In addition, exome-sequencing studies have identified variants with predicted influence on protein structure and function at \textit{TNFSF15}, a TNF ligand family protein which regulates NF-$\kappa$B and MAP kinases activation in endothelial cells \parencite{Dand2017, Wang2014}.

%The psoriasis and PsA GWAS associations with other members of these pathways, such as the NF-$\kappa$B inhibitor \textit{NFKBIA} and the NF-$\kappa$B subunit \textit{REL}, are solely supported by proximity to nearby intergenic SNPs, with no direct experimental evidence for a role of these genetic variants in regulating the expression of either gene \parencite{GWAS studies}. Moreover, the latest psoriasis and PsA meta-analysis study revealed three additional associations with genes belonging to the NF-$\kappa$B pathway (\textit{CHUK}, \textit{IKBKE} and \textit{FASLG}), further implicating NF-$\kappa$B activation in psoriasis and PsA development \parencite{Tsoi2017}.

%The hypothesised psoriasis and PsA GWAS association with the NF-$\kappa$B inhibitor \textit{NFKBIA} and the NF-$\kappa$B subunit \textit{REL} are solely supported by proximity to nearby intergenic SNPs, with no direct experimental evidence for a role of these genetic variants in regulating the expression of either gene \parencite{GWAS studies}. \textit{REL} has been associated with other inflammatory diseases, including CD and RA \parencite{ImmunoBase} although interestingly, the RA risk allele has a protective effect in PsA \parencite{Bowes2012}.

%The relevance of genes downstream of TNF-$\alpha$ signalling is highlighted by GWAS associations with \textit{TNIP1} and \textit{TNFAIP3}, which participate in the regulation of NF-$\kappa$B activation. For example, knock-in of a region including \textit{Tnfaip3} induces psoriasis in mice and increases the risk of athresoclerosis, one of the most prevalent co-morbidities in psoriasis and PsA \parencite{Wang2008, Idel2003}. Nevertheless, reported immune related pathologies due to constitutive deficiency in NF-$\kappa$B highlights the lack of drugability of this target \parencite{Orange2005,Puel2004}

\subsubsection\*{Type I IFN and innate host defense}

Members of the type-I IFN signalling pathway have also been associated with psoriasis and PsA, highlighting the role of genes contributing to the host response to viruses and bacteria in the disease pathophysiology.

%Mapping of several GWAS loci to genes from the type-I IFN signalling pathway together with clinical and experimental data has reinforced the role of pathogen response in psoriasis and PsA \parencite{Nestle2005}. GWAS associations involved in this IFN response include \textit{IL28RA}, \textit{IFIH1}, \textit{TYK2}, \textit{RNF114}, \textit{ELMO1} and \textit{DDX58}, some of which have been previously reported as susceptibility loci for other immune-mediated diseases (\ref{tab:GWAS\_summary}). For instance, GWAS-lead SNPs causing missense mutations in \textit{TYK2} have been identified in CD, IBD, T1D, RA and MS, in addition to psoriasis and PsA (\url{https://www.immunobase.org}). % \textit{TYK2} is a Janus kinases (JAK) protein member that initiates the IFN type I downstream response \parencite{Calamonici1994}.

Exome-sequencing and GWAS have identified two independent protective missense mutations predicted to impair the catalytic activity of the Janus kinases (JAK) protein member TYK2, and thus the initiation of the IFN-I downstream inflammatory cascade in psoriasis and PsA \parencite{Strange2010, Tsoi2012, Dand2017}. A JAK inhibitor approved for RA is currently undergoing clinical trials in psoriasis and PsA, alongside with development of more specific JAK inhibitors and drugs targeting upstream type I IFN pathway members, such as \textit{TLR7} and \parencite{TLR9} \parencite{Yogo2016,Baker2017}.

%For example, monoclonal Ab against IFN-$\alpha$ subtypes have failed to suppress the IFN gene signature in psoriasis patients and new approaches towards blocking the IFN-$\alpha$ receptor have shown greater efficacy in SLE \parencite{Furie2017}. Psoriasis and PsA GWAS associations with upstream elements of the IFN I pathway such as intronic variants in \textit{ELMO1} gene may distort the activation of the pathogen-sensing receptors \textit{TLR7} and \parencite{TLR9} and hence IFN-$\alpha$ production in pDC \parencite{Tsoi2012}.Clinical trials testing inhibitors of these TLR receptors are being conducted in SLE \parencite{Baker2017}.

%IFNGR for type II IFN inhibition could be added

\subsubsection\*{IL-17/IL-23 axis}

Together with the TNF pathway, the IL-17/IL-23 axis is the most common target of biological therapeutics. In fact, some studies have reported greater efficacy of individual IL-17A or IL-23 blockade compared to TNF inhibition in the treatment of psoriasis and PsA \parencite{Griffiths2015,Blauvelt2017}.

The cytokine IL-23 is formed of two subunits: IL-23A/p19 and IL-12B/p40. Transcriptional studies have shown increased levels of p40 and p19 in psoriasis lesional skin and a role for both subunits in abnormal KC differentiation \parencite{Lee2004,Zhu2011}. Psoriasis and PsA GWAS associations with \textit{IL23R} have been reported, including a protective two SNP haplotype shared with CD \parencite{Nair2008, Strange2010, Tsoi2012}. GWAS associations have also been established implicating \textit{IL-23A} and \textit{IL-12} \parencite{Cargill2007,Strange2010,Tsoi2012}.

%Under inflammatory conditions, the arginine to glutamine (Arg381Gln) exchange in the IL23R showed a protective effect in CD \parencite{Duerr2006}. Conversely, this haplotype is not associated with psoriasis risk in Chinese population where a different non-synonymous potentially damaging variant has been reported as the putative mechanisms \parencite{Tang2014}.

