



Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

Advanced genomics and clinical phenotypes in psoriatic arthritis

Matteo Vecellio^{a,b,*}, Stefano Rodolfi^{a,c}, Carlo Selmi^{a,c,*}^a Department of Rheumatology and Clinical Immunology, IRCCS Humanitas Research Hospital, Via Manzoni 56, 20089, Rozzano, Milan, Italy^b Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Botnar Research Centre, University of Oxford, Oxford, United Kingdom^c Department of Biomedical Sciences, Humanitas University, Via Rita Levi Montalcini 4, 20072, Pieve Emanuele, Milan, Italy

ARTICLE INFO

Keywords:

Psoriatic arthritis
Genomics
Genetics
SNP
Methylation

ABSTRACT

Psoriatic Arthritis (PsA) is a complex polygenic inflammatory disease showing a variable musculoskeletal involvement in patients with skin psoriasis. PsA coexist in 25–40 % of patients with the dermatological manifestations, but PsA may also predate the appearance of psoriasis. Nonetheless, the immunopathogenesis of psoriasis and PsA manifest significant similarities, with a major role of the individual susceptibility in both cases. Genome wide association studies (GWAS) identified several genes/loci associated with the risk to develop PsA, both dependent and independent of psoriasis. The major challenge is thus represented by the need to translate the identification of functional polymorphisms and other genetics findings into biological mechanisms along with the identification of novel putative drug targets. A functional genomics approach aims to increase GWAS power and recent evidence supports the use of a multilayer process, including eQTL, methylome, chromatin conformation analysis and genome editing to discover novel genes that can be affected by disease-associated variants, such as PsA. The available data have considered PsA as a unique homogeneous clinical entity while the clinical experience supports a wide variability of skin and joint manifestations coexisting in diverse patients with different mechanisms underlying the musculoskeletal and dermatological domains. A better discrimination of the patient features is encouraged by the limited data on functional genomics. We provide herein a review of the latest findings on PsA functional genomics highlighting the exciting developments in the field and how these might lead to a better understanding of gene regulation underpinning disease mechanisms and ultimately refine clinical phenotyping.

1. Introduction

Psoriatic arthritis (PsA) is a chronic inflammatory and autoimmune disease affecting the joints and it develops in 20–40 % of patients with skin or nail psoriasis [1]. It represents the second most frequent form of inflammatory arthritis after rheumatoid arthritis (RA) and it has a major impact to the patients' quality of life, affecting society both socially and economically [2]. PsA is a clinically heterogeneous disease, and it is characterised by a variable combination of peripheral arthritis, enthesitis, dactylitis, and axial involvement [1,3], which represent the four musculoskeletal domains of PsA.

The biological mechanisms behind PsA remain poorly understood, with significant evidence supporting a mechanistic interaction between genetic and environmental factors, via gut microbiome alterations, biomechanical stress and chronic inflammation mediated by both innate and adaptive immune systems, with the latter two elements contributing to the resulting mechano-inflammation [1]. The immunopathogenesis of

PsA is thought to be initially localized within the fibrocartilaginous entheses, the specialized interface between tendons/ligaments and bones which is most influenced by the biomechanical stimuli. Recent pathophysiological models propose that the stress at the level of entheses in genetically predisposed individuals results in production of IL-23 by resident dendritic cells, T-cells and macrophages [4,5]. IL-23 then orchestrates the secretion of IL-17, IL-22 and other proinflammatory cytokines by, among all, type III innate lymphoid cells, mucosal associated invariant T (MAIT) cells and resident $\gamma\delta$ T cells [5]. The accumulation of IL-17 producing cells, facilitated by neoangiogenesis across the bone cortex at the enthesal site induces the activation of synovial fibroblasts, osteoclasts and osteoblasts, resulting in cartilage degradation, and both bone resorption and new bone formation, typical features of PsA [1].

It is well established that PsA has a strong genetic component, with a very high degree of heritability, considered to be higher than psoriasis, although the bias of a disease found within another disease may

* Corresponding authors at: Department of Rheumatology and Clinical Immunology, Humanitas Research Hospital, IRCCS, Rozzano, Milan, Italy
E-mail addresses: matteo.vecellio@ndorms.ox.ac.uk (M. Vecellio), carlo.selmi@hunimed.eu (C. Selmi).

<https://doi.org/10.1016/j.smim.2022.101665>

undermine this observation [6]. Nonetheless, a significant overlap of genetic predisposition between PsA, psoriasis, and ankylosing spondylitis (AS) has been demonstrated [7] and the risk associated with a positive family history for PsA or psoriasis, as recognized also in classification criteria for PsA [8], strongly supports the role of shared genetics in determining disease susceptibility.

The genetic heritability of PsA been investigated through twin studies and family-based approaches. Following the CASPAR criteria, a set of classification criteria for the definition of PsA, a family history of psoriasis contributes to obtain a positive score [8] the psoriasis concordance rate in monozygotic twins is the highest for chronic inflammatory and autoimmune diseases while twin data on PsA are limited to a small number of sets [9]. Family studies demonstrated that having a first degree affected by PsA increases the risk of developing the disease by roughly 50 times more compared to the general population [10].

Genome Wide Association Studies (GWAS) have identified numerous regions that confer the increased susceptibility to develop PsA making it a polygenic immune-mediated disease [11–13]. PsA most robust association is with HLA class I family genes, which account for 30–50 % of PsA heritability [14], in particular with HLA-B* 08, HLA-B* 27, HLA-B* 38, and HLA-B* 39, while HLA-B* 44 seems to reduce the risk of PsA development [15]. As mentioned, PsA is a highly heterogeneous disease and extended class I MHC haplotypes have been documented to be preferentially linked to specific disease phenotypes, especially when patients are arrayed based on the dermatological or rheumatological manifestations. For example, HLA-Cw6 is strongly associated with early onset psoriasis, while it has limited penetrance in PsA and is associated to a milder and late-onset form of arthritis, despite increasing the risk of its appearance in patients with skin manifestations [16]. More specifically, the frequency of the HLA-C* 06:02 allele in patients with only skin psoriasis, without peripheral joints or axial involvement, accounts for roughly 60 %, while is below 30 % in PsA patients [14]. The frequency of HLA-C* 06:02 has been found negatively associated with the presence of dactylitis and onychopathy [17]. Further, it has been demonstrated that the presence of HLA-B* 27:05 is indeed associated with an axial PsA phenotype, including spondylitis, enthesitis, symmetric sacroiliitis and dactylitis [18], while HLA-B* 08:01 is predominantly associated with peripheral arthritis and asymmetrical sacroiliitis [19]. Last, HLA-DR4 haplotype has not been found increased in PsA patients when compared with healthy controls but was found enriched when considering the subpopulation of PsA patients having RA-like symmetric polyarthritis suggesting that the “polyarticular phenotype” of PsA shares a similar genetic predisposition with RA [20].

Most non-HLA genes overlap between PsA and psoriasis, in several cases with clear functional consequences or rationale. Among genetic variants specifically associated with PsA and independent of those identified in psoriasis alone, the *IL23R* (Interleukin 23 receptor) and *TNFAIP3* (TNF α Induced Protein 3) genomic loci have significant immunological connections with the pathogenesis of the disease [13]. This finding highlights the plausibility of a different mechanisms acting at these loci in PsA which might be different from the ones acting in psoriasis.

Understanding the biological mechanism of disease-associated genetic variants it's not trivial as the majority of associated SNPs map to non-coding, often regulatory, portions of the genome, which physically interact with their target promoters in cell-type specific manner. The genome is organized in a very dynamic way and its 3D architecture is essential in facilitating different processes occurring in the cell nucleus, from transcriptional regulation to DNA damage and replication [21]. This is what has been hypothesized to occur also in PsA.

