\section{Discussion}

\subsection{Characterising chromatin accessibility in PsA samples}

Technological advances in the field of epigenetics has enabled characterization of the epigenetic landscape in immune cells isolated from PB and SF in PsA patients. The cell types included in this study (CD14$^+$ monocytes, mCD4$^+$, mCD8$^+$ and NK) represent key players in the innate and adaptive immune response dysregulated in PsA pathophysiology. In particular, expansion of mCD4$^+$ and mCD8$^+$ cells in the synovium of PsA patients has been described, and GWAS have highlighted significant association with MHC-I and other pivotal genes involved in T cell immune response. \parencite{Taams2018}.

In this chapter I have used ATAC to identify genome-wide differences between PB and SF in four disease-relevant cell types from PsA patients. CD14$^+$ monocytes demonstrated the largest number of DARs between SF and PB (23.3\% of the investigated regions). For all the cell types, the majority of the DARs were located at intergenic and intronic regions annotated as enhancers (weak or strong), and were also enriched for eRNAs identified in each cell type by the FANTOM project \parencite{ FANTOM2014}. Altogether these findings may suggest a role for the differential regions in long-range regulation of gene expression \parencite{Qu2015}. Pathway enrichment analysis of the genes proximal to the DARs revealed both commonalities and differences across cell types. In SF, enrichment for genes in the NF-$\kappa$B pathway was found in CD14$^+$ monocytes, consistent with the TNF-$\alpha$ signature and the efficacy of anti-TNF therapies already reported for PsA \parencite{Ahil2016}. Interestingly, in mCD8$^+$ T cells enrichment of DARs open in PB was found proximal to genes in the Wnt signaling pathway, such as \textit{SMAD3}. Wnt signalling is involved in many biological processes and its dysregulation is associated with a number of autoimmune diseases, such as RA \parencite{Miao2013}. In this data, the significantly increased chromatin accessibility near Wnt signalling genes in PB mCD8$^+$ cells may be related to an increased recall proliferation capacity of the circulating cells compared to the tissue-resident \parencite{Boudousquie2014}. In NK cells, increased accessibility proximal to gene members of the NK-mediated cytotoxicity pathway was found in PB compared to SF cells. NK CD56$^{bright}$ cells resident at sites of inflammation are more specialised for cytokine production than cytotoxicity \parencite{Michel2016}. Notably, matched mass cytometry data in these patients have shown a reduced proportion of CD56$^{bright}$ cells in PB compared to SF which would be consistent with the NK-mediated cytotoxicity enrichment.

Overall, this approach has identified robust differences in chromatin accessibility between SF and PB in relevant immune cells isolated from PsA patients. This is in line with other studies that have revealed changes in chromatin accessibility between patients and healthy controls and also across different tissues involved in disease \parencite{Scharer2016,Wang2018,Corces2016}. Although these findings suggest meaningful functional differences in chromatin accessibility between circulating and affected tissue cell populations, a limitation of pathway analysis in ATAC is linking putative regulatory regions to genes. Annotation of ATAC peaks with genes in proximity has been widely used in the literature \parencite{Scharer2016,Ackermann2016, Corces2016, Wang2018}. However, this approach fails to evaluate long range interactions, assuming accessible chromatin regulates neighbouring regions. Moreover, accessible chromatin is not a definitive marker for regulatory regions and mapping of histone marks such as H3K4me1 and H3K27ac together with eRNA quantification will help to refine the functional relevance of the identified DARs.

%\subsection{Biological insights in the integration of chromatin accessibility, gene expression and proteomics data in PsA}

\subsection{Bulk gene expression profiling and integration with chromatin accessibility data}

In contrast to chromatin accessibility profiling, recent research has investigated differences in the transcriptional profile between PBMCs, bulk T cells, SFMCs and synovial tissue from PsA patients \parencite{Dolcino2015, Fiocco2015}. However, comparative analysis in matched discrete cell subpopulations from SF and PB from the same patient have yet to be reported.