Interestingly, an \textit{IL-23} signal secondary to that reported by Tsoi \textit{et al.,} 2012 has been specifically associated with PsA \parencite{Tsoi2012,Bowes2015}. Regarding the genetics of the Th-17 pathway, its relevance is partly explained through the cross-talk with the IL-23 response, which mediates Th-17 cell differentiation and activation. Additionally, GWAS associations implicating TFs regulating Th-17 polarisation, such as \textit{IRF4} and \textit{STAT3}, have also been identified for psoriasis and PsA \parencite{ Tsoi2012,Huber2008,Harris2007}.

%These GWAS associations are also reported for other immune-mediated disease including CD and MS \parencite{, Immunobase}. Moreover, previously mentioned GWAS associations in the NF-$\kapa$B and TNF pathways such as \textit{TRAF3IP2}, \textit{NFKBIZ} and \textit{TYK2} are also shared with the IL-23/IL-17 axis, stressing not only the relevance of this pathway but also the importance of pathway cross-talk.

% Interestingly, the inhibition of IL-17A using secukinumab is effective in the treatment of psoriasis, PsA and AS, whereas it worsens CD for which the treatment using antibodies against IL-12/23p40, as the previously mentioned ustekinumab, have a much prolonged benefit compared to the other diseases \parencite{Patel2012,Hueber2012,Blauvelt2017b} .Overall, this stresses the importance of the Th17/IL-23 axis in inflammation and demonstrates that blocking the pathway at different levels translates into different effects within and across inflammatory diseases.

\subsubsection{Genome-wide pathway enrichment analysis and intergenic regions}

New approaches using genetic association data have disclosed relevant biological processes by conducting genome-wide pathway analysis. %This analysis represents a more powerful and biological meaningful way than GWAS to study the association of functionally related genes with disease risk.

In psoriasis, this has revealed association of novel processes, such as retinol metabolism, transport of inorganic ions and aminoacids and post-translational protein modifications (PTMs) \parencite{Aterido2015}.

As previously mentioned, the majority of the non-coding GWAS associations are located in intergenic regions and often lack functional characterisation. Therefore these variants tend to be associated to the nearest gene but may occur in intergenic regions at a distance from any gene, including chr1p36.23, chr2p15 and chr9q31.2 in psoriasis and PsA. One of the most interesting regions the chr1p36.23, shared with UC and proximal to a number of gene candidates including \textit{RERE}, \textit{SLC45A1}, \textit{ERRFI1} and \textit{TNFRSF9} \parencite{Tsoi2012}. Unpublished capture-HiC data using the immortalised keratinocyte cell line HaCaT has revealed interaction of SNPs in this locus with the promoter of the \textit{ERRFI1} gene, an inhibitor of the epidermal growth factor receptor signalling required for normal keratinocyte proliferation \parencite{Ray-Jones2017}. %Nevertheless, the same locus could be an enhancer for other nearby genes depending on the cell type, reinforcing the importance of the cell type specificity in functional studies.

\subsection{Limitations and future of GWAS}

GWAS have made a great contribution to our understanding of the genetic basis of complex diseases. However, this approach has a number of limitations that need to be considered.

One of the major limitations is the challenge of fine-mapping due to linkage disequilibrium (LD). An association between a genetic locus and a trait does not reveal the causal variant, which could potentially be any of the highly correlated SNPs in the same LD block at the lead SNP. This can be addressed in part by dense genotyping, statistical fine-mapping methods and incorporation of epigenetic data while ultimately application of genome-wide editing may be needed to define the GWAS causal SNP.

Another concern is the missing heritability when that explained by the GWAS is compared to the estimated heritability from twin and family studies \parencite{Ku2010, Yang2010}. Since complex traits are influenced by polygenic effects, where the genetic contribution is driven by multiple variants with small effect size, larger experimental cohorts have led to the discovery of new genome-wide significant associations \parencite{Visscher2017}. For example, in human height, most of the missing heritability could be explained by GWAS associated variants with nominal significance that failed to pass the stringent threshold due to their small effect size \parencite{Yang2010}.

Another source of unexplained heritability may be rare putative causal variants poorly tagged by common SNPs \parencite{Wray2005}. Such limitations have partly been overcome by improved genotyping arrays like Immunochip, which incorporates SNPs with MAF${<}$1\% \parencite{Cortes2011}. Moreover, exome studies have also demonstrated the contribution of coding and intronic rare variants (MAF${<}$5\%) in the genetic architecture of complex traits such as height or psoriasis \parencite{ Marouli2017, Dand2017}. In addition to rare variants, other sources of structural variation such as copy number variants (CNVs), small ($<$1Kb) insertions/deletions (indels) and inversions could all contribute to missing heritability. Incorporation of new genotyping platforms has allowed the genome-wide identification of CNV while the accurate detection of translocations and inversions relies on the implementation of long read whole genome sequencing (WGS) technologies \parencite{Glessner2009,Marshall2017,Visscher2017}. Lastly, the missing heritability may also be the consequence of the overestimated heritability in complex traits as the result of assuming additive genetic effect instead of epistatic interaction between the different associated loci \parencite{Zuk2012}.