Therefore, in this review we will focus on such dynamic processes highlighting the most recent advances in epigenomics effects, covering the progresses made on understanding the contribution of DNA methylation, mapping target genes relevant to PsA using chromosome conformation capture methods, elucidating the validation of the

findings using gene editing approaches and, finally, examining the overall impact of these investigations on target discovery and drug development.

2. Understanding the link between PsA-associated genetic variants to function

Moving from genetics to function is challenging, as this process requires a robust model for validation of functional polymorphisms. It is also crucial to identify the relevant cell types where the genetic variants mapped through GWAS may act [22]. Almost 90 % of autoimmune diseases-associated SNPs identified by GWAS lie within non-coding regions of the genome and most of them act by enhancing function in immune cells [23,24].

In complex disease like PsA it is not always clear which cells are causal, similar to RA where different immune cells, from T-cells to B cells and macrophages, and synovial fibroblasts have been demonstrated to have a strong involvement in the inflammation process believed to cause the disease [25,26].

Expression quantitative trait loci (eQTL) studies are the most common and simplest tools to link phenotype-associated genetic variants with function, as they correlate gene expression levels with the presence of a specific genetic makeup in selected cell types. Bowes and colleagues performed the fine-mapping of immune-related susceptibility loci and found a correlation with the increased gene expression for Solute Carrier Family 22 Member 5 (*SLC22A5*) and the SNP *rs10065787* in CD8 + and CD4 + T-cells obtained from PsA patients (cell type specific eQTL) [11, 27]. Several eQTL databases (eQTL Gen, GTEx portal, eQTL catalogue) provide gene expression profiling of trait-related genetic variants in different human tissues [28–30]. For example, DICE (Database of Immune Cell Expression, Expression quantitative trait loci (eQTLs) and Epigenomics) offers transcriptomic and epigenomic maps in a plethora of human immune cell types to understand how genetic variations may act [31].

In a seminal work, Fairfax and colleagues showed the impact of immune stimulation (interferon- γ and lipopolysaccharide for 2 and 24 h) upon regulatory variant activity on primary monocytes gene expression [32]. One of the major limitations of eQTL studies, however, is the need of large samples to achieve an adequate statistical power: for this reason, many studies in autoimmune diseases have been conducted with whole blood or specific immune cells populations derived from patients' samples, as described extensively elsewhere [33,34]. For example, back in 2010, Ding and colleagues performed a refined statistical analysis to quantify the specificity of selected eQTLs in psoriasis and skin tissues compared with a published panel of eQTLs in lymphoblastoid cell line. The authors provided a comprehensive list of cis-eQTLs specific for skin, including significant signals in *FUT2*, *ERAP1* and *ERAP2* [35].

A very recent work from the Barton's group in the UK described two separate genome-wide meta-analyses conducted on more than 5000 patients with PsA and 21,000 healthy controls and for 4340 patients with PsA and roughly 6500 patients with skin psoriasis. They calculated the heritability of PsA as a SNP-based heritability estimate (h^2 SNP) and with Priority Index they identified the biological pathways that discriminate between the two conditions. In addition, the authors developed a PsA risk prediction model. Novel genomic loci were identified and among others, the Wnt/ β -catenin and NF- κ B pathways were demonstrated as crucial signalling that differentiate PsA from cutaneous psoriasis [36]. An up-to-date list of PsA associated SNPs is shown in Table 1.

To fully dissect the mechanism by which genetic variants act, a comprehensive functional genomics approach is necessary, including chromosome conformation capture (3 C) techniques and DNA methylation and chromatin accessibility profiling. This will facilitate the understanding of disease-associated variants in their specific spatial contexts and the physical link of precise polymorphisms to target genes,

Table 1

Genome-wide significant genetic loci associated with susceptibility for PsA ($p < 5 \times 10^{-8}$) outside the HLA locus.

Chromosome	SNP	Putative candidate gene	OR	P value	Reference
1	rs12044149	<i>IL23R</i>	1.4	2.25×10^{-15}	[11]
1	rs2477077	<i>DENND1B</i>	1.23	1.20×10^{-06}	[11]
1	rs2476601	<i>PTPN22</i>	1.32	1.49×10^{-10}	[101]
1	rs7552167	<i>IFNLRI</i>	1.21	3.04×10^{-08}	[13]
5	rs715285	<i>P4HA2</i>	1.25	2.65×10^{-10}	[11]
5	rs76956521	<i>TNIP1</i>	1.5	4.98×10^{-09}	[11]
5	rs918520	<i>IL12B</i>	1.5	9.32×10^{-10}	[13]
6	rs12191877	<i>RPL3P2</i> , <i>WASF5P</i>	1.73	4.87×10^{-10}	[13]
6	rs33980500	<i>TRAF3IP2</i>	1.74	4.20×10^{-9}	[13]
12	rs2020854	<i>STAT2</i>	1.7	7.73×10^{-10}	[11]
14	rs8016947	<i>PSMA6</i>	1.2	9.65×10^{-05}	[13,27]
19	rs35251378	<i>TYK2</i>	1.41	3.5×10^{-8}	[13]

as well as the elucidation of cell-type specificity.

3. Chromosome conformation capture approaches to map target genes in PsA

As previously stated, most disease-associated genetic variants are predicted to affect regulatory regions, such as enhancers [24]. The genome is not linear but packaged and characterised by a complex 3D structure, where enhancers and their target genes may be far apart but are brought together by chromatin loop interactions [37].

Chromosomes are folded into compartment domains, which are often called A and B, where chromatin is compartmentalised [38,39], while topologically associated domains (TADs) are highly interacting local domains of megabase size, which play a crucial role in regulating gene expression [40,41]. Genome folding has a critical role in gene regulation and transcription, and thanks to chromosome conformation capture techniques it was possible to demonstrate the enhancer-promoter physical interaction which basically occurs in a cell type specific manner [42]. Enhancers are characterised by specific histone modifications (H3K4Me1, H3K27Ac, H3.3, H2Az) [43], and the presence of transcription factors bound to specific DNA sequences (including Mediator complex, RNA pol-II, p300, architectural proteins and chromatin remodelling complexes) [44].

In the neighbouring field of AS, a chronic inflammatory arthritis of the spine sharing various clinical features and susceptibility factors with PsA, our group has recently demonstrated with a 3 C approach chromatin looping between non-coding AS-associated SNPs and the distal promoter of the *RUNX3* gene, confirming the involvement of this transcription factor as possible causal mechanism [45].

Over the last two decades, different specific methods have been developed to investigate the 3D genome architecture and estimate the frequency of interaction of multiple genomic loci. In particular, the most recent include high-throughput chromosome conformation capture (Hi-C), Hi-ChIP and Tiled Capture [46–48]. Hi-C was introduced in 2009 and it recapitulates the “all versus all” unbiased approach to investigate genome wide interactions through sequencing [47]. A seminal paper by Javierre and colleagues demonstrated that, through a modification of Hi-C (i.e. Capture Hi-C), it was possible to identify the interaction regions of 31,253 promoters in 17 human primary hematopoietic cell

types. The authors were also able to connect non-coding variants associated to different diseases to putative target promoters [49]. In addition to Hi-C, Hi-ChIP has been developed to delineate promoter-enhancer interactions by leveraging principles of in situ Hi-C [48], combining long-range contacts investigation with enrichment of specific histone proteins (i.e. H3K27Ac), which are normally associated with active regulatory regions of the genome. Recently, Chandra and colleagues performed HiChIP to provide evidence of non-coding genetic variants having effect on gene expression (i.e. cis-eQTL) and cell-specific gene regulation in five immune cell types [31].