In this chapter, I have presented a pilot study characterising expression of relevant immune genes in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ cells isolated from SF and PB and integrated this with paired chromatin accessibility data from the same samples. CD14$^+$ monocytes and mCD8$^+$ presented the largest number of consistently modulated genes between SF and PB in this pilot analysis. The most significantly dysregulated genes between SF and PB in the four cell types were \texit{SPP1} and \textit{FN1}, the same as reported by Dolcino \textit{et al.}. Other highly DEGs in Dolcino \textit{et al.}’s study, including \textit{TNFA}, \textit{CXCL13} or \textit{CCL18}, were also found to be modulated between SF and PB in at least one of the cell types in this pilot data. Consistent with their role in Th17 cell biology, \textit{CXCL13} and \textit{IL26} appeared significantly up-regulated in SF mCD4$^+$ and/or mCD8$^+$ cells but not in CD14$^+$ monocytes \parencite{Takagi2008}.

The subsequent integration of transcriptome profiling with paired-ATAC data in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ revealed that genes with differentially modulated expression in SF and PB corresponded with nearby DARs showing changes in the same direction. Some of those DARs had also been identified as eRNAs in CD14$^+$ monocytes (e.g \textit{NFKB1}) and mCD4$^+$ cells (e.g \textit{CCR6}) by the FANTOM project. Although the characterization of those DARs as eRNAs evidences the downstream role of those regions in active transcriptional regulation, it does not unequivocally link this regulatory role to the proximal gene found to be differentially expressed between tissues by qPCR.

The integration of differences in PB gene expression between PsA patients and controls with the cross-tissue comparison within patients led to identification of systemic, tissue-specific and putative-disease specific modulated genes in this pilot data. According to my data, more profound transcriptional changes across PsA tissues (tissue-specific genes) were identified when compared to changes in expression between PsA patients and controls in PB for CD14$^+$ monocytes and mCD8$^+$ cells.

Systemic genes for mCD8$^+$ cells included for example \textit{CCR10}, a chemokine receptor co-expressed by a subset of memory cells that preferentially migrate to skin, which has also been identified as an up-regulated gene in tCD8$^+$ cells in the psoriasis cohort (Chapter \ref{ch:Results2} and in patients with atopic dermatitis \parencite{Hijnen2005}. Interestingly, \textit{SPP1} and \textit{FN1} appeared in the tissue-specific category in the three cell types. Dolcino and colleagues had reported these genes as the two most dysregulated when comparing bulk synovial membrane transcriptome from PsA patients and healthy individuals \parencite{Dolcino2015}. My data suggests that CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ cells may all contribute to the up-regulation of \textit{SPP1} and \textit{FN1} in the PsA synovium membranes. The \textit{SPP1} protein product, osteopontin, is a cytokine and chemokine expressed by many cell types, including monocytes/macrophages and T cells. It is involved in cell migration, adhesion and cell-mediated immune response through regulation of T cells, importantly in Th-17 \parencite{Morimoto2010}. \textit{SPP1} also plays a role in other chronic inflammatory and autoimmune diseases \parencite{Rittling2015}. \textit{FN1} encodes fibronectin-1, a main component of the cartilage matrix, involved in cell adhesion, migration, growth and differentiation and found to be highly expressed in RA inflamed synovium \parencite{Chang2005}. Moreover, \textit{FN1} has been shown to induce bone resorption mediated by pro-inflammatory mediators, such as nitric oxyde and IL-1$\beta$ \parencite{Gramoun2010}. In Dolcino’s data \textit{FN1} up-regulation was only found when comparing synovial membranes of PsA versus controls, and no changes were reported in PB samples, altogether suggesting the tissue-specificity of this dysregulation in PsA. Furthermore, the identification of DARs open in SF at the promoter and 3’ downstream of \textit{FN1} in CD14$^+$ monocytes may suggest a link between changes in modulation of gene expression and chromatin accessibility in this particular cell type.

In contrast to the observation in my pilot study, Dolcino also showed moderate up-regulation of \textit{SPP1} expression in PB from PsA patients compared to controls. This could be explained by the fact that Dolcino's study was performed in bulk PBMCs and dysregulation of \textit{SPP1} in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ cells may be tissue-specific. However, this could also be the result of the small number of qPCR replicates and \textit{SPP1} dysregulation in PB between PsA and healthy samples failing to reach significance in my study. Overall, up-regulation of these two genes in SF cells reflect the activation of chemotaxis and infiltration of circulating monocytes and T cells, activation of the Th-17 immune response and dysregulation of osteoclast bone remodeling , all of which are pivotal in PsA pathophysiology, particularly at the inflamed tissue \parencite{Durham2015, Mensah2017}.

