One SNP at a Time: Moving beyond GWAS in Psoriasis

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Although genome-wide association studies have revealed important insights into the global genetic basis of psoriasis, the findings require further investigation. At present, the known genetic risk loci are largely uncharacterized in terms of the variant or gene responsible for the association, the biological pathway involved, and the main cell type driving the pathology. This review primarily focuses on current approaches toward gaining a complete understanding of how these known genetic loci contribute to an increased disease risk in psoriasis.

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INTRODUCTION

Psoriasis is thought to be dependent on a complex interplay between many genetic loci and environmental factors. The development of sophisticated methods for rapid genotyping of DNA has led to the era of high-powered genome-wide association studies (GWASs), which have revolutionized our understanding of complex trait genetics (Stranger et al., 2011). GWAS and more targeted candidate gene approaches [Immunochip; Tsoi et al. (2012)] have identified more than 40 single nucleotide polymorphisms (SNPs) associated with psoriasis at a genome-wide significance level ($P < 5 \times 10^{-8}$), many of which are situated near genes involved in adaptive and innate immunity pathways, which are summarized in Supplementary Table S1 (online) and reviewed by Mahil et al. (2015). In the "post-GWAS" era, many challenges remain before the full genetic component of disease association can be understood. One of these challenges is to better understand how the known genetic loci confer risk to disease, which is the primary focus of this review.

GENETICS OF PSORIASIS: A BRIEF OVERVIEW

The genetic locus conferring the greatest risk for psoriasis susceptibility in both European and Chinese populations is the major histocompatibility complex (MHC) class I, implicating the involvement of the adaptive immune system in psoriasis pathology (Ellinghaus et al., 2010; Liu et al., 2008; Nair et al., 2009; Strange et al., 2010; Stuart et al., 2010; Tsoi et al., 2012; Zhang et al., 2009). Within the MHC, an allele at the *HLA* gene, *HLA-C*06:02*, shows the strongest association with psoriasis. However, it is evident that independent risk associations exist across the MHC (Feng et al., 2009), including ethnicity-specific signals (Yin et al., 2015). A recent fine mapping study confirmed the presence of independent signals at *HLA-C*12:03*, *HLA-B*, *HLA-A*, and *HLA-DQA1* through conditional analysis (Okada et al., 2014). Outside of the MHC, a second well-established risk locus resides at the gene for endoplasmic reticulum aminopeptidase 1 (Strange et al., 2010). The endoplasmic reticulum aminopeptidase 1 protein is thought to be responsible for *N*-terminal trimming of peptides allowing binding to the MHC class I molecule (Alvarez-Navarro and de Castro, 2014; Saric et al., 2002); therefore, this signal further implicates the involvement of the adaptive immune system in psoriasis.

Many SNPs have also implicated gene candidates from innate immunity pathways in European cohorts (Capon et al., 2008; Cargill et al., 2007; Ellinghaus et al., 2012; Nair et al., 2009; Strange et al., 2010; Stuart et al., 2010; Tsoi et al., 2012, 2015b). These include NF-κB signaling (e.g., *REL, TNIP1, NFKBIA*, and *CARD14*), IFN signaling (e.g., *IL28RA* and *TYK2*), T-cell regulation (e.g., *RUNX3, IL13, TAGAP, ETS1,* and *MBD2*), and antiviral signaling (e.g., *IFIH1, DDX58*, and *RNF114*). Multiple loci containing genes involved in the IL-23 pathway specifically implicate a role for Th17 cells (e.g., *TNFAIP3, IL23R, IL12B, TRAF3IP2, IL23A*, and *STAT3*).

Aside from the immune system, skin barrier regulatory genes of the late cornified envelope (LCE) within the epidermal differentiation complex are associated with psoriasis in both European and Chinese populations (de Cid et al., 2009; Strange et al., 2010; Tsoi et al., 2012; Zhang et al., 2009). The variants in this region likely tag a 30-kb deletion including the genes *LCE3C* and *LCE3B*. The loss of these genes is thought to impair reparation of the skin barrier after injury (Bergboer et al., 2011, 2012). Alternatively, the loss of an epidermal-specific enhancer element within the

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Abbreviations: ChIP, chromatin immunoprecipitation; eQTL, expression quantitative trait locus; GPP, generalized pustular psoriasis; GWAS, genome-wide association study; LCE, late cornified envelope; LD, linkage dysequilibrium; MHC, major histocompatibility complex; SNP, single nucleotide polymorphism

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deleted region could be causing aberrant global transcription of epidermal differentiation complex genes (de Guzman Strong et al., 2010).