Capture Hi-C has been used by Martin and colleagues to investigate four autoimmune diseases associated loci (including PsA) and identify genomic interactions between associated variants and their functional targets in T- and B-cell lines. They demonstrated more than 8000 interactions, with 372 showing evidence of interaction with a promoter within 500 kb. Most of these interactions result highly cell-type specific [50].

Further, McGovern et al. have shown that the locus 6q23 which contains genetic variants associated with a plethora of autoimmune diseases including PsA, RA and systemic lupus erythematosus, interacts with *IL20RA* displaying also a strong eQTL effect [51].

HiChIP was recently performed in two different cell lines (HaCaT, immortalised keratinocytes and skin lymphoma-derived CD8 + T cells) to identify chromatin looping between disease-associated loci with target genes in specific dermatological traits including, psoriasis, PsA, atopic dermatitis, melanoma, and systemic sclerosis [52]. Very interestingly, the genetic variant *rs10794648*, which is generally allocated to the gene *IFNLRI* (Interferon Lambda Receptor 1), was linked to *GRHL3* (Grainyhead Like Transcription Factor 3), a gene which has crucial role in skin repair and development, and it plausibly might have a role in dermatological diseases.

4. DNA methylation and PsA

It has been postulated that epigenetic mechanisms contribute to gene expression changes in PsA, as recently pointed out by a transcriptomic profiling performed in PsA MZ twins concordant for clinical presentation, but discordant for radiographic progression, demonstrating 36 coding genes differentially expressed between healthy and affected twins [53].

Epigenetics mechanisms include DNA methylation, histone modifications and non-coding RNA [54]. These changes concur at variable levels to regulate the functions of different types of cells including immune cell in autoimmune diseases, affecting differentiation, gene expression and plasticity [55]. The identification of the so-called methylation quantitative trait loci (meQTLs) and the integration of these data with disease-associated variants from GWAS may further outline functional mechanisms which underlie genetic variant-disease association [56].

DNA methylation is a conserved epigenetic mark, and it consists in the transfer of a methyl group (-CH₃) onto the C5 position of a cytosine to form 5-methylcytosine (5mC). This reaction is catalysed by specific enzymes called DNA methyltransferases (DNMTs) which transfer the methyl group from the S-adenyl methionine [57]. DNA methylation is typically associated with gene silencing and repression of transcription, as it can reduce DNA accessibility for transcription factors binding and RNA polymerases. Further, methyl-CpG binding domain (MBD)-containing proteins (i.e. MECP2) which bind to 5mC particularly at promoter regions, can recruit both histone methyltransferases and deacetylases causing gene silencing [58].

Back in 1996, the first DNA methylation study in PsA patients suggested that PBMCs obtained from PsA patients have a specific methylation signature, although the study included a small number of patients [59]. Pollock and colleagues have recently studied DNA methylation variation in the sperm of PsA patients, showing significant changes in the methylation levels of genes associated with skin and joint disease,

including *MBP*, *OSBPL5*, *IL22*, *ELF5*, and *PTPRN2* [60].

Three studies have evaluated the DNA methylation contribution at genome-wide level in psoriasis compared to healthy skin. A 2012 epigenome-wide study focusing on altered CpG methylation in the psoriatic skin from 20 patients, identified 1108 sites differentially methylated compared to unaffected skin samples [61]. Zhang and colleagues performed a methylated DNA immunoprecipitation sequencing (MeDIP-Seq) in skin lesions affected and unaffected by psoriasis to characterize whole-genome DNA methylation patterns. They demonstrated that Programmed Cell Death 5 (*PDCD5*) and Tissue Inhibitor of Metalloproteinases 2 (*TIMP2*) resulted significantly hyper methylated in psoriatic skin samples compared to healthy skin [62]. In 2016, Zhou and colleagues completed an epigenome-wide association study on 262 skin and 48 peripheral blood mononuclear cell samples obtained from psoriatic patients and identified a specific pattern of differentially methylated sites associated with psoriasis. Specifically, they identified a strong psoriasis association for nine differentially methylated sites only in skin, including Cytochrome P450 Family 2 Subfamily S Member 1 (*CYP2S1*) and Protein Argonaute-2 (*EIF2C2*), confirming these sites acting in a skin-specific manner [63].

Our group recently performed a genome-wide DNA methylation study on whole blood of monozygotic twins discordant for psoriasis and PsA, called for simplicity psoriatic disease, using the Illumina Infinium Methylation platform. We identified 2564 differentially methylated positions (DMPs) between psoriatic disease and controls, and 19 regions with at least two DMPs within 1 kb of distance and significant within-pair $\Delta\beta$ -values ($p < 0.005$). Transcriptomic analysis identified IL-6/JAK/STAT3 and TNF- α pathways as central signalling axes involved in psoriatic disease. Moreover, this analysis demonstrated an enrichment in pathways related to oxidative phosphorylation, suggesting a role for altered glucose metabolism in the immune cells of patients with PsA, as already described in proinflammatory effector CD4 T cells [64]. Another group has recently investigated DNA methylation in peripheral blood CD8 + T cells, comparing 7 PsA patients, 10 patients with psoriasis and 9 healthy controls [65]. Of note, the study demonstrated that it is possible to obtain a DNA methylation profile in CD8 + T cells associated with psoriasis patients compared to controls, in particular with 397 DMPs and 9 Differentially Methylated Regions (DMRs). Further, PsA patients can be discriminated from patients with skin psoriasis using 1861 DMPs and more than 20 DMRs.

It has been further demonstrated by several studies the importance of coupling DNA methylation analysis with other functional genomics approaches such as the investigation of open chromatin [66,67]. Recently, a comprehensive study on different malignancy grade human gliomas profiling chromatin accessibility, DNA methylation, histone modifications and gene expression patterns, provided an inclusive atlas of brain specific enhancers and promoters having a role in brain cancer. These findings could potentially be a valuable tool for translation into effective clinical treatments [68].

The use of DNA methylation and open chromatin together with the annotation of ChIP-seq chromatin state has been recently instrumental to shedding light on novel mechanisms, for example in the cardiac QT interval length (found in the Long QT syndrome) and in type 2 diabetes expanding knowledge on the pancreatic islet regulome [69,70]. In particular, Wang and colleagues used a combination of genome-wide maps of cardiac enhancer activity together with the investigation of specific epigenetic signals (i.e. number of hypo/hypermethylated CpG at left ventricle, DNase I hypersensitivity at foetal heart and 3 C combined with high-throughput sequencing [4 C] in human induced pluripotent stem cells-derived cardiomyocytes) to prioritize loci with molecular functions in the regulation of genes associated with cardiac traits, such as the QRS duration reflecting cardiac conduction and the electrocardiographic QT interval reflecting myocardial repolarization. While, Thurner et al. demonstrated that genetic signals increasing the predisposition for type 2 diabetes are enriched predominantly in low methylated regions of the genome also characterised by higher chromatin

accessibility, specifically in human islet. To infer a complete epigenomic annotation for a specific cell type, the Chrom Hidden Markov Model (HMM) multivariate tool proves capable to predict the enrichment of the different chromatin-states genome wide, helping in the functional interpretation of the noncoding genome [71].

Though DNA methylation data need prospective validation, they are potentially very important for translation into clinical care, as a sizable minority (15–25 %) of patients with PsA develop arthritis and joints inflammation many years before the onset of skin involvement. The understanding of DNA methylation patterns and how these might change from skin disease to progression to PsA, and vice versa, is relevant both to understand the biological mechanisms and for the development of drugs that may act at different stage of psoriasis/PsA and/or PsA/psoriasis progression. At present, the number of studies investigating DNA methylation remain limited, but their expansion will facilitate also addressing other relevant questions, such as their role on therapeutic response to different systemic treatments and on differences in paternal versus maternal transmission of PsA [72,73]. Ultimately, the relationship between DNA methylation and chromatin accessibility remains to be elucidated but will influence the 3D architecture of the genome and, consequently, the functional role on diverse clinical phenotypes and treatment response.