An example of an interesting putatively disease-specific gene identified by this study was \textit{GPR68}, up-regulated in mCD4$^+$ PsA PB compared to controls with expression further increased in SF. \textit{GPR68} is a G protein-coupled receptor (GPCR), expressed in T cells and others, that undergoes activation through pH acidification, characteristic of synovial tissues under inflammation \parencite{Biniecka2016, Saxena2011}. Interestingly, \textit{GPR65}, another member of the acid-sensing GPCR family, has been associated with a number of immune mediated diseases, including AS, CD and MS \parencite{Cortes2013,Lassen2016,Wirasinha2018}. GPR65 was found to be a marker of pathogenic Th17 cells in the murine and human systems \parencite{Gaublomme2015, Al-Mossawi 2017}. Unfortunately, GPR65 was not included in the gene array used in this study. Indeed, the use of a gene array rather than a transcriptomics approach using RNA sequencing is a limitation of this study.

In terms of relevant biological processes, pathway analysis using consistently modulated genes between SF and PB in this data revealed enrichment for TLR and NOD-like signalling pathways in CD14$^+$ monocytes. This was consistent with the relevance of TLR and NOD-like receptors for rheumatic diseases \parencite{McCormack2009}. Up-regulated expression of \textit{TLR1} and \textit{TLR2} was significant in SF CD14$^+$ monocytes compared to PB and a similar trend in \textit{TLR4} expression was also observed but failed to reach significance in this pilot study. These finding were in line with some studies that have identified increased \textit{TLR-2} and \textit{TLR-4} expression in SFMCs compared to PBMCs in patients with juvenile idiopathic arthritis \parencite{Myles2011}. The relevance of NOD-like signaling has also been highlighted in the genome-wide trancriptomic analysis in lesional and uninvolved psoriatic skin presented in Chapter \ref{ch:Results2}, reinforcing the role of NOD-like signalling in the inflammatory response at the site of inflammation. The cross-talk between the TLR and NOD-like signalling pathways was further evidenced by network-based analysis in this data highlighting increased activation of the NF$\kappa$B TF in SF, particularly in CD14$^+$ monocytes. In my data, enrichment of DARs open in SF in the proximity of genes within the NF-$\kappa$B pathway was also found and further supported transcriptionally by the consistent up-regulation of downstream genes such as \textit{TNFA}, textit{CCL2} and \textit{CCL5} in SF CD14$^+$ monocytes. Moreover, analysis for conserved TFBS motifs in the ATAC data revealed enrichment for NF-$\kappa$B binding motifs within DARs open in SF CD14$^+$ monocytes but not in DARs open in PB (data not shown).

The enrichment of differentially modulated genes between SF and PB in mCD4$^+$ for the IL-10 signalling pathway was particularly interesting in the context of flow cytometry from the same three patients evealing expansion of Tregs in SF compared to PB (data not shown). Tregs are characterised by the expression of anti-inflammatory cytokines, including IL-10 \parencite{O’Garra2004}. The qPCR transcriptomic data showed in SF mCD4$^+$ and mCD8$^+$ a significant increase in expression of the IL-10 receptor subunit $\alpha$ \textit{IL10RA} and a similar trend for \textit{IL10} up-regulation (pval=0.14 and 0.07, respectively) compared to PB. Altogether, this could suggest that inflammation in PsA is refractory to the immunomodulatory effects for IL-10 signalling in SF o counterbalanced by the immunostimulatory properties of this cytokine, which could be one of the reason for failure of IL-10 agonist therapy in CD and psoriasis \parencite{Marlow2013, Kimball2002}.

%Overall, this work sought to characterise gene expression and chromatin accessibility differences between SF and PB, and has revealed interesting observations across all cell types.

\subsection{The relevance of monocytes and investigation of other cell types}

In this exploratory data, CD14$^+$ monocytes showed the most DARs and confidently modulated genes between SF and PB as well as functionally relevant pathway enrichment. The work presented is part of a collaborative multi-omics PsA pilot study. In light of this thesis, and for the cohort size available at the time of analysis, CD14$^+$ monocytes were chosen as the cell type to further explore by scRNA-seq and integrate with mass cytometry data. mCD4$^+$ and mCD8$^+$ also showed relevant changes in chromatin accessibility and gene expression, and have been identified as the two cell types undergoing greater expansion in the synovium of PsA patients. Colleagues involved in this project have since performed differential TCR clonality analysis between SF and PB using PsA samples included in this thesis. Interestingly, a significant number of mCD8$^+$ TCR clones with potentially shared antigen recognition across patients were found to be enriched in SF compared to PB (manuscript in preparation).

