\section{Discussion}

\subsection{Characterising chromatin accessibility in PsA samples}

The technological advances in the epigenetics field have allowed to map the epigenetic landscape in particular relevant 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 of the dysregulated innate and adaptive immune response involved in PsA pathophysiology. Particularly, expansion for mCD4$^+$ and mCD8$^+$ in the synovium from PsA patients has been described, and many of the GWAS associated genes represent pivotal components in orchestrating the immune function in those specific cellular types. \parencite{Taams2018}.

In this thesis, for the first time I have used ATAC to characterise the chromatin landscape of four relevant immune cell types isolated from PB and SF of PsA patients, revealing genome-wide differences between the two tissues for all of them. CD14$^+$ monocytes was the cell type presenting the largest number of DARs between SF and PB (23.3\% of the investigated regions). The majority of the DARs were located at intergenic and intronic regions and annotated as enhancers (weak or strong). Interestingly, DARs were also enriched for eRNAs identified in each of the four relevant cell types by the FANTOM project, reinforcing the functional role of the differential regions in active regulation of gene expression \parencite{ FANTOM2014}. ~~At this respect, the enrichment found for NK DARs in neutrophils eRNAs may be consequence of the lower signal-to-noise found in the NK ATAC libraries, as previously commented and which may limit the reliability of the findings made in this cell type.~~

Pathway enrichment analysis for the genes placed proximal to the DARs revealed both commonalities and differences between regions with increased accessibility in SF or PB for each of the cell types. In SF, enrichment for open DARs proximal to genes in the NF-$\kappa$B pathway was found in CD14$^+$ monocytes, consistent with the TNF-$\alpha$ signature of PsA and the efficacy of anti-TNF therapies \parencite{ Ahil2016}. Interestingly, in mCD8$^+$ cells enrichment of DARs open in PB was found proximal to genes involved in Wnt signaling, such as \textit{SMAD3}. Wnt signalling is involved in many biological processes and its dysregulation is associated to a number of autoimmune diseases, such as RA \parencite{Miao2013}. In this case, the increased chromatin accessibility nearby 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 memory cells which has been previously described \parencite{Boudousquie2014}. In NK cells from PB, mapping the chromatin landscape revealed increased accessibility proximal to gene members of the NK-mediated cytotoxicity pathway. NK CD56$^{bright}$ resident at sites of inflammation have been described to be more specialised for cytokine production than cytotoxicity \parencite{Michel2016}. Notably, mass cytometry data in this patients have shown a reduced proportion of CD56$^{bright}$ cells in PB compared to SF consistently with the enrichment of DARs open in PB for the NK-mediated cytotoxicity pathway.

Overall, this approach has identified robust differences in chromatin accessibility between SF and PB in relevant immune cells isolated from PsA patients. Although these differences may suggest meaningful functional differences, a limitation of pathway analysis in ATAC is linking putative regulatory regions to genes. Annotation of ATAC peaks with genes in the proximity has been widely used in the literature \parencite{Scharer2016,Ackermann2016, Corces2016,Wang2018}. However, this approach fails to evaluate long range interactions and assumes accessible chromatin to regulate expression of the 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}

\subsubsection{Chromatin accessibility landscape and bulk gene expression profiling in paired samples}

Alike for chromatin accessibility profiling, recent research has been conducted to deepen into the characterisation of transcriptional profiles of PBMCs from PsA patients, with some studies even including synovial membrane biopsies from PsA patients and controls \parencite{Dolcino2015}. In this chapter, I performed a pilot study characterising the transcriptomic profile for relevant immune genes in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ cells isolated from SF and PB and integrating it, for the first time, with paired chromatin accessibility data form the PsA samples. CD14$^+$ monocytes and mCD8$^+$ presented the largest number of consistently modulated genes between SF and PB in this pilot analysis. The top most significantly dysregulated genes between SF and PB in the four cell types, \texit{SPP1} and \textit{FN1}, were found to be the same as the reported by Dolcino \textit{et al.} in their study. Other highly DEGs in Dolcino \textit{et al.} study including \textit{TNFA}, \textit{CXCL13} or \textit{CCL18} were found also to be modulated between SF and PB at least one of the cell types in this pilot data. Consistently with their role in Th-17 cell biology, \textit{CXCL13} and \textit{IL26} only 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 presenting 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, this overlap may suggest a downstream role of those regions in active transcriptional regulation of nearby genes. Moreover, further exploratory integration of differences in gene expression in PB between PsA patients and controls with the previous cross-tissue comparison within matched PsA samples from SF and PB led to definition of systemic, tissue-specific and putative-disease specific modulated according to my data.