5. Validation of findings using gene editing approach

Since its earliest phases, CRISPR/Cas9 represents a ground-breaking technique which allows the precise deletion of specific sections of the genome [74]. It was adapted from the naturally occurring RNA-mediated adaptive immune system editing process first identified in archaea and in bacteria [75]. Ten years have gone from the first CRISPR/Cas9 gene editing demonstration and the techniques has been constantly improved making the CRISPR/Cas9 system widely adopted and applied in several organisms. The importance of using gene-editing approaches to study the non-coding genome is highlighted by the fact that the CRISPR/Cas system might be used to map the function of the regulatory elements associated with disease: CRISPR-mediated gene interference (CRISPRi) or gene activation (CRISPRa) have been developed for this specific purpose. Specifically, CRISPRi uses a faulty version of the Cas9 enzyme to avoid the contact between regulatory elements and their target genes [76], while CRISPRa applies a transcriptional activator which is fused to the Cas9 protein to enhance transcription [77,78]. In general terms, both techniques are able to regulate cis-regulatory elements by changing or sterically blocking epigenetic modifications causing loss or gain of function.

A recent systematic review identified 309 experimentally validated non-coding GWAS polymorphisms, in turn regulating 252 genes across 130 human disease traits. Among the validation approaches, genome editing was used in 96 articles [79]. Numerous studies have been published in the context of T-cell biology: CRISPR screenings have been performed to investigate T-cell activation and identify essential master regulators after systematic genes knock-down [80,81]. The data provided by these studies are relevant for complex immune-mediated diseases such as PsA, RA and AS, where the genetic variants identified by GWAS are likely to play a role during T cell activation and differentiation [82,83].

Thus, coupling a CRISPR genomic approach with other functional analysis represents a powerful approach to validate GWAS findings (see Fig. 1). For example, single cell sequencing coupled with CRISPR technology is a real promising tool for genotype-phenotype mapping [84]. Another example comes from Perturb-seq which can provide information on genetic perturbations and single-cell gene expression, a readout for CRISPR-screens [85,86]. Ideally, genome editing approaches might be performed on primary cells from disease relevant tissue to really dissect the role of disease associated SNPs, but most of the current studies are limited to cell lines, because of the large number of cells required for this sort of experiments and the need to maintain cells in

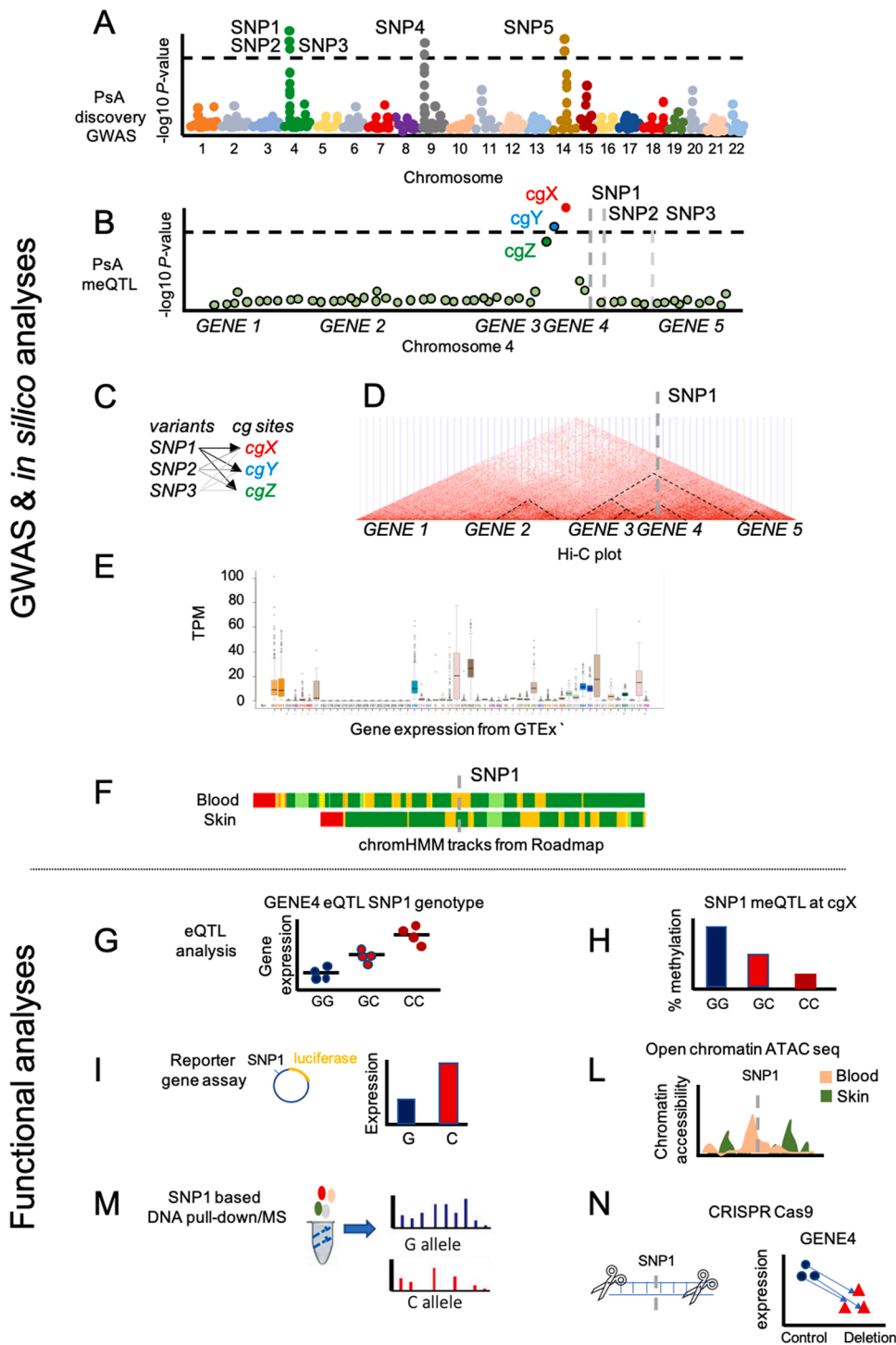


Fig. 1. Experimental workflow for the identification and analysis of a PsA risk SNP leading to a possible mechanistic effect. (A) Manhattan plot for a PsA GWAS. Every dot represents a SNP. Dotted line indicates genome wide significance threshold. SNP1 to SNP5 are significant genetic variants with increased risk for PsA. (B) meQTL for SNP1, 2 and 3. Each light green circle is a CpG site; the red dot (cgX) is the one strongly associated with SNP1 genotype, located at *GENE4* genomic locus. Blue and bright green dots (cgY and cgZ) show CpG sites weakly associated with SNP1 genotype. Dotted line indicates statistical significance threshold after multiple testing correction. (C) Linear regression model to associate significant SNPs and profiled CpG sites. (D) Hi-C plot showing interaction frequency at topologically associated domains (TADs), subTADs and chromosome organization within the genomic region of interest. Location of SNP1 is shown within *GENE4*. (E) GTEx showing expression of *GENE4* in different human cells and tissues. (F) ChromHMM data showing chromatin states in relevant tissues for PsA, in specific for skin and whole blood. The position of SNP1 is shown, overlapping enhancer region (yellow) in blood, and a transcription site in skin (green). Red represent transcription start sites. (G) Association between mRNA expression levels of *GENE4* and SNP1 genotype. The allele C is associated with increased expression (H) Plot showing inverse correlation between SNP1 genotype and cgX methylation levels. The allele C is associated with decreased methylation levels (I) Luciferase reporter assay to investigate the expression of target *GENE4* in the presence of protective and risk allele for SNP1. C allele increases enhancer activity. (L) ATAC-seq analysis showing open chromatin levels around the locus encompassing SNP1 in blood and skin tissues. (M) DNA pull-down approach followed by mass spectrometry (MS) analysis to identify the transcription factors differentially bound to SNP1 protective and risk alleles. (N) *In vitro* genomic deletion of SNP1 encompassing region with CRISPR/Cas9 approach and related *GENE4* expression analysis.

culture for long periods of time. Recent studies reported p53-dependent cellular toxicity following the Cas9-induced double-strand breaks [87, 88], which is an important limitation for its application. Further methodological improvements are needed to apply gene-editing approach as a routine validation technique for GWAS.