%In addition to rare variants, other sources of common variation such as CNV, small (<1Kb) insertions/deletions (indels) and inversions could contribute to the missing heritability. The 1000 Genome Project and HapMap have helped to better understand these other sources of variation and later genotyping platforms such as the Illumina Human 1M Beadchip, the Affymetrix 6.0 and the Immunochip have included probes for CNV and small indels \parencite{Ku2010}. Incorporation of new genotyping platforms have has allowed to identifythe identification of genome-wide associations of CNV with autism and schizophrenia, among others \parencite{Glessner2009,Marshall2017}. CNV in \textit{LCE} has been proved to be the causal for the association to psoriasis and PsA, as previously mentioned \parencite{Cid2009}. However, genome-wide studies have failed to yield reproducible results \parencite{Uebe2017}.

%%Examples of CNV (mention psoriasis LCE and CARD14 and also big study about CNV) and also explanation of translocations in the heritability

%%Talk about exome sequencing and WGS

%It is clear that as the whole-genome sequencing (WGS) technologies became more affordable they will naturally replace to the genotyping arrays in the GWAS. Currently, examples of some diseases whole genome sequencing

%%Case of the genome rearrangements

%In the case of translocations and inversions, neither arrays nor widely-used short reads NGS technologies are appropriate to detect this type of variation. Although this type of variation has a role in several disease genotypes \parencite{Feuk2010}, detection of translocations and inversions at a genome-wide scale is still very and their real frequency understimated \parencite{Ku2010}. There are some statistical methods that use dense SNP genotyping to detect an unusual LD pattern among the SNPs as a read oy for chromosome rearrangements \parencite{Bansal2007}. Nevertheless, implementation of WGS using long reads sequencing technologies are the best tool to accurately assess this genetic variability \parencite{Visscher2017}.

%From omnigenic to polygenic

%Talk about Andy/Ola project with nanopore...: nanopore gives longer reads to build the structure of the genome in terms of positions of the fragments and then you can do WGS illumina+10X and then you can incorporate nanostring where you have sequence tags across the entire chromosome and runs the entire chromosome through the sequencing chip to get those and then place the sequences in right orientation and order. Overall this tries to uncover the role of structural variation in complex diseases in particular regions such as the MHC. In the OBB some individuals have very particular MHC haplotypes that may suggest structural variation can play a role in this region. also interesting to identify changes of structural variation between cell types (may be somatic) could affect the role of different cell types in disease

\section{Functional interpretation of GWAS in complex diseases}

\subsection{Overcoming the limitations of GWAS: post-GWAS studies}

\textcolor[rgb]{1,0,0}{GWAS report associations with disease for particular locus but typically fail to identify the true causal variant(s) within the haplotype block, yielding a large number of SNPs in high LD with the lead variant \parencite{Edwards2013}. Thus, refinement of the number of putative causal variants for each GWAS association is required previous to functional validation of their putative pathogenic effect through molecular and cellular assays and \textit{in vivo} models. Statistical fine-mapping partially overcomes some of the GWAS limitations by further refining the number of most likely causal variants driving disease association within each GWAS LD block. The integration of statistical fine-mapping with cell type and context specific epigenetic data, including chromatin accessibility, histone modifications and DNA methylation, can help to determine the chromatin state where the fine-mapped variants are located and its potential in regulating gene expression \parencite{Petronis2010}. Additionally, the incorporation of gene expression, eQTL analysis and chromatin interaction data can establish a relationship between non-coding variants and putative gene targets.}

\subsection{Understanding the epigenetic landscape in complex diseases}

\label{subsec:Epigenetics}

Epigenetic modifications consist of heritable changes in the phenotype and/or gene expression that do not involve changes in the DNA sequence \parencite{Feil2012}. These changes include a wide range of modification in the proteins which serve as scaffold for the DNA, known as histones, as well as DNA methylation and non-coding RNAs. Environmental and intrinsic factors can trigger changes in the epigenome that result in dysregulation of gene expression and, consequently, in alteration of gene function.

Genetic background can increase the predisposition to epigenetic changes caused by extrinsic factors. Studies have demonstrated differences in response to environmental factors by different mice breeds as well as greater differences in the epigenetic landscape between human dizygotic twins when compared to monozygotic \parencite{Pogribny2009,Kaminsky2009}. Importantly, disease-associated GWAS variants have consistently shown enrichment for DNA regulatory elements, characterised by the combination of epigenetic marks, including accessible chromatin, histone modifications and DNA methylation \parencite{Trynka2013,Trynka2013b,Gusev2014}.

The plasticity of the epigenetic landscape is required for cell differentiation and identity and particularly important in the immune system to ensure adaptation and response to different pathogen infections \parencite{Yosef2016}. The role of cell type specificity in the epigenetic landscape has been demonstrated in eQTL studies, where 50 to 90\% the genetic variants regulating gene expression are cell type and stimulus dependent \parencite{Dimas2009,Nica2011,Fairfax2012,Fairfax2014,Raj2014,Naranbhai2015,Kasela2017}. Recent methodological advances have made the personalised study and understanding of the epigenome possible by the implementation of low-cell-input high-throughput techniques coupled to next generation sequencing (NGS) \parencite{Buenrostro2013, Schmidl2016,Oudelaar2017}. Understanding of cell-to-cell epigenomic heterogeneity is also being addressed with single-cell methods and may help to elucidate the impact of genetic variability in regulation of gene expression and disease mechanisms \parencite{Buenrostro2015, Cusanovich2015,Rotem2015,Nagano2013,Smallwood2014}.

%The functional relevance of epigenetic changes in the regulation of gene expression has stressed the relevance of performing epigenome-wide association studies(EWAS), which also allow a more cell type specific approach, instrumental to understand complex diseases. These studies are particularly relevant given the plasticity of the epigenome that would allow using those risk associated changes as potential drug targets, alike genetic variants that are more challenging for alteration. As an example, a DNA methylation EWAS in psoriasis skin samples revealed nine disease-associated differentially methylated sites as result of disease status and environmental factors rather than genetic effects \parencite{Zhou2016}.