CD14$^+$ monocytes and mCD8$^+$ presented a larger number of genes modulated between SF and PB in PsA patients only (tissue-specific genes) than genes changing only expression in PB when comparing PsA versus control samples (systemic genes). Interestingly, \textit{CCR10} gene was one of the systemic genes for mCD8$^+$ cells. \textit{CCR10} is a chemokine receptor co-expressed by subset of memory cell which preferentially migrate to skin, and has also been identified as an up-regulated gene in tCD8$^+$ cells in the psoriasis cohort (Chapter \ref{ch:Results1} and in patients with atopic dermatitis \parencite{Hijnen2005}. Interestingly, \textit{SPP1} and \textit{FN1}, which are the top two modulated genes between SF and PB in all cell types appeared in the tissue-specific category for all of them. These two genes were reported by Dolcino and colleagues as the two top most dysregulated genes when comparing bulk synovial membrane transcriptome from PsA patients and healthy individuals (undergoing post-traumatic surgery) \parencite{Dolcino2015}. The data from my thesis suggests that CD14, mCD4$^+$ and mCD8$^+$ cells may all be contributing to the up-regulation \textit{SPP1} and \textit{FN1} in PsA synovium membranes observed by Dolcino and colleagues. \textit{SPP1} known as osteopontin is a cytokine and chemokine expressed by many cell types (including monocytes/macrophages and T cells) and is involved in cell migration, adhesion and cell-mediated immune response through regulation of T cells, particularly Th-17 \parencite{Morimoto2010}. \textit{SPP1} has also been described to play a role in other chronic inflammatory and autoimmune diseases \parencite{Rittling2015}. On the other hand, \textit{FN1} is the gene coding for 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 NO 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, but no changes were reported between PsA and healthy controls 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 suggest a link between changes in modulation of gene expression and chromatin accessibility in this particular cell type. In contrast 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 thus my pilot data may suggest tissue-specificity for \textit{SPP1} dysregulation in CD14$^+$ monocytes, mCD4$^+$ and mCD8$^+$ cells. 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 them pivotal in PsA pathophysiology \parencite{Durham2015, Mensah2017}.

A particularly interesting gene amongst the defined as putative-tissue specific was \textit{GPR68} , which up-regulation in mCD4$^+$ PsA PB compared to controls was further exacerbated in SF. \textit{GPR68} is a G protein-coupled receptor (GPCR), expressed in T cells, amongst other cells, that undergoes activation through pH acidification, characteristic of synovial tissues under inflammation, yielding to increased Ca$^2$$^+$ levels and subsequent activation of immune-related pathways \parencite{Biniecka2016, Saxena2011}. Interestingly, \textit{GPR65}, another member of the GPCR family, has been associated with a number of immune mediated diseases, including AS, CD and MS \parencite{Cortes2013,Lassen2016,Wirasinha2018}.

Pathway analysis using the genes identified to be consistently modulated between SF and PB in this pilot data revealed enrichment for relevant pathways in CD14$^+$ monocytes and mCD4$^+$ cells. In in CD14$^+$ monocytes, TLR and NOD-like signalling pathways were enriched, consistently with the relevance of TLR and NOD-like receptors for rheumatic diseases. Up-regulated expression of \textit{TLR1} and \textit{TLR2} presented significant differential modulation in CD14$^+$ monocytes in SF; however a similar trend in \textit{TLR4} expression failed to reach significance in this pilot study. This is consistent with some studies that have identified increased in SFMCs compared to PBMCs in patients with juvenile idiopathic arthritis \parencite{Miles2011}. Moreover, the transcritomic analysis in lesional and uninvolved psoriatic skin presented in Chapter \ref{ch:Results2} also highlighted 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, a well-known phenomenon in inflammation, was further evidenced by network-based analysis in this data. TLR and NOD-like signalling pathways lead to activation of the NF$\kappa$B TF, which appeared transcriptionally up-regulated in SF CD14$^+$ monocytes in this data, and to subsequent transcription of pro-inflammatory cytokines and chemokines \parencite{Mitchell2010}. In this pilot data, enrichment of DARs open in SF in the proximity of genes within the NF-$\kappa$B pathway was found and further supported at the transcriptional level by the consistent up-regulation of \textit{TNFA}, textit{CCL2} and \textit{CCL5} expression 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 (data not shown).