6. Impact on drug discovery

Only 10 % of drugs starting clinical trials reach patients, with a very high degree of failure due to insufficient efficacy [89], a problem that is also a major issue for PsA and other spondyloarthritides. Genetics

studies obtained some good success in the spondyloarthritis field, with the identification of the contribution of the IL-17/IL-23 molecular axis and pathogenic Th-17 cells to disease pathogenesis and the development of biological drugs blocking IL-17 (i.e. secukinumab or ixekizumab), or IL-23 (i.e. ustekinumab, guselkumab, risankizumab, tildrakizumab in psoriasis/PsA) [90]. Notably, while spectacular responses are achieved with these agents in cutaneous response, with 65–80 % of patients experiencing almost complete skin clearance, over 50 % of patients do not achieve minimal disease activity in joint response indicating disease heterogeneity in the two compartments even within the same individuals [91,92]. In that context gene expression

profiling of matched skin and synovial biopsies have demonstrated divergent expression of the IL23 and IL17 pathways with high expression of the IL23/IL17 axis in the skin of almost all individuals but variable expression in the joint, suggesting the drug-target expression levels may influence therapeutic response to the cognate biologic targeted therapy [93,94]. Thus, new therapeutic approaches are needed to move away from “trial and error” to rational patient prescription, based on disease mechanisms and drug mode of action.

The identification of PsA-associated genetic variants has the power to find genes or pathways that may contain optimal targets for drug therapy. GWAS results have the power to be translated into clinics: a careful and systematic analysis of the top candidate genes from GWAS together with a drug-drug and disease-disease similarities investigation and the evaluation of gene expression profiles and biological networks analysis will facilitate the identification of plausible drug target candidates. Fang and colleagues have developed a priority index pipeline which analyses 30 immune trait-associated variants obtained from GWAS data and prioritize putative drug targets through a gene-centric (via a genomic-features model) or a pathway-centric approach (via protein-protein interactions) [95]. This approach applied to disease-specific pathways might significantly ameliorate the translation between genome-scale data and drug target discovery.

Martin et al. analysed existing chromatin interaction data [50] to identify potential drug targets in PsA, RA and juvenile idiopathic arthritis [96]. The authors identified 454 high confidence genes, including 48 drug targets, where 108 drugs could have been effective (they also found 11 existing therapies used in the treatment of rheumatic diseases) [96]. Finally, polygenic risk score (PRS) which combines thousands of genetic variants to quantify an individual's genetic risk for a specific disease, it has been recently used to discriminate AS from other causes of chronic back pain [97]. Coupling functional annotations analysis with PRS may also improve its power (with the inclusion of functional SNPs relevant for different populations) and its transferability across different populations, where most casual variants are thought to be shared [98].

Altogether, the integration of functional genomics, immune-related functional annotation, chromosome conformation capture approach and the knowledge of network connectivity can maximize the information given by genetics to prioritize genes and pathways for target validation.

7. Conclusions

PsA is a clinically and biologically heterogeneous disease, with a stronger heritability compared to other chronic inflammatory diseases, possibly including psoriasis. Large scale comprehensive genomic studies have been successfully employed in genetically complex diseases, such as type I diabetes mellitus and RA, to identify genomic loci with plausible candidate drug targets. Using functional genomics, it has been then possible to improve the understanding of the biological basis of several diseases including PsA, which facilitates translation into target discovery and drug development. The approaches enlisted in this review still bear several limitations and improvements in these techniques are warranted to provide conclusive evidence to allow the identifications of patients at risk to develop PsA, especially in the absence of a pre-existing psoriasis. In addition, the available data do not account for the numerous clinical manifestations encountered in clinical practice, thus it remains unclear whether the different PsA domains are based on specific genetic backgrounds or if the resulting phenotype is derived solely from environmental stimuli. Though the latter hypothesis is supported by the role of biomechanical stress and the microbiome [99, 100], it is still possible that different disease manifestations such as enthesitis or skin psoriasis, two features sharing similar Koebner phenomena, may be predisposed by genetic susceptibility and demand dedicated studies in the future to overcome the complexity of the clinical presentations in daily practice.

In that context, the application of combined advance functional genomics methodologies may enhance the investigational power to be able to make an earlier diagnosis and unravel the mechanisms underlying the development of different clinical phenotypes and the heterogeneity of treatment response.