Several recent studies have demonstrated that Chinese populations display a number of unique genetic associations with psoriasis, such as *NFKB1*, *PTTG1*, *MTHFR*, and *CCDC129* (Sun et al., 2010; Zuo et al., 2015), and share some loci with European populations (Cheng et al., 2014; Y Li et al., 2013; Sheng et al., 2014; Tang et al., 2014; Zhang et al., 2009). Recently a transethnic psoriasis GWAS, including both Chinese and European cohorts, identified four novel loci in European patients (*LOC144817, COG6, RUNX1*, and *TP63*) and population-specific effects at several loci (Yin et al., 2015). Further transethnic GWAS studies that compare allele frequencies, odds ratios, and disease pathways between different populations will advance the current understanding of global psoriasis pathogenesis.

The genetics of late-onset psoriasis, in which disease occurs after 40 years of age, substantially overlaps with that of early-onset psoriasis. In a GWAS, the known type I psoriasis risk loci *IL12B* and *HLA-C* reached genome-wide significance, and six more known loci reached study-wide significance (*IL23R, TRAF3IP2, IL23A, IFIH1, RNF114*, and *HLA-A*) (Hebert et al., 2015). However, late-onset psoriasis may also have unique risk loci at *IL1B* and *IL1R1* (Hebert et al., 2014, 2015; Reich et al., 2002). Subsequent well-powered studies will be required to determine how an increasing age of onset affects the strength of these genetic associations.

The genetic architecture of psoriasis subtypes are gradually being defined; for example, generalized pustular psoriasis (GPP) is associated with protein-coding mutations in *CARD14* (Jordan et al., 2012b; Qin et al., 2014; Sugiura et al., 2014) and *IL36RN* (Hayashi et al., 2014; Korber et al., 2013; M Li et al., 2013; Sugiura et al., 2013). In *CARD14*, the de novo mutation p.Glu138Ala was found in a child with GPP (Jordan et al., 2012b), and the rare variant p.Asp176His was shown to predispose to GPP with plaque psoriasis in Japanese patients (Sugiura et al., 2014). In *IL36RN*, protein modeling and biochemical analyses showed that the GPP-associated mutation p.L27P reduced the stability of IL36RN protein and decreased its expression and potency, leading to increased proinflammatory signaling (Marrakchi et al., 2011). Rare *IL36RN* mutations are thought to be uniquely associated with GPP (Capon, 2013; Sugiura et al., 2013) and are linked with a more severe disease phenotype and earlier age of onset (Hussain et al., 2015).

WHY HASN'T GWAS PROVIDED ALL THE ANSWERS?

Missing heritability

To date GWAS has only revealed a small proportion of the genetic component of psoriasis. In Europeans, the proportion of psoriasis heritability explained by GWAS variants was most recently estimated at 22% (Tsoi et al., 2012), whereas in Chinese it is reportedly 45.7% (Jiang et al., 2015). Several reasons have been proposed for the apparent missing heritability in complex disease, including gene-gene and gene-environment interactions and the existence of highly deleterious rare variants, although the latter may not greatly impact on psoriasis heritability (Hunt et al., 2013; Tang et al., 2014). Ultimately, it is likely that more genetic signals will be discovered along with the increased use of next-generation sequencing technology that encompasses whole genomes or exomes. Additionally, increased study power through large sample sets and refined statistical methods (fine mapping, genotype calling, and imputation) can identify common novel loci, strengthen known signals, and find independent effects at known loci (Tsoi et al., 2015b; Yin et al., 2015). To detect rare variants, however, novel statistical analysis techniques such as burden testing may be required.