\subsection{Characterisation of monocytes by scRNA-seq}

Monocytes are very plastic cell populations that undergo cell differentiation at the site of inflammation, with differences that may be better captured at the single-cell level. In this pilot experiment, cluster identification in scRNA-seq from combined SF and PB monocytes using a conservative approach (see \ref{Discussion\_scRNAseq) revealed two main subpopulations (CC-mixed and CC-IL7R). CC-mixed appeared as a large heterogenous cluster in contrast to the CC-IL7R subpopulation, characterised by cells consistently expressing \textit{IL7R}, \textit{IL32} and textit{CCL5}, amongst other markers. According to the scRNA-seq data, the CC-IL7R cluster represented around 3\% of the total monocytes and had approximately the same number of cells from SF and PB (3 and 2.7\%, respectively). Conversely, flow cytometry data in PsA and AS patients have shown up to 35\% of the total SF CD14$^+$ monocyte to be IL-7R$^+$ versus approximately 1\% in PB (in revision \parencite{Al-Mossawi2018}). This could be due to discrepancies between gene expression and protein translation acknowledged in the literature but may also be a consequence of lower sensitivity of scRNA-seq in quantifying gene expression \parencite{Liu2016,Islam2014}. This may be leading to underestimate the CC-IL7R cluster size and the predominance of SF monocytes IL-7R$^+$ in the contribution towards this cluster. Differences in sensitivity between qPCR and scRNA-seq may also partly explain the limited overlap of differentially expressed genes between SF and PB when using qPCR and scRNA-seq. Although the same top dysregulated genes (including \textit{SPP1}, \textit{FN1} or \textit{OLR1}) were identified by both techniques, a modest number of significantly modulated genes found by qPCR were reproduced by the scRNA-seq analysis in the CC-mixed cluster. In terms of chromatin accessibility, the comparison of FCs from contrasting SF and PB chromatin accessibility in CD14$^+$ monocytes and scRNA-seq expression in the CC-mixed cluster only showed moderate correlation. This limited correspondence between chromatin accessibility and gene expression has also been reported by other studies and may be also the result of aforementioned limitations in annotating accessible chromatin with a putative target gene \parencite{Wang2018}.

The identification of sub-populations of monocytes expressing IL-7R$^+$ is of biological interest as \textit{IL7R} polymorphisms are associated with a number of chronic inflammatory diseases, including AS and MS \parencite{Gregory2007, Cortes2007}. Although the role of IL-7 and IL-7R in mediating the immune response was only characterised in T cells, the relevance of IL-7R in CD14$^+$ monocytes under LPS stimulation has been demonstrated in eQTL studies and also at the protein level in a manuscript under review, to which I have contributed \parencite{Fairfax2014, Al-Mossawi2018}. Al-Mossawi and colleagues identified a distinct transcriptional profile of the IL7R cluster in PsA SF monocytes very similar to the gene expression profile from IL7R$^+$ \textit{in vitro} stimulated monocytes. Interestingly the \textit{IL-7R} locus showed differentially accessible chromatin in PsA SF and was one of the top DEGs in the CD14$^+$ monocyte qPCR array. Moreover, my analysis \textit{CD44} appeared to be up-regulated in SF compared to PB in the CC-IL7R cluster. Interestingly, \textit{CD44} is the receptor for osteopontin and this observation may suggest that the SF CC-IL7R cells may be more responsive to this cytokine. Taken together, this data supports a role for IL-7 signalling in PsA circulating and tissue monocytes in chronic inflammation.

Pathway enrichment analysis using scRNA-seq DEGs between SF and PB in the CC-mixed cluster identified biologically relevant processes, ncluding MHC-II Ag processing, IFN signaling and extracellular-matrix components, amongst others. Interestingly, up-regulation of \textit{IFI6} and \textit{IFITM3}, two of the genes contributing to the enrichment of this pathway, have also been identified as markers of a subpopulation of IFN-$\gamma$ activated monocytes in from RA synovial tissue using scRNA-seq \parencite{Zhang2018}. In addition to the IFN-$\gamma$ activated, this study identified other three cluster within RA and osteoarthritis (OA) patients monocytes; however IL-7R$^+$ were not explicitly mentioned. Genes enriched for the extracellular-matrix pathway included members of the S100 protein family, previously reported to be dysregulated in lesional skin from psoriasis patients (Chapter \ref{ch:Results2}), which are also involved in joint erosion and development of arthritis \parencite{Raghunatha2012}. Two genes of this family, \textit{S100A8} and \textit{S100A9}, were up-regulated in lesional skin compared to uninvolved, but down-regulated in SF CC-mixed monocytes in this data. The lack of overlap between the pathways identified for the DEGs in the CC-mixed and those found by qPCR in bulk CD14$^+$ monocytes could be due the result of the qPCR array being biased to a small number of genes versus the unbiased approach of the scRNA-seq.

\subsection{Mass cytometry in CD14$^+$ monocytes and the integration with chromatin accessibility and transcriptomic data}

Single-cell mass cytometry in matched PB and SF was first conducted in the same three PsA with available paired ATAC and/or qPCR data. Despite technical limitations, mass cytometry in this samples identified a greater percentage of CD14$^+$ monocytes producing TNF-$\alpha$ in the SF compared to PB, in lines with the chromatin accessibility and transcriptomic data suggesting increased NF-$\kappa$B activation in this tissue. A cohort with additional ten SF and PB PsA samples validated with statistical significance the TNF-$\alpha$ observation. Moreover, the expanded cohort also demonstrated increased percentage of CD14$^+$ monocytes actively producing MCP-1 and osteopontin in SF compared to PB, consistently with the up-regulation in \textit{CCL2} and \textit{SPP1} expression in SF. A study using quantitative mass cytometry comparing SF from PsA and OA as control did not identify any of these three proteins to be up-regulated \parencite{Cretu2014}. Conversely, another study using enzyme-linked immunosorbent assay (ELISA) reported an increased production of TNF-$\alpha$, amongst other cytokines, in PsA SF compared to OA \parencite{Partsch1997}.

Notably, \textit{CCL2}/MCP-1 represented a good example of correlating differences between SF and PB monocytes across chromatin accessibility, gene expression and protein production data. Dolcino’s study did not identify up-regulation of \textit{CCL2} expression in PsA synovial membranes when compared to controls, which could be due to these differences being masked by the mix of cell populations in this tissue. Interestingly, increased levels of MCP-1 in SF, similarly to the observation made by our collaborators in Basle, were previously reported, and correlation with the infiltrated levels of T cells was also demonstrated \parencite{Ross2000}. Regarding the open DAR in SF proximal to \textit{CCL2}, no eQTL or chromatin conformation data in CD14$^+$ monocytes has revealed direct evidence for a relationship between differential expression of \textit{CCL2} and changes in chromatin accessibility in this nearby region.

\subsection{Challenges and future perspectives in multi-omic approaches}

\label{Discussion\_scRNAseq}

The work presented in this chapter is an exploratory study and a proof of principle for the implementation of a multi-omics approach, which represents a very powerful strategy to dissect disease pathophysiology in a cell type specific manner. Nevertheless, a number of limitations and challenges were encountered and need to be taken into account to contextualise these results. One limitation is the small sample size (n=3) and the lack of paired data across all the techniques presented. This results from difficulties of recruiting PsA patients na\'{i}ve for any treatment, the logistical difficulties to coordinate all of the techniques from the same sample, and the high cost of this approach. A further limitation in this study is the lack of PB from healthy controls or SF from another autoimmune or non-inflammatory joint disease, as included by other studies \parencite{Fumitaka2018, Dolcino2015,Zhang2018}. The definition and categorisation of the qPCR significantly modulated genes into systemic, tissue-specific and putative disease-specific was particularly limited by a lack of control samples to compare to the SF cells and the use of a biased transcriptomic analysis using a qPCR array.