References

- [1] D.J. Veale, U. Fearon, The pathogenesis of psoriatic arthritis, *Lancet* 391 (10136) (2018) 2273–2284.
- [2] A. Zink, K. Thiele, D. Huscher, J. Listing, J. Sieper, A. Krause, et al., Healthcare and burden of disease in psoriatic arthritis. A comparison with rheumatoid arthritis and ankylosing spondylitis, *J. Rheuma* 33 (1) (2006) 86–90.
- [3] F. Van den Bosch, L. Coates, Clinical management of psoriatic arthritis, *Lancet* 391 (10136) (2018) 2285–2294.
- [4] C. Bridgwood, K. Sharif, J. Sherlock, A. Watad, D. McGonagle, Interleukin-23 pathway at the enthesis: the emerging story of enthesitis in spondyloarthritis, *Immunol. Rev.* 294 (1) (2020) 27–47.
- [5] J.P. Sherlock, B. Joyce-Shaikh, S.P. Turner, C.C. Chao, M. Sathe, J. Grein, et al., IL-23 induces spondyloarthritis by acting on ROR-gammat+ CD3+CD4-CD8-entheseal resident T cells, *Nat. Med.* 18 (7) (2012) 1069–1076.
- [6] D.D. O’Rielly, P. Rahman, Clinical and molecular significance of genetic loci associated with psoriatic arthritis, *Best. Pr. Res. Clin. Rheuma* 35 (2) (2021), 101691.
- [7] J. Feld, V. Chandran, N. Haroon, R. Inman, D. Gladman, Axial disease in psoriatic arthritis and ankylosing spondylitis: a critical comparison, *Nat. Rev. Rheuma* 14 (6) (2018) 363–371.
- [8] W. Taylor, D. Gladman, P. Helliwell, A. Marchesoni, P. Mease, H. Mielants, et al., Classification criteria for psoriatic arthritis: development of new criteria from a large international study, *Arthritis Rheum.* 54 (8) (2006) 2665–2673.
- [9] O.B. Pedersen, A.J. Svendsen, L. Ejstrup, A. Skytthe, P. Junker, On the heritability of psoriatic arthritis. Disease concordance among monozygotic and dizygotic twins, *Ann. Rheum. Dis.* 67 (10) (2008) 1417–1421.
- [10] V. Chandran, C.T. Schentag, J.E. Brockbank, F.J. Pellett, S. Shanmugarajah, S. M. Tolosa, et al., Familial aggregation of psoriatic arthritis, *Ann. Rheum. Dis.* 68 (5) (2009) 664–667.
- [11] J. Bowes, A. Budu-Aggrey, U. Huffmeier, S. Uebe, K. Steel, H.L. Hebert, et al., Dense genotyping of immune-related susceptibility loci reveals new insights into the genetics of psoriatic arthritis, *Nat. Commun.* 6 (2015) 6046.
- [12] E. Ellinghaus, P.E. Stuart, D. Ellinghaus, R.P. Nair, S. Debrus, J.V. Raelson, et al., Genome-wide meta-analysis of psoriatic arthritis identifies susceptibility locus at REL, *J. Invest. Dermatol.* 132 (4) (2012) 1133–1140.
- [13] P.E. Stuart, R.P. Nair, L.C. Tsoi, T. Tejasvi, S. Das, H.M. Kang, et al., Genome-wide association analysis of psoriatic arthritis and cutaneous psoriasis reveals differences in their genetic architecture, *Am. J. Hum. Genet.* 97 (6) (2015) 816–836.
- [14] R. Winchester, G. Minevich, V. Steshenko, B. Kirby, D. Kane, D.A. Greenberg, et al., HLA associations reveal genetic heterogeneity in psoriatic arthritis and in the psoriasis phenotype, *Arthritis Rheum.* 64 (4) (2012) 1134–1144.
- [15] M. Haroon, R. Winchester, J.T. Giles, E. Heffernan, O. FitzGerald, Certain class I HLA alleles and haplotypes implicated in susceptibility play a role in determining specific features of the psoriatic arthritis phenotype, *Ann. Rheum. Dis.* 75 (1) (2016) 155–162.
- [16] M. Haroon, B. Kirby, O. FitzGerald, High prevalence of psoriatic arthritis in patients with severe psoriasis with suboptimal performance of screening questionnaires, *Ann. Rheum. Dis.* 72 (5) (2013) 736–740.
- [17] R. Winchester, O. FitzGerald, The many faces of psoriatic arthritis: their genetic determinism, *Rheumatology (Oxford)* 59 (Suppl 1) (2020) i4–i9.
- [18] S. Rahmati, L. Tsoi, D. O’Rielly, V. Chandran, P. Rahman, Complexities in genetics of psoriatic arthritis, *Curr. Rheuma Rep.* 22 (4) (2020) 10.
- [19] O. FitzGerald, M. Haroon, J.T. Giles, R. Winchester, Concepts of pathogenesis in psoriatic arthritis: genotype determines clinical phenotype, *Arthritis Res. Ther.* 17 (2015) 115.
- [20] D.D. Gladman, V.T. Farewell, P. Rahman, C.T. Schentag, F. Pellett, C.M. Ng, et al., HLA-DRB1*04 alleles in psoriatic arthritis: comparison with rheumatoid arthritis and healthy controls, *Hum. Immunol.* 62 (11) (2001) 1239–1244.
- [21] R.P. McCord, N. Kaplan, L. Giorgetti, Chromosome conformation capture and beyond: toward an integrative view of chromosome structure and function, *Mol. Cell* 77 (4) (2020) 688–708.
- [22] C. Shi, M. Rattray, A. Barton, J. Bowes, G. Orozco, Using functional genomics to advance the understanding of psoriatic arthritis, *Rheumatology (Oxford)* 59 (11) (2020) 3137–3146.
- [23] S.L. Edwards, J. Beesley, J.D. French, A.M. Dunning, Beyond GWAS: illuminating the dark road from association to function, *Am. J. Hum. Genet.* 93 (5) (2013) 779–797.
- [24] K.K. Farh, A. Marson, J. Zhu, M. Kleinewietfeld, W.J. Housley, S. Beik, et al., Genetic and epigenetic fine mapping of causal autoimmune disease variants, *Nature* 518 (7539) (2015) 337–343.
- [25] A.P. Cope, H. Schulze-Koops, M. Aringer, The central role of T cells in rheumatoid arthritis, *Clin. Exp. Rheuma* 25 (5 Suppl 46) (2007) S4–S11.
- [26] A.P. Croft, J. Campos, K. Jansen, J.D. Turner, J. Marshall, M. Attar, et al., Distinct fibroblast subsets drive inflammation and damage in arthritis, *Nature* 570 (7760) (2019) 246–251.