Interpreting association signals

GWAS-associated variants usually require further extensive interrogation for a full interpretation of the data. In part, this is because of the number of highly correlated genetic variants in linkage dysequilibrium (LD) that may be causal. Research has shown that only 5% of lead GWAS SNPs are likely to be causal and tend to lie an average distance of 14 kb from the probable causal SNP (Farh et al., 2015). Thus, the first task after GWAS is to perform dense genotyping, resequencing or imputation, to test the association of all variants in LD with the lead variant and gain a detailed picture of potentially causal variants. The remainder of this review addresses the question of how to best utilize the current gains made by GWAS by identifying the function of putative causal variants, particularly in noncoding regions (Figure 1).

CONSIDERATIONS FOR FUNCTIONAL ANNOTATION OF GWAS VARIANTS

In a minority of psoriasis susceptibility loci, the GWAS signal intersects with coding regions of genes (e.g., *IL23R* and *CARD14*). In these cases, the function of the variants can be readily assessed (di Meglio et al., 2013; Jordan et al., 2012b; Sarin et al., 2011). The genetic association of psoriasis with *CARD14* was initially discovered through linkage mapping (Tomfohrde et al., 1994,) followed by positional cloning using next-generation sequencing that identified an excess of rare missense variants in families affected by the disease and in psoriasis cohorts (Jordan et al., 2012a, 2012b). After these studies, a psoriasis-associated common missense variant discovered by Jordan et al. (2012a) achieved genome-wide significance in cohorts of European and Chinese ancestry (Tang et al., 2014; Tsoi et al., 2012). CARD14 is a scaffolding protein that has a role in NF-κB activation. In functional experiments, some of the associated rare variants were found to affect *CARD14* splicing, leading to increased downstream NF-κB expression in keratinocytes (Jordan et al., 2012a, 2012b).

The majority of psoriasis-associated GWAS loci are located outside of traditional gene coding regions, often in regulatory enhancer regions characterized by open areas of accessible chromatin that are sensitive to DNAse I and contain modified histone marks (Ernst et al., 2011). Here, identification of the causal SNP becomes more challenging; bioinformatic evidence is first used to form a hypothesis about which SNPs are likely to be causal. The hypothesis should then be tested directly with functional experiments to show the mechanism by which the putative causal SNP affects gene expression or function.

Bioinformatic and experimental approaches must take into account relevant cell types and stimulatory factors that may

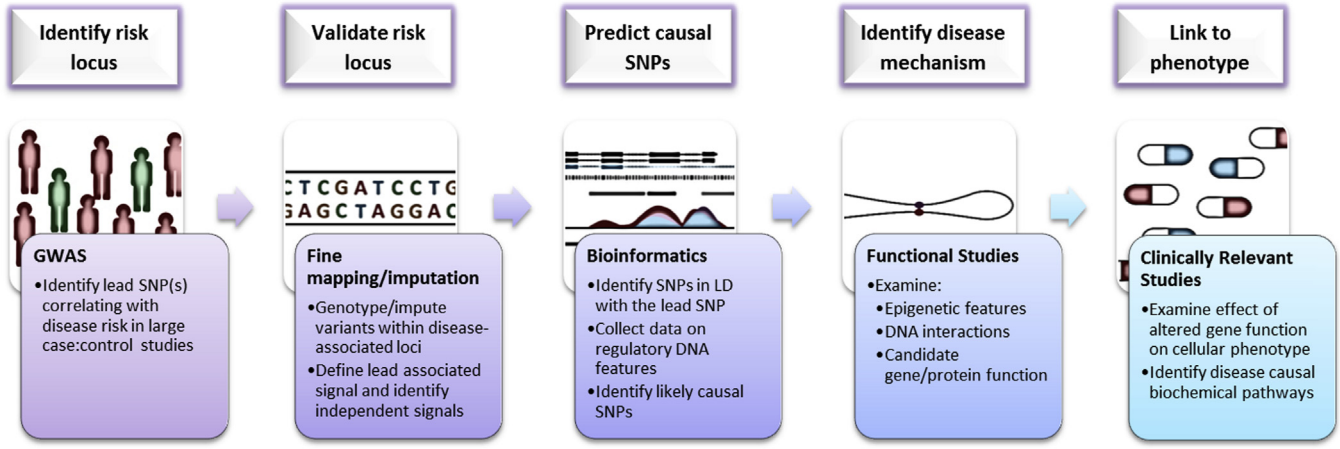

Figure 1. Workflow for identification of putative causal variants and the genes they affect. Genome-wide association study (