\subsection{The chromatin landscape}

In the cell nucleus, DNA is compacted into a highly organised structure known as chromatin. The nucleosome is the basic repeating unit of chromatin and is formed by a 147bp segment of DNA wrapped around an octamere core of histone proteins regularly spaced by 10bp of linker DNA \parencite{Luger1997}. In general, highly compacted DNA will remain more inaccessible for the assembly of the transcriptional machinery, consequently preventing gene expression. Chromatin accessibility can be altered by PTM of the histone proteins that affects their affinity with the DNA within the nulcleosome as well as the interaction between nucleosomes in the vicinity \parencite{Polach2000,Pepenella2014}. Additionally, chromatin structure can also be influenced by adenosin triphosphate (ATP)-remodelling complexes that facilitate sliding of individual nucleosomes to neighboring DNA segments, increasing temporary chromatin accessibility at particular sites \parencite{Cosma1999}. From the biochemical point of view, the signature of chromatin accessibility, histone modifications, transcription factor occupancy and DNA methylation has been used to identifying \textit{cis}-regulatory elements such as promoters, enhancers, silencers, insulators and locus control regions, and define the cellular chromatin landscape \parencite{Boyle2012,Kundaje2015}.

\subsubsection{Methods to ascertain chromatin accessibility}

Accessible chromatin constitutes about 1\% of the human genome and represents a very robust marker for histone modifications, early replication regions, transcription start sites (TSS) and TF binding sites (TFBSs) \parencite{ENCODE2007}. The informativeness of chromatin accessibility for understanding gene regulation has driven the development of several high-throughput techniques for accurately tagging these regions. Amongst those techniques, the \textquotedblleft gold standard \textquotedblright is DNase I hypersensitive sites sequencing (DNase-seq), which uses the non-specific double strand endonuclease DNase I to preferentially cut on nucleosome-free regions known as DNase hypersensitive sites (DHSs). In this approach, isolation of the chromatin-free DNA is followed by further enzymatic digestion and DNA library preparation prior to NGS \parencite{John2013}. DNase-seq also provides high quality information regarding TFBS, generating footprints that identify TF binding in relation to chromatin structure \parencite{Hesselberth2009,Boyle2010}.

Another method to interrogate the accessible genome is formaldehyde-assisted isolation of regulatory elements (FAIRE-seq), which uses formaldehyde cross-linking, sonication and phenol-chloroform extraction to remove the DNA-protein complexes and retain only the nucleosome-depleted regions that undergo NGS \parencite{Giresi2006}. Both methods have enabled ENCODE to map regulatory elements in several cell lines, primary cells and tissues, revealing that 76.6\% of all non-coding GWAS SNPs together with those in complete LD are located within broadly accessible chromatin tagged by DHSs \parencite{ENCODE2007,Buck2014,Gaulton2010, Maurano2012}.

Indirect measurement of the chromatin accessibility has also been performed using micrococcal nuclease-sequencing (MNase-seq), which retains nucleosome-bound material for downstream sequencing, providing a qualitative and quantitative comprehensive map for nucleosome positioning and also TF occupancy \parencite{Axel1975,Ponts2010}. The high number of cells (5 to 10 millions or more) required by these assays for good quality data limits their application to particular biological and clinical samples.

Recently, a new technique assay for transposase-accessible chromatin using sequencing (ATAC-seq) has represented a groundbreaking step in characterisation of the genomic regulatory landscape \parencite{Buenrostro2013}. ATAC-seq is based on an engineered hyperactive transposase enzyme, known as Tn5, that preferentially accesses and tags nucleosome-free and inter-nucleosomal DNA using lower number of cells and shorter processing times compared to DNase-seq \parencite{Gradman2008, Adey2010}. This makes ATAC-seq a very versatile technique to interrogate the chromatin landscape in a clinical set-up, where sample availability and time-efficiency are key factors \parencite{Scharer2016,Qu2015,Qu2017}.

\subsubsection{The role of histone modifications and TF occupancy in the chromatin landscape}

Identifying the combination of histone modifications and binding of TF is essential to characterise regulatory regions of the genome. Histone modifications take place in the NH$\_2$ terminal tail that protrudes from the nucleosome, the most common modifications being acetylation, phosphorylation and methylation. The co-localisation of different histone marks modulate the affinity for DNA-binding proteins and the interaction with neighboring nucleosomes in varied manners, contributing to the overall chromatin accessibility landscape of the cells \parencite{Jenuwein2001, Bannister2011}.

The combination of histone modifications can be used to broadly divide chromatin into condensed non-transcribed heterochromatin and accessible transcriptionally active euchromatin. Further studies have identified facultative and constitutive heterochromatin, which distinguishes spatially and temporally regulated genes from those permanent silenced, respectively. Facultative heterochromatin is enriched for H3K27me3 and the polycomb repressor complexes (PRCs), whilst constitutive heterochromatin is marked by H3K9me3 \parencite{Hansen2008,Bannister2001}.

Several types of chromatin corresponding to different regulatory elements have also been defined. Enhancers and promoters, regardless of their activation state, are tagged by high levels of H3K4me1 or H3K4me3, respectively, and both features co-localise with H3K4me2 modifications \parencite{Heintzman2007,Hon2009}. H3K9ac is specifically enriched at active promoters whereas H3K27ac generally designates activation at both promoters and enhancers \parencite{Hon2009,Creyghton2010}. Conversely, H3K27me3 together with the heterochromatin mark H3K9me3 indicates gene repression at promoter elements \parencite{Hansen2008,Bannister2001,Pan2007}. Interestingly, GWAS variants for different complex diseases have demonstrated to be relatively enriched for some of those modifications, importantly H3K4me3, H3K9ac, H3K79me2, H3K4me1 and H3K36me3 \parencite{Ernst2011, Trynka2013}. Overall, functional understanding and interpretation of histone mark co-localisation remains challenging and incorporation of additional epigenetic information is usually required.

Together with histone modifications, TF also play a role in nucleosome positioning as well as in acting as boundary elements to separate chromatin states \parencite{Vierstra2014,Zhang2009,Bell2000}. TF occupancy is indirectly tagged by chromatin accessibility assays, such as DHS, through reduced cutting sensitivity of DNase I due to protein binding and steric hindrance.

Chromatin immunoprecipitation sequencing (ChIP-seq) has been widely to precisely locate histone modifications and TF binding in the genome. This technique assays protein-DNA binding \textit{in vivo} using Abs that specifically recognise histone modifications or TF after DNA-protein cross-linking and sonication. Following immunoprecipitation of the desired DNA-protein complexes with the appropriate Ab, the cross-linking is reversed and the proteins digested prior to DNA library preparation and sequencing \parencite{Solomon1988,Barski2007,Johnson2007}. ChIP-seq has been used to analyse a wide range of histone modifications and TF binding in different cell lines, primary cells and tissues \parencite{ENCODE2012,Bernstein2010,Adams2012}. Similarly to the first generation of chromatin accessibility techniques, ChIP-seq requires at least between 5 to 10 million cells per experiment, restricting its application to the availability of biological material. In order to overcome this limitation, a wide range of protocols have been developed, of which ChIPmentation (ChIPm) appears as the simplest and most cost-effective method, only requiring 10,000 and 100,000 cells to assay histone modifications or TF binding, respectively \parencite{Schmidl2016}. ChIPm involves the use of the Tn5 transposase, accelerating library preparation and increasing the sensitivity of the results.

\subsubsection{DNA methylation}

DNA methylation involves the transferal of a methyl group to the 5' carbon of a cytosine that precedes a guanine nucleotide (CpG sites) by a group of enzymes known as DNA methyl-transferase (DNMTs). CpG islands are found along the entire genome and their methylation generally associates with repression of gene expression \parencite{Herman2003}. Together with histone modifications, DNA methylation has a pivotal role in the differentiation of haematopoietic stem cells and the maturation and activation of immune cells \parencite{Sellars2015,Lai2013}.

%Whole-genome bisulfite sequencing (WGBS) and bead array hybridisation are currently the most widely used methods to characterise DNA methylation. Both are based on bisulfite treatment of DNA, whichconverts cytosine into uracil prior to sequencing or probe hybridisation \parencite{Frommer1992,Miura2014,Dedeurwaerder2013}. The use of methylome arrays such as the Illumina HumanMethylation450 Bead Chip is the most cost-effective strategy to detect functionally relevant differences in methylation focusing on the major known regulatory CpG islands \parencite{Tserel2015,Bonder2017}.

The pathogenicity of changes in the methylome has been studied in a range of diseases including RA, systemic lupus erythematosus (SLE), psoriasis and PsA \parencite{Lei2009,Liu2013,Zhang2010}. For example, regulation of TNF-$\alpha$ production upon inflammatory stimuli involves a complex network of DNMTs that alter the methylation signature at the locus \parencite{Sullivan2007}. % Interestingly, DNA methylation is tightly coordinated with histone methylation and the repressive mark H3K9me3 is involved in driving DNA methylation at the same site \parencite{Rottach2009}.

%This ability to ascertain the epigenome profile in clinical samples has also enabled epigenome-wide association studies (EWAS) that identify CpG methylation changes between patients and controls in a cell type specific manner \parencite{Zhou2016}.

\subsubsection{Chromatin interactions and gene expression}

The functional understanding of non-coding variants has benefited from eQTL studies. Nevertheless, eQTLs only provide indirect evidence of the effect of a SNP on regulating expression of a particular gene. Since enhancers may not control expression of the closest gene, functional interpretation of GWAS variants requires genome-wide mapping of those chromatin interactions \parencite{Smemo2014}. Chromatin is organised into topologically associating domains (TADs) of several hundred kb insulated from other TADs by the binding of CTCF protein, amongst others \parencite{Nora2017}. Chromatin loops between promoters and the corresponding regulatory elements mostly take place within the same TAD and are highly cell- and context-specific \parencite{Smith2016}. Hence, interrogation of chromatin interactions provides additional evidence for physical contact between enhancers and gene promoters coordinating assembly of the transcriptional machinery and consequently regulating expression. As an example, obesity risk non-coding variants located within the \textit{FTO} gene appeared to regulate expression through chromatin looping of the \textit{IRX3} gene, located 1Mb downstream \parencite{Smemo2014}.

A wide range of genome-wide and high-throughput methods to investigate the 3D chromatin conformation have been developed, being of particular interest Capture-C, as simultaneously scales up the number of interactions investigated at high resolution and minimises the number of cells required\parencite{Davies2017,Oudelaar2017}. Other techniques such as promoter capture HiC have yielded comprehensive immune-specific maps of promoter-enhancer interactions in seventeen human primary hematopoietic cell types \parencite{Javierre2016}. %Lately, HiChIP has improved the integration of ChIP and chromatin interaction methods to enhance the specificity of the assay while reducing sequencing depth and input material \parencite{Mumbach2016}.

\subsection{Transcriptional profiles in disease}

The role of environmental and genetic factors in altering gene expression regulation in complex diseases has been investigated through extensive comparison of case-control transcriptional profiles. The informativeness of this approach is conditional on studying the relevant disease tissue, which sometimes remains challenging due to a lack of pathophysiological understanding of disease mechanisms or difficulties in accessing it. In immune-mediated diseases, peripheral blood mononuclear cells (PBMCs) differential gene expression (DGE) analysis between patients and controls has enabled identification on relevant pathways and biochemical functions in a number of chronic immune diseases, including psoriasis and PsA (further detailed in Chapter \ref{ch:Results2} and \ref{ch:Results3}, respectively) \parencite{Miao2013,Junta2009,Baechler2003,Assassi2010,Batliwalla2005}. The growing evidence supporting cell type and context specificity has also prioritised the use of disease-specific affected tissue over PBMCs, when available, including skin biopsies in psoriasis, synovial-isolated macrophages in RA and B cells and monocytes in SLE \parencite{Katschke2001,Dozmorov2015,Jabbari2012}.

Likewise, the extensive overlap of GWAS variants with non-coding regions potentially dysregulating gene expression has highlighted the importance of performing context-specific eQTL studies. In this respect, consortia such as the Genotype Tissue Expression (GTEx) have generated publicly accessible comprehensive tissue-specific eQTL studies that have greatly contributed to the functional understanding of GWAS risk alleles in many complex diseases \parencite{Lonsdale2013,Fagny2017}. %Lately, eQTL studies have also expanded in the disease context. For instance, an eQTL study in five immune relevant cell types isolated from IBD and anti-neutrophil cytoplasmic antibody-associated vasculitis patients have revealed disease specific eQTLs, some of which disappear following treatment \parencite{Peters2016}.

\subsubsection{Long non-coding RNAs and enhancer RNAs}

In addition to protein coding mRNAs, non-coding RNAs have been demonstrated to have a role in regulation of gene expression. One category of non-coding RNAs are the long non-coding RNAs (lncRNAs), transcripts between 200 and 100Kb long that undergo splicing, 5' capping and 3' poly-adenylation \parencite{Derrien2012}. LncRNAs can positively and negatively regulate transcription through different mechanisms including guidance of chromatin modifiers such as DMTs and PRCs to specific loci, alteration of mRNA stability, translational control, and acting as a decoy for other non-coding RNAs and regulatory proteins \parencite{Pandey2008,Faghihi2008,Gong2011,Carrieri2012, Kino2010}. %A large number of lncRNAs with different functions have been identified and different categories have been established based on location (nucleus or cytoplasm) and mechanism of action \parencite{Rinn2012, Fatica2014}.

Amongst the characterised lncRNAs, many have been demonstrated to play a role in the regulation of the innate and adaptive immune response, for example in T cell activation and host-pathogen interactions \parencite{Pang2009, Rossetto2012}. Moreover, differential case-control gene expression analyses have underscored the contribution of lncRNAs in several chronic inflammatory conditions, including RA, SLE and psoriasis \parencite{Mueller2014,Shi2014,Li2014,Ahn2016}.

A particularly relevant type of lncRNAs are the enhancer RNAs (eRNAs), shorter molecules compared to the canonical lncRNAs (approximately 346 nucleotides) that do not undergo splicing or poly-adenylation \parencite{FANTOM2014}. Although traditionally chromatin segmentation maps have defined enhancers as DNA regions with particular epigenetic characteristics, later studies have shown their ability to be bi-directionally transcribed into eRNAs molecules \parencite{De Santa2010, Kim2010}. Importantly, the transcriptional activity of enhancers has been demonstrated to be an excellent proxy to identify functionally active regulatory region, which have also been successfully validated by reporter assays \parencite{FANTOM2014, Anderssen2014}.

Another class of non-coding RNAs are micro-RNAs (miRNAs), between 21 to 24 nucleotides long \parencite{Lee2002}. Under particular conditions, expression of genes containing complementary sequences to miRNAs are commonly negatively regulated through assembly of the miRNA-induced silencing complex followed by mRNA degradation, mRNA destabilisation or translational repression with 30 and 80\% of human genes predicted to be under transcriptional control of miRNAs \parencite{Ameres2010,Braun2011,Petersen2006,Lewis2005, Friedman2008}. %Joint efforts have resulted into experimental characterisation of a number of miRNAs that are catalogued and updated in the publicly accessible miRNA Registry \parencite{Griffiths-Jones2004}. Moreover, several studies highlighting the role of miRNA transcriptional regulation in disease have been conducted, particularly in the context of immune-mediated complex diseases, including psoriasis \parencite{Lerman2011}.

\subsubsection{Methods to assay gene expression}

RNA sequencing (RNA-seq) involves reverse-transcription of the extracted RNA into cDNA and PCR amplification preserving relative abundance of each transcript, followed by library preparation and NGS \parencite{Mortazavi2008}. Systematic comparison has shown superior dynamic range of detection for RNA-seq compared to micro-arrays, particularly for low abundance transcripts \parencite{Zhao2014}. Furthermore, RNA-seq allows the capture of additional information to the expression profile, including the identification of new exons, alternative splicing events and allele-specific expression (ASE).

%For example, regulation of gene activity through differential isoform usage is very common between different tissues and during particular biological processes. RNA-seq isoform quantification has highlighted that differentiation of CD4$^+$ T cells into the pro-inflammatory Th-17 is particularly driven by one of the nuclear receptor RORC$\gamma$ isoforms \parencite{Zhao2014}. %Several methods have been developed to perform differential exon and isoform quantification with different strengths and limitations in their performance \parencite{Steijger2013,Ding2017}.

Quantification of ASE through RNA-seq has provided direct evidence for local/\textit{cis}-eQTLs driven by allele-specific mechanism in up to 88\% of the genes with an associated \textit{cis}-eQTL\parencite{Yan2002,Pickrell2010}.%It is in the aPsA intro

%scRNA-seq does not require prior isolation of populations based on a panel of surface or intra-cellular molecular markers by FACS and identifies cell-to-cell variation and rare populations using the transcriptomic profiles of thousand of cells. scRNA-seq has importantly contributed to the field of immunology identifying new subsets of DCs and monocyte populations involved in mounting the immune response and re-defining the atlas of human blood myeloid cells \parencite{Jaitin2014, Villani2017}.

Additionally, 5’end RNA-sequencing methods such as cap analysis of gene expression (CAGE) has been used to quantify eRNAs by the functional annotation of the mammalian genome 5' (FANTOM5) Consortium, contributing to a better definition of enhancers and their spatial and temporal specificity in hundreds of human primary cells and tissues \parencite{FANTOM2014,Andersson2014}.

%Importantly, this data has stressed the relevance of mapping not only histone marks and DHSs but also eRNAs to confidently distinguish active enhancers from putative regulatory regions non-functional in a particular cell type, tissue or condition.

Lastly, development of single-cell RNA-seq (scRNA-seq) has enabled the identification of cell sub-populations within a tissue in an unbiased way \parencite{Tang2009, Tang2010}.

\subsection{Transcriptional regulation in complex diseases}

Non-coding GWAS variants can exert pathogenic effects by affecting one or many of the previously described mechanisms responsible for the fine regulation of gene expression in homeostatic conditions. For example, intronic SNPs can influence mRNA splicing through exon skipping, resulting in truncated but functional proteins. For instance, exon skipping caused by an intronic risk allele at the TNF Receptor Superfamily Member 1A (\textit{TNFRSF1A}) associated with MS results in a soluble isoform of the TNFRS1A protein with TNF antagonistic function \parencite{Gregory2012}. On the other hands, non-coding variants at enhancers, silencers and promoters can dysregulate gene expression by altering affinity at TFBS, histone modifications and chromatin accessibility. For instance, in thyroid autoimmunity, the risk allele of an intronic SNP in the thyroid stimulating hormone receptor \textit(TSHR) gene reduces \textit{TSHR} protein expression in IFN-$\alpha$ stimulated thyroid cells \parencite{Stefan2014}. The risk SNP increases the affinity of the repressor promyelocytic leukemia zinc finger protein (\textit{PLZF}) that recruits histone acetylases (HDACs) to the locus, resulting in impaired tolerance to thyroid auto-antigens. Alterations in TF binding can also affect looping and long-range chromatin interactions between enhancers and promoters. For instance, in prostate cancer this phenomenon causes upregulated expression of the oncogene \textit{SOX9} due to increased enhancer activity and enhancer-promoter interaction \parencite{Zhang2012}.

Alternatively, non-coding SNPs can regulate gene expression by creating a new promoter-like element, as in the $\alpha$- thalassemia disease, where this phenomenon leads to dysregulated downstream activation of all $\alpha$-like globin genes in erythroid cells \parencite{Gobbi2006}. Genetic variants at eRNAs can also affect regulation of gene expression as it has been demonstrated in the nuclear receptor for anti-diabetic drugs PPAR$\gamma$ in mice \parencite{Soccio2015}. Lastly, non-coding variants placed in UTRs and intergenic regions can affect binding of miRNAs and lncRNA to the target genes. This is the case of a CD associated variant at the 3'UTR of the gene immunity related GTPase M \textit{IRGM} which reduces binding of the miR-196, increasing its mRNA stability and translation, ultimately resulting in disrrupted autophagy \parencite{Brest2011}. In psoriasis and PsA, some specific SNPs located at 3' UTR of genes such as \textit{IL-23}, \textit{TRAF3IP2} or \textit{SOCS1} have been hypothesised to disrupt or create \textit{de novo} miRNA binding sites, but no experimental evidence has been provided yet \parencite{Pivarcsi2014}.

\subsection{The use of fine-mapping to prioritise functional causal variants}

The aim of fine-mapping is to reduce the size of GWAS genomic intervals and yield a minimal set of SNPs containing the causal variant that will explain most of the association for that particular locus \parencite{Spain2015}. Fine-mapping studies require extensive genotyping to meet the assumption that the putative causal variant will be likely interrogated in the analysis. This can be achieved by WGS, dense genotyping arrays and \textit{in silico} imputation using publicly available data. The use of the Immunochip array across most of the immune-mediated inflammatory diseases has increased the genotyping density at previously associated immune-relevant loci in a cost-effective manner \parencite{Trynka2011}. Similarly, imputation methods using WGS reference panels, such as HapMap and 1000 Genomes Project, have offered genome-wide coverage for SNPs and CNVs with MAF $>$1\% across different ancestry groups \parencite{Abecasis2012}. More recently, the UK10K project has improved the quality of imputation specifically for rare variants with MAF between 0.01 and 0.5\% \parencite{Chou2016}.

%Interestingly, exhaustive fine-mapping using a customised genotyping array has been conducted for eight psoriasis GWAS loci using a frequentist approach which measure the association of each SNP through p-values \parencite{Das2014}.

Bayesian statistical analysis has been chosen over the frequentist approach (based on p-value calculations) to increase the resolution of the GWAS associations and facilitate the identification of relevant genes and disease mechanism. Bayesian fine-mapping quantifies the evidence of association for each of the genotyped or imputed SNPs as Bayes Factor (BF). BFs are later used to calculate posterior probabilities (PP) which represent the probability of each SNP to drive a particular association \parencite{Wakefield2007}. Since including only the most significant fine-mapped SNP would miss the causal variant in approximately 97.6\% of loci, the Bayesian strategies report a credible set of SNPs which includes those variants capturing 95 or 99\% of the cumulative PP in each loci \parencite{Bunt2015}. This strategy has proven further refinement and reduction of false-positives compared to inclusion of all the SNPs in high LD (e.g r$^2\geq$0.8) with the lead variant\parencite{Bunt2015}. Furthermore, inclusion of functional data from publicly available sources as priors of the approximate Bayesian model has demonstrated a reduction of the number of SNPs in the credible set and also increased the proportion of successfully fine-mapped loci \parencite{Bunt2015, Kichaev2015}. The integration of fine-mapping data generated with the Bayesian probabilistic identification of causal SNPs (PICS) method and a map of genomic regulatory elements, revealed that approximately 60\% of the top fine-mapped SNPs overlapped enhancer elements (importantly stimulus-specific) and were very close but not within TF binding sites (TFBS) \parencite{Farh2015}.

%Fine-mapping can also benefit from the integration of epigenetic data generated in clinical samples and rare populations using the latest methodological improvements previously reviewed. The advances in the field of epigenetics have also led to the generation of more accurate chromatin states maps based on larger number of samples that have been integrated with fine-mapping strategies highlighted promising potential GWAS causal variants in T2D \parencite{Thurner2018}

%with \textit{cis}-regulatory elements for thirty-three immune cell types \parencite{Farh2015}. Interestingly, the top fine-mapped causal variants presented the greatest enrichment ($\sim$60\%) for enhancer elements, particularly for those in activated cell types and also for DHS and TF binding sites. In the particular case of psoriasis, PICS prioritised SNPs showed enrichment for Th0 na\"{i}ve CD4$^+$ T cells followed by Th1, Th2 and Th17 CD4$^+$ subsets. Recently, publicly available tools such as fGWAS and PAINTOR have leveraged cell type-specific annotation to inform the Bayesian analysis and output a further refine credible set of SNPs with functional relevance \parencite{Pickrell2014,Kichaev2015}.

%Specific case for psoriasis https://academic.oup.com/nar/article/44/18/e144/2468351 maybe to include in the specific chapter?

\subsection{Integration and interpretation of genomic data}

The evolution of different –omics methods towards generation of paired datasets at a high-throughput scale presents a challenge in terms of interpretation and integration. This is particularly important in the field of complex diseases resulting from the interaction of many risk variants, with small or moderate effect, that involve several genes and signalling pathways through alteration of epigenetic features and dysregulation of gene expression.

Tools such as RegulomeDB allow the querying of a large number of publicly available epigenetic and functional datasets, including DHSs, TFBS, histone modification and DNA-protein interactions, at the SNP level \parencite{Boyle2012}. Other powerful tools include the University of California Santa Cruz (UCSC) genome browser, a resource to display in-house and publicly accessible annotation data \parencite{Kent2002}. In addition to this, international consortia generating large-scale epigenetic and expression data such as ENCODE, Blueprint, the Roadmap Epigenomics Project, GTEx or FANTOM have created comprehensive website resources for browsing and downloading data \parencite{ENCODE2007,Lonsdale2013, FANTOM2014,Adams2012 }. These collaborations have also led to the integration of epigenetic datasets and assembling of cell type specific chromatin states maps. This consists of the segmentation and labelling of the genome with a chromatin state based on concurrence of several epigenetic marks using Hidden Markov Model algorithms such as ChromHMM, amongst others \parencite{Ernst2010, Ernst2011,Hoffman2013, Kundaje2015 }.

%Amongst the most comprehensive chromatin segmentation maps, The Roadmap Epigenome Project has released chromatin state maps defining eight active and seven repressed regulatory states for a total of 111 elements, including primary cells and tissues \parencite{Kundaje2015, Ernst2011}.

In addition to data integration, the other main bottleneck encountered by functional genomics is determining the clinical relevance of GWAS SNPs, eQTLs, differentially expressed genes or differentially epigenetic modified regions. This can be addressed by performing enrichment analysis, which tests for statistically significant over-representation of particular annotation terms (e.g ontologies, signalling pathways or functional elements) within the entities of interest. For instance, pathway enrichment analysis uses functional units containing related genes defined by prior knowledge. Amongst the most comprehensive and informative pathways sources are The Kyoto Encyclopedia of Genes and Genomes (KEGG) and the REACTOME, which also considers biochemical reactions such as binding, activation or protein translocation \parencite{Kanehisa2000, Fabregat2018}. Such annotation sources may be used to interpret, for example, a set of differentially expressed genes or a list of genes obtained through annotation of non-coding regions using proximity, chromatin interaction data or eQTL studies. Similarly, this type of analysis can be used to find enrichment of genomic regions of interest for a varied collection of epigenomic features tagging regulatory elements in relevant cell types.% For example, of the latest meta-analysis psoriasis GWAS hits were enriched for enhancers in Th-1,Th-17 and CD8$^+$ T cells \parencite{Tsoi2017}.

From the number of tools designed to perform this type of analysis, eXploring Genomic Relations (XGR) is particularly powerful \parencite{Fang2016}. XGR is an open source R package and web-app that allows handling of different types of input data (SNPs, genes and regions). XGR integrates a wide range of ontologies and up to date publicly available functional data to perform different types of annotation and enrichment analysis, facilitating background customisation for reliable and meaningful output results. Moreover, XGR also performs gene network analysis from the same inputs as the pathway analysis. This leverages experimentally validated interaction information to identify gene networks modulated by putative pathogenic variants, improving interpretation through consideration of network connectivity.