- [27] A. Budu-Aggrey, J. Bowes, A. Barton, Identifying a novel locus for psoriatic arthritis, *Rheumatology (Oxford)* 55 (1) (2016) 25–32.
- [28] G.T. Consortium, The genotype-tissue expression (GTEx) project, *Nat. Genet.* 45 (6) (2013) 580–585.
- [29] G.T. Consortium, The GTEx Consortium atlas of genetic regulatory effects across human tissues, *Science* 369 (6509) (2020) 1318–1330.
- [30] N. Kerimov, J.D. Hayhurst, K. Peikova, J.R. Manning, P. Walter, L. Kolberg, et al., A compendium of uniformly processed human gene expression and splicing quantitative trait loci, *Nat. Genet.* 53 (9) (2021) 1290–1299.
- [31] V. Chandra, S. Bhattacharyya, B.J. Schmedel, A. Madrigal, C. Gonzalez-Colin, S. Fotsing, et al., Promoter-interacting expression quantitative trait loci are enriched for functional genetic variants, *Nat. Genet.* 53 (1) (2021) 110–119.
- [32] B.P. Fairfax, P. Humburg, S. Makino, V. Naranbhai, D. Wong, E. Lau, et al., Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression, *Science* 343 (6175) (2014), 1246949.
- [33] B.J. Schmedel, D. Singh, A. Madrigal, A.G. Valdivino-Gonzalez, B.M. White, J. Zapardiel-Gonzalo, et al., Impact of genetic polymorphisms on human immune cell gene expression, *Cell* 175 (6) (2018) 1701–1715, e16.
- [34] H.J. Westra, M.J. Peters, T. Esko, H. Yaghootkar, C. Schurmann, J. Kettunen, et al., Systematic identification of trans eQTLs as putative drivers of known disease associations, *Nat. Genet.* 45 (10) (2013) 1238–1243.
- [35] J. Ding, J.E. Gudjonsson, L. Liang, P.E. Stuart, Y. Li, W. Chen, et al., Gene expression in skin and lymphoblastoid cells: refined statistical method reveals extensive overlap in cis-eQTL signals, *Am. J. Hum. Genet.* 87 (6) (2010) 779–789.
- [36] M. Somero, M. Stadler, N. Dand, J. Bluett, D. Jadon, F. Jalali-Najafabadi, et al., Comparative genetic analysis of psoriatic arthritis and psoriasis for the discovery of genetic risk factors and risk prediction modeling, *Arthritis Rheuma* 74 (9) (2022) 1535–1543.
- [37] H. Zheng, W. Xie, The role of 3D genome organization in development and cell differentiation, *Nat. Rev. Mol. Cell Biol.* 20 (9) (2019) 535–550.
- [38] E.M. Hildebrand, J. Dekker, Mechanisms and functions of chromosome compartmentalization, *Trends Biochem. Sci.* 45 (5) (2020) 385–396.
- [39] Q. Szabo, F. Bantignies, G. Cavalli, Principles of genome folding into topologically associating domains, *Sci. Adv.* 5 (4) (2019) eaaw1668.
- [40] E. de Wit, E.S. Vos, S.J. Holwerda, C. Valdes-Quezada, M.J. Versteegen, H. Teunissen, et al., CTCF binding polarity determines chromatin looping, *Mol. Cell* 60 (4) (2015) 676–684.
- [41] V.Y. Goel, A.S. Hansen, The macro and micro of chromosome conformation capture, *Wiley Inter. Rev. Dev. Biol.* 10 (6) (2021), e395.
- [42] B. van Steensel, E.E.M. Furlong, The role of transcription in shaping the spatial organization of the genome, *Nat. Rev. Mol. Cell Biol.* 20 (6) (2019) 327–337.
- [43] E. Calo, J. Wysocka, Modification of enhancer chromatin: what, how, and why? *Mol. Cell* 49 (5) (2013) 825–837.
- [44] A. Panigrahi, B.W. O'Malley, Mechanisms of enhancer action: the known and the unknown, *Genome Biol.* 22 (1) (2021) 108.
- [45] C.J. Cohen, C. Davidson, C. Selmi, P. Bowness, J.C. Knight, B.P. Wordsworth, et al., Disruption of c-MYC binding and chromosomal looping involving genetic variants associated with ankylosing spondylitis upstream of the RUNX3 promoter, *Front Genet.* 12 (2021), 741867.
- [46] D.J. Downes, A.L. Smith, M.A. Karpinska, T. Velychko, K. Rue-Albrecht, D. Sims, et al., Capture-C: a modular and flexible approach for high-resolution chromosome conformation capture, *Nat. Protoc.* 17 (2) (2022) 445–475.
- [47] E. Lieberman-Aiden, N.L. van Berkum, L. Williams, M. Imakaev, T. Ragoczy, A. Telling, et al., Comprehensive mapping of long-range interactions reveals folding principles of the human genome, *Science* 326 (5950) (2009) 289–293.
- [48] M.R. Mumbach, A.J. Rubin, R.A. Flynn, C. Dai, P.A. Khavari, W.J. Greenleaf, et al., HiChIP: efficient and sensitive analysis of protein-directed genome architecture, *Nat. Methods* 13 (11) (2016) 919–922.
- [49] B.M. Javierre, O.S. Burren, S.P. Wilder, R. Kreuzhuber, S.M. Hill, S. Sewitz, et al., Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters, *Cell* 167 (5) (2016) 1369–1384, e19.
- [50] P. Martin, A. McGovern, G. Orozco, K. Duffus, A. Yarwood, S. Schoenfelder, et al., Capture Hi-C reveals novel candidate genes and complex long-range interactions with related autoimmune risk loci, *Nat. Commun.* 6 (2015) 10069.
- [51] A. McGovern, S. Schoenfelder, P. Martin, J. Massey, K. Duffus, D. Plant, et al., Capture Hi-C identifies a novel causal gene, IL20RA, in the pan-autoimmune genetic susceptibility region 6q23, *Genome Biol.* 17 (1) (2016) 212.
- [52] C. Shi, H. Ray-Jones, J. Ding, K. Duffus, Y. Fu, V.P. Gaddi, et al., Chromatin looping links target genes with genetic risk loci for dermatological traits, *J. Invest. Dermatol.* 141 (8) (2021) 1975–1984.
- [53] M.M. Angioni, A. Floris, I. Cangemi, M. Congia, E. Chessa, S. Orru, et al., Gene expression profiling of monozygotic twins affected by psoriatic arthritis, *Open Access Rheuma* 13 (2021) 23–29.
- [54] J.M. Greally, A user's guide to the ambiguous word 'epigenetics', *Nat. Rev. Mol. Cell Biol.* 19 (4) (2018) 207–208.
- [55] M. Vecellio, H. Wu, Q. Lu, C. Selmi, The multifaceted functional role of DNA methylation in immune-mediated rheumatic diseases, *Clin. Rheuma* 40 (2) (2021) 459–476.
- [56] M.J. Bonder, R. Luijk, D.V. Zhernakova, M. Moed, P. Deelen, M. Vermaat, et al., Disease variants alter transcription factor levels and methylation of their binding sites, *Nat. Genet.* 49 (1) (2017) 131–138.
- [57] L.D. Moore, T. Le, G. Fan, DNA methylation and its basic function, *Neuropsychopharmacology* 38 (1) (2013) 23–38.
- [58] A.E.A. Surace, C.M. Hedrich, The role of epigenetics in autoimmune/inflammatory disease, *Front Immunol.* 10 (2019) 1525.
- [59] Y.I. Kim, J.W. Logan, J.B. Mason, R. Roubenoff, DNA hypomethylation in inflammatory arthritis: reversal with methotrexate, *J. Lab. Clin. Med.* 128 (2) (1996) 165–172.
- [60] R.A. Pollock, L. Zaman, V. Chandran, D.D. Gladman, Epigenome-wide analysis of sperm cells identifies IL22 as a possible germ line risk locus for psoriatic arthritis, *PLoS One* 14 (2) (2019), e0212043.
- [61] E.D. Roberson, Y. Liu, C. Ryan, C.E. Joyce, S. Duan, L. Cao, et al., A subset of methylated CpG sites differentiate psoriatic from normal skin, *J. Invest. Dermatol.* 132 (3 Pt 1) (2012) 583–592.
- [62] P. Zhang, M. Zhao, G. Liang, G. Yin, D. Huang, F. Su, et al., Whole-genome DNA methylation in skin lesions from patients with psoriasis vulgaris, *J. Autoimmun.* 41 (2013) 17–24.
- [63] F. Zhou, W. Wang, C. Shen, H. Li, X. Zuo, X. Zheng, et al., Epigenome-wide association analysis identified nine skin DNA methylation loci for psoriasis, *J. Invest. Dermatol.* 136 (4) (2016) 779–787.
- [64] M. Vecellio, E.M. Paraboschi, A. Ceribelli, N. Isailovic, F. Motta, G. Cardamone, et al., DNA methylation signature in monozygotic twins discordant for psoriatic disease, *Front Cell Dev. Biol.* 9 (2021), 778677.
- [65] A. Charras, J. Garau, S.R. Hofmann, E. Carlsson, C. Cereda, S. Russ, et al., DNA Methylation patterns in CD8(+) T cells discern psoriasis from psoriatic arthritis and correlate with cutaneous disease activity, *Front Cell Dev. Biol.* 9 (2021), 746145.
- [66] K.R. Barnett, B.E. Decato, T.J. Scott, T.J. Hansen, B. Chen, J. Attalla, et al., ATAC-Seq captures prolonged DNA methylation of dynamic chromatin accessibility loci during cell fate transitions, *Mol. Cell* 77 (6) (2020) 1350–1364, e6.
- [67] S. Pott, Simultaneous measurement of chromatin accessibility, DNA methylation, and nucleosome phasing in single cells, *Elife* (2017) 6.
- [68] K. Stepniak, M.A. Machnicka, J. Mieczkowski, A. Macioszek, B. Wojtas, B. Gielniewski, et al., Mapping chromatin accessibility and active regulatory elements reveals pathological mechanisms in human gliomas, *Nat. Commun.* 12 (1) (2021) 3621.
- [69] M. Thurner, M. van de Bunt, J.M. Torres, A. Mahajan, V. Nylander, A.J. Bennett, et al., Integration of human pancreatic islet genomic data refines regulatory mechanisms at Type 2 Diabetes susceptibility loci, *Elife* (2018) 7.
- [70] X. Wang, N.R. Tucker, G. Rizki, R. Mills, P.H. Krijger, E. de Wit, et al., Discovery and validation of sub-threshold genome-wide association study loci using epigenomic signatures, *Elife* (2016) 5.
- [71] J. Ernst, M. Kellis, Chromatin-state discovery and genome annotation with ChromHMM, *Nat. Protoc.* 12 (12) (2017) 2478–2492.
- [72] A.L. Carvalho, C.M. Hedrich, The molecular pathophysiology of psoriatic arthritis-the complex interplay between genetic predisposition, epigenetics factors, and the microbiome, *Front Mol. Biosci.* 8 (2021), 662047.
- [73] C.M. Nguyen, W. Liao, Genomic imprinting in psoriasis and atopic dermatitis: a review, *J. Dermatol. Sci.* 80 (2) (2015) 89–93.
- [74] H. Wang, M. La Russa, L.S. Qi, CRISPR/Cas9 in genome editing and beyond, *Annu. Rev. Biochem.* 85 (2016) 227–264.
- [75] J.E. Garneau, M.E. Dupuis, M. Villion, D.A. Romero, R. Barrangou, P. Boyaval, et al., The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA, *Nature* 468 (7320) (2010) 67–71.
- [76] L.S. Qi, M.H. Larson, L.A. Gilbert, J.A. Doudna, J.S. Weissman, A.P. Arkin, et al., Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression, *Cell* 152 (5) (2013) 1173–1183.
- [77] D. Bikard, W. Jiang, P. Samai, A. Hochschild, F. Zhang, L.A. Marraffini, Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system, *Nucleic Acids Res.* 41 (15) (2013) 7429–7437.
- [78] W. Jiang, D. Bikard, D. Cox, F. Zhang, L.A. Marraffini, RNA-guided editing of bacterial genomes using CRISPR-Cas systems, *Nat. Biotechnol.* 31 (3) (2013) 233–239.
- [79] A.J. Alsheikh, S. Wollenhaupt, E.A. King, J. Reeb, S. Ghosh, L.R. Stolzenburg, et al., The landscape of GWAS validation: systematic review identifying 309 validated non-coding variants across 130 human diseases, *BMC Med. Genom.* 15 (1) (2022) 74.
- [80] J. Henriksson, X. Chen, T. Gomes, U. Ullah, K.B. Meyer, R. Miragaia, et al., Genome-wide CRISPR screens in T helper cells reveal pervasive crosstalk between activation and differentiation, *Cell* 176 (4) (2019) 882–896, e18.
- [81] E. Shifrut, J. Carnevale, V. Tobin, T.L. Roth, J.M. Woo, C.T. Bui, et al., Genome-wide CRISPR screens in primary human T cells reveal key regulators of immune function, *Cell* 175 (7) (2018) 1958–1971, e15.
- [82] D. Calderon, M.L.T. Nguyen, A. Mezger, A. Kathiria, F. Muller, V. Nguyen, et al., Landscape of stimulation-responsive chromatin across diverse human immune cells, *Nat. Genet.* 51 (10) (2019) 1494–1505.
- [83] B. Soskic, E. Cano-Gamez, D.J. Smyth, W.C. Rowan, N. Nakic, J. Esparza-Gordillo, et al., Chromatin activity at GWAS loci identifies T cell states driving complex immune diseases, *Nat. Genet.* 51 (10) (2019) 1486–1493.
- [84] E. Cano-Gamez, G. Trynka, From GWAS to function: using functional genomics to identify the mechanisms underlying complex diseases, *Front Genet* 11 (2020) 424.
- [85] L. Brunello, Genome-scale single-cell CRISPR screens, *Nat. Rev. Genet.* 23 (8) (2022) 459.
- [86] A. Dixit, O. Parnas, B. Li, J. Chen, C.P. Fulco, L. Jerby-Arnon, et al., Perturb-Seq: dissecting molecular circuits with scalable single-cell RNA profiling of pooled genetic screens, *Cell* 167 (7) (2016) 1853–1866, e17.
- [87] E. Haapaniemi, S. Botla, J. Persson, B. Schmierer, J. Taipale, CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response, *Nat. Med.* 24 (7) (2018) 927–930.