Another challenge in this study relates to the analysis and integration of scRNA-seq and mass cytometry data. Both techniques still represent emerging fields were no consensus has been reached on the best strategy to combine samples across patients and experiments, accounting for batch effect. In this exploratory study, monocytes were identified from each SFMCs and PBMCs scRNA-seq sample and combined using CCA for further subpopulation identification. However standard resolution for cluster identification yielded potentially spurious subpopulations, leading to the adoption of a more conservative approach to define clusters in this particular analysis. This may be the consequence of remaining batch effects, and alternative methods of combining samples from the different experiments should be investigated. In this respect, the identification of robust and stable subpopulations through cluster analysis will benefit from the implementation of algorithms designed for cluster validation such as Silhouettes, which has recently been used successfully in the field of single-cell \parencite{Rousseeuw1987, Zhang2018}. In addition to this, incorporation of bulk RNA-seq data from CD14$^+$ monocytes will help interpretation and validation of the scRNA-seq results. In mass cytometry, to reduce batch effects patient samples are undergoing \textit{ex-vivo} fixation and cryopreservation followed by simultaneous staining and barcoding. Moreover, different methodologies for cluster identification and annotation are also being explored and so far no clear clusters have confidently been found.

In terms of data integration, this pilot study used relatively simplistic multi-omics data integration limited by sample size, technical aspects and time scale, and provides a platform for future validation studies. A more systematic integrative approach should be implemented for the expanded cohort to establish appropriate correlation across datasets. Currently Zhang and colleagues have presented the most exhaustive methodology to integrate bulk-RNA-seq, scRNA-seq, mass cytometry and flow cytometry into multi-modal transcriptomic and proteomics profiles, but their work is still under peer review \parencite{Zhang2018}. This strategy has revealed disease-specific functional expanded subpopulations amongst the most relevant cell types in RA pathophysiology. Additionally, the correlation between bulk ATAC and scRNA-seq is clearly limited by the different scales of the two approaches. Therefore, generation of scATAC-seq data, identification of clusters based on chromatin profiling and appropriate methods for the overlap with scRNA-seq populations should be used to have a better understanding of the correlation between chromatin accessibility and gene expression at the single-cell level \parencite{Duren2018}.

\subsection{The use of PsA functional data to inform fine-mapping GWAS loci}

Integration of epigenetic data with fine-mapped SNPs from GWAS studies has been widely demonstrated to be a powerful tool to further narrow down candidate causal variants, particularly for intergenic or intronic signals not driven by LD with missense coding SNPs \parencite{Bunt2015,Farh2014}. Although DARs between SF and PB in four cell types did not show any overlap with SNPs from the credible set, significant enrichment of fine-mapped SNPs for accessible chromatin was demonstrated in all four cell types. A number of SNPs from the 5q31 credible set in my analysis overlap accessible chromatin and eQTL signals in the same cell type. Integration of tCD4$^+$ and tCD8$^+$ eQTL from Kasela \textit{et al.} confirmed the association between SNPs from the 5q31 GWAS signal and T cells \textit{SLC22A5} expression previously reported in a smaller study by Bowes \textit{et al.}, and also suggested a potential role for the 5q31 PsA-specific GWAS association in regulating \textit{P4HA2} and \textit{SLC22A5} expression in unstimulated and stimulated monocytes. \textit{SLC22A5} is a cell membrane transporter of carnitine involved in fatty acids metabolism and has been prioritised by an in-house pipeline as the third most promising druggable candidate for the treatment of psoriasis. Supporting evidence highlights the implications of this gene in other inflammatory conditions such as CD and the potential as a druggable therapeutic target in PsA \parencite{Leung2006}.

Contrary to the initial hypothesis, the integration of fine-mapping data and DARs between SF and PB in PsA relevant cell types failed to show overlap in any of the four studied cell types. %For associations driven by exonic non-synonymous missense mutations, such as \textit{TRAIF3IP2}, changes in chromatin accessibility are not necessarily expected.

For non-coding signals hypothesised to have a regulatory role, these results may suggest that fine-mapped SNPs from PsA GWAS loci do not have a tissue specific effect in chromatin accessibility changes for any of these cell types. These results may also be biased by the small size in my differential chromatin accessibility analysis and the limited power of the fine-mapping analysis using only a subset of the PsA GWAS cohort. Studying variation in chromatin accessibility upon genotype of the fine-mapped SNPs, similar to the example of chr2p15 presented in Chapter \ref{ch:Results2}, may be more informative when integrating epigenetics at a GWAS associated locus.