~~The enrichment of chemokine signalling at the transcriptional level in CD14$^+$ monocytes was also closely related to the increased transcript levels in SF of these chemotractant molecules, involved in the recruitment of T cells to the site of inflammation, as well as reactive oxygen species (ROS) which exert their function through Ca$^{2^+}$ mobilisation, one of the pathways enriched for DARs open in SF open in CD14$^+$ monocytes.~~

In mCD4$^+$, the IL-10 signalling was the only pathway enriched for significantly modulated genes between SF and PB. This result was of particular interest in the context of flow cytometry data in matched PBMCs and SFMCs from the same three patients, which revealed an expansion of Tregs in SF compared to PB (data not shown). Tregs are characterised by expression of anti-inflammatory cytokines, including IL-10 \parencite{O’Garra2004}. qPCR analysis showed significant increased expression of the IL-10 receptor subunit \textit{IL10RA} in mCD4$^+$ from SF and a trend that, although not statistically significant in this data, was inclined towards increased expression of \textit{IL10} for all three cell types in SF compared to PB. Together, this could suggest a refractory effect for IL-10 signalling in SF or a counterbalance of IL-10 immunosupressive effects by the immunostimulatory properties, hypothesised as one of the reasons for failure of IL-10 agonist therapy in CD \parencite{Marlow2013}.

Altogether, this pilot study aiming to characterise gene expression and chromatin accessibility differences between SF and PB has revealed interesting observations across all cell types. In this exploratory data, CD14$^+$ monocytes showed the larger number of DARs and confidently modulated genes between SF and PB as well as functional relevant pathway enrichment for those changes. The work here presented is part of a multi-omics PsA pilot study in a collaborative effort. In light of this thesis and the results presented for the cohort size available at the time of performing data analysis, CD14$^+$ monocytes were chosen as the cell type to further explore by scRNA-seq and further 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. In fact, other members involved in this project have focused on performing differential TCR clonality analysis between T cells in SF and PB some of the PsA samples included in this thesis. Interestingly, eleven mCD8$^+$ TCR clones recognizing five putative antigen peptide sequences were found to be enriched in SF compared to PB (manuscript in preparation).

\subsubsection{Further characterisation of CD14$^+$ monocytes by scRNA-seq and mass cytometry}

Monocytes are very plastic cell populations that undergo cell differentiation at the site of inflammation and these differences may be better captured at the single-cell level. In this pilot experiment, cluster identification in scRNA-seq from combined SF and PB CD14$^+$ monocytes data using a conservative approach (see \ref{Discussion\_scRNAseq) revealed two main subopulations (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. The equal contribution from SF and PB monocytes to the CC-IL7R cluster identified by scRNA-seq (3 and 2.7\%, respectively) contrasted with observations made by flow cytometry from the same three PsA patients, which showed approximately 35\% of the total SF CD14$^+$ monocyte population to be IL-7R$^+$ (in revision \parencite{Al-Mossawi2018}). This could be due to well characterised dicrepancies between gene expression and protein translation but may also be a consequence of lower sensitivity of scRNA-seq in quantifying gene expression, as only 20\% of the transcriptome of a cell is reverse-transcribed \parencite{Liu2016,Islam2014}. Thus the number of cells expression \textit{IL7R} may be underestimated by scRNA-seq and the size of this cluster may be larger, accordingly to observed differences in qPCR gene expression and chromatin accessibility between SF and PB in bulk CD14$^+$ monocytes. These 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 discrete 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 SF versus PB FCs between ATAC in bulk CD14$^+$ monocytes and scRNA-seq expression in the CC-mixed cluster only showed moderate correlation. This discrete correspondence between chromatin accessibility and gene expression has also been reported by other studies and may be likely affected by the aforementioned limitations in annotating accessible chromatin with a putative target gene \parencite{Wang2018}.

The identification of sub-populations of CD14$^+$ monocytes IL-7R$^+$ entails biological interest as \textit{IL7R} is 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 IL7-R in CD14$^+$ monocytes under LPS stimulation has been demonstrated in eQTL studies and also at the protein level in a manuscript under review towards which I have contributed \parencite{Fairfax2014, Al-Mossawi2018}. In this work, a distinct transcriptional profile of the IL-7R cluster in PsA SF CD14$^+$ monocytes was found to be very similar to the gene expression profile from IL7R$^+$ \textit{in vitro} stimulated monocytes, supporting altogether a role of IL-7 signalling in the innate immune response and chronic inflammation.

Despite the relevance of the CC-IL7R cluster marker genes in the immune response (e.g \textit{IL32} and \textit{CCL5}), most of the DEGs between SF and PB in the CC-IL7R cluster (30 out of 37) were shared with the CC-mixed subpopulation. Interestingly, one of the unique seven DEGs in the CC-IL7R subpopulation was \textit{CD44}, a receptor of hialuronic acid and osteopontin. Although \textit{SPP1} is up-regulated in SF compared to PB in the CC-mixed and CC-IL7R clusters, SFCD14$^+$ monocytes from the CC-IL7R subpopulation may be more responsive to this chemokine. Altogether, the extensive overlap of DEGs between SF and PB for the two clusters in this pilot study does not reveal marked tissue-specific intrinsic differences in the CC-IL7R cluster.

As for the qPCR modulated genes, pathway enrichment analysis using scRNA-seq DEGs between SF and PB in the CC-mixed cluster also identified a number of significantly enriched and biologically relevant pathways, including MHC-II Ag processing, IFN signalling 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}. 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, whereas they appeared as down-regulated in SF CC-mixed monocytes in this data. ~~None of the pathways identified for the DEGs in the CC-mixed cluster were common to the pathways identified by qPCR analysis, which could be expected due to the already mentioned limited overlap between dysregulated genes across the two assays as well as the nature of the pathway enrichment analysis regarding the background list of genes to compute the enrichment.~~

%Follow to overlap with ATAC and qPCR example CCL2

Single-cell mass cytometry characterization in matched PBMCs and SFMCs from the same patients was conducted in order identify monocytes subpopulations at the proteomic level. The pilot data from these three PsA patients has only revealed clear separation between classical, intermediate and non-classical monocytes. However, no further subpopulations within the classical and intermediate monocytes (CD14$^+$ monocytes investigated at by scRNA-seq) have been distinguished. Similarly to the scRNA-seq, this analysis has also been under limitations (see \ref{Discussion\_scRNAseq}). Despite these limitations, mass cytometry data has confirmed the increases chemokine and cytokine production abilities of the SF CD14$^+$ monocytes previously suggested by chromatin accessibility and transcriptomic data. For example, up-regulation of \textit{SPP1} in SF CD14$^+$ monocytes was validated at the protein level though increased increased levels of osteopontin in this cell type. Moreover, NF-$\kappa$B activation in SF compared to PB was confirmed by increased number of SF CD14$^+$ monocytes producing TNF-$\alpha$ compared to the PB counterpart.

\subsection{Challenges and future perspectives in the multi-omics approach}

\label{Discussion\_scRNAseq}

The advances in epigenetic and single-cell techniques has enabled the investigation of the chromatin accessibility, transcriptomic and proteomic profiles in clinical samples. This multi-omics approach represent a very powerful strategy to dissect disease pathophysiology in a cell type specific manner. The work presented in this chapter is a pilot exploratory study and a proof of principle for the implementation of a multi-omics approach. Nevertheless, a number of limitations and challenges have been encountered and need to be taken into account to contextualise these results. One of the limitations is the small sample size (n=3) and the lack of paired data across all the techniques here presented. This has been caused by the difficulties of recruiting samples from PsA patients na\'{i}ve for any treatment , the logistic and difficulties to coordinate the performance of all the techniques from the same sample and the financial constraints. This has also directly impacted in the lack of PB from healthy controls or SF from patients suffering from an autoimmune or non-inflammatory joint disease, similarly to other studies \parencite{Fumitaka2018, Dolcino2015,Zhang2018}. The definition and categorisation of the qPCR significantly modulated genes into systemic, tissue-specific and putative disease-specific involves limitations due to lack of control samples to compare to the SF cells. As a result, further analysis using the three list of genes has not been performed but may be of interest for future analysis when performing genome-wide gene expression analysis (see \ref{Discussion\_scRNAseq}).

Another challenge in this pilot study related to the strategy to analyse and integrate scRNA-seq and mass cytometry data. Both techniques still represent an emerging field were no consensus has been reached on the best strategy combine samples from different patients and experiments accounting for batch effect. This is particularly relevant when aiming to identify further subpopulations within cell types, where the defined clusters may be the result of differences from unsuccessful correction of this batch effect. In this exploratory study, CCA was chosen as the best strategy to combine CD14$^+$ monocytes scRNA-seq data first identified individually in each sample the six samples. However standard resolution for cluster identification yielded spurious subpopulations, leading to adopt a more conservative approach to define clusters in this particular analysis. This may the consequence of remaining batch effect and alternative ways 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 design for cluster validation such as Silhouettes, which has been successfully used in the field of single-cell \parencite{Rousseeuw1987, Zhang2018}. In addition to this, incorporation of bulk RNA-seq data to from CD14$^+$ monocytes will help to interpret and validate the scRNA-seq results. ~~Bulk-RNA-seq is less sensitive to technical artifacts and can be used in combination with the scRNA-seq to identify shared sources of variation and also identify populations from the scRNA-seq data that drive changes in the bulk transcriptomic profile.~~ Similarly to scRNA-seq, Dr Nicole Yager has also tested different ways to analyse the data using different approached for cluster identification and annotation, including Cytoskape and FlowSOM. To avoid batch effect in mass cytometry, subsequent recruited patient samples are undergoing fixation to perform staining and mass cytometry analysis simultaneously in all of them. Likewise, technical problems regarding to staining for some of the markers have also been encountered. Importantly, identification of an IL-7R$^+$ cluster in monocytes by mass cytometry has been halted by technical problems for detection of IL-7R.

In terms of data integration, this pilot study shows a basic integration of the multi-omics data adapted to the sample size and quality of the data. However, a more systematic approach for data integration will require to be implemented for the expanded cohort to establish appropriate correlation between robust and confidently identified scRNA-seq and mass cytometry subpopulations in an unbiased way. 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 in RA \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 here presented is clearly limited by the different scales of the two approached and also the simplicity in the methodology used to perform such integration. 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 conducted to have a better understating 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}

The integration of epigenetic data with fine-mapped SNPs from GWAS studies have been widely demonstrated to be a powerful tool to further narrow down the candidate putative causal variants, particularly for intergenic or intronic signals not driven by LD with coding SNPs \parencite{Bunt2015,Farh2014}. In this case, DARs between SF and PB in four cell types did not show any overlap with SNPs from the credible set identified by fine-mapping at eight PsA GWAS loci. Conversely, overlap of these SNPs were found with accessible chromatin across (ATAC peaks), with significant enrichment of fine-mapped SNPs for ATAC accessible regions compared to GWAS Catalogue SNPs. Further investigation of SNPs from the 5q31 credible set identified by fine-mapping have revealed particularly interesting SNPs overlapping chromatin accessibility and eQTL signals in the same cell type. Incorporation of eQTL datasets also confirmed the association between SNPs in the credible set and \textit{SLC22A5} expression in T cells found by Bowes \textit{et al.} in a larger cohort of tCD4$^+$ and tCD8$^+$ \parencite{Kasela2016}. Moreover, this integration also highlighted a potential role for the 5q31 PsA-specific GWAS association also in regulating expression of \textit{P4HA2} and \textit{SLC22A5} in unstimulated and stimulated monocytes. \textit{SLC22A5} is a cell membrane transported of carnitine that is involved in fatty acids metabolism. Interestingly \textit{SLC22A5} has been prioritised by our in-house pipeline as the third most promising druggable candidate for the treatment of psoriasis with supporting evidence for implication of this gene in other inflammatory conditions such as CD \parencite{Leung2006}.

Unlike initially hypothesised in this chapter, the integration of fine-mapping data and DARs between SF and PB in PsA relevant cell types failed to show any overlap-mapping credible set of SNPs with the epigenetic data generated in SF and PB from PsA patients in any of the four studied cell types. This limited success in this integration could be partly affected by the small sample size used for the identification of DARs (n=3) or the reduced power of the fine-mapping analysis using only a subset of the PsA GWAS cohort. For those associations which have been described to be driven by the GWAS LD SNP being in high LD with a non-synonymous missense mutation in an exon, for example \textit{TRAIF3IP2}, changes in chromatin accessibility are not necessarily expected. In other instances, 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. Thus, an approach focused on variation of chromatin accessibility upon genotyping data, similarly to the example of chr2p15 presented in Chapter \ref{ch:Results2}, may be more informative when integrating epigenetic at GWAS associated locus.