- [88] R.J. Ihry, M.R. Salick, D.J. Ho, M. Sondey, S. Kommineni, S. Paula, et al., Genome-scale CRISPR screens identify human pluripotency-specific genes, *Cell Rep.* 27 (2) (2019) 616–630, e6.
- [89] T.J. Hwang, D. Carpenter, J.C. Lauffenburger, B. Wang, J.M. Franklin, A. S. Kesselheim, Failure of investigational drugs in late-stage clinical development and publication of trial results, *JAMA Intern. Med.* 176 (12) (2016) 1826–1833.
- [90] M. Vecellio, V.X. Hake, C. Davidson, M.C. Carena, B.P. Wordsworth, C. Selmi, The IL-17/IL-23 axis and its genetic contribution to psoriatic arthritis, *Front Immunol.* 11 (2020), 596086.
- [91] I.B. McInnes, F. Behrens, P.J. Mease, A. Kavanaugh, C. Ritchlin, P. Nash, et al., Secukinumab versus adalimumab for treatment of active psoriatic arthritis (EXCEED): a double-blind, parallel-group, randomised, active-controlled, phase 3b trial, *Lancet* 395 (10235) (2020) 1496–1505.
- [92] P.J. Mease, P. Rahman, A.B. Gottlieb, A.P. Kollmeier, E.C. Hsia, X.L. Xu, et al., Guselkumab in biologic-naïve patients with active psoriatic arthritis (DISCOVER-2): a double-blind, randomised, placebo-controlled phase 3 trial, *Lancet* 395 (10230) (2020) 1126–1136.
- [93] J. Belasco, J.S. Louie, N. Gulati, N. Wei, K. Nograles, J. Fuentes-Duculan, et al., Comparative genomic profiling of synovium versus skin lesions in psoriatic arthritis, *Arthritis Rheuma* 67 (4) (2015) 934–944.
- [94] A. Nerviani, M.A. Boutet, W.S.G. Tan, K. Goldmann, N. Purkayastha, T.A. Lajtos, et al., IL-23 skin and joint profiling in psoriatic arthritis: novel perspectives in understanding clinical responses to IL-23 inhibitors, *Ann. Rheum. Dis.* 80 (5) (2021) 591–597.
- [95] H. Fang, U.-D. Consortium, H. De Wolf, B. Knezevic, K.L. Burnham, J. Osgood, et al., A genetics-led approach defines the drug target landscape of 30 immune-related traits, *Nat. Genet.* 51 (7) (2019) 1082–1091.
- [96] P. Martin, J. Ding, K. Duffus, V.P. Gaddi, A. McGovern, H. Ray-Jones, et al., Chromatin interactions reveal novel gene targets for drug repositioning in rheumatic diseases, *Ann. Rheum. Dis.* 78 (8) (2019) 1127–1134.
- [97] Z. Li, X. Wu, P.J. Leo, E. De Guzman, N. Akkoc, M. Breban, et al., Polygenic risk scores have high diagnostic capacity in ankylosing spondylitis, *Ann. Rheum. Dis.* 80 (9) (2021) 1168–1174.
- [98] U.M. Marigorta, A. Navarro, High trans-ethnic replicability of GWAS results implies common causal variants, *PLoS Genet.* 9 (6) (2013), e1003566.
- [99] K. Sharif, C. Bridgewood, S. Dubash, D. McGonagle, Intestinal and enthesitis innate immunity in early axial spondyloarthritis, *Rheumatology (Oxford)* 59 (Suppl4) (2020) iv67–iv78.
- [100] D. Simon, A. Kleyer, S. Bayat, J. Knitz, L. Valor-Mendez, M. Schweiger, et al., Biomechanical stress in the context of competitive sports training triggers enthesitis, *Arthritis Res. Ther.* 23 (1) (2021) 172.
- [101] J. Bowes, S. Loefer, A. Budu-Aggrey, S. Uebe, I.N. Bruce, M. Feletar, et al., PTPN22 is associated with susceptibility to psoriatic arthritis but not psoriasis: evidence for a further PsA-specific risk locus, *Ann. Rheum. Dis.* 74 (10) (2015) 1882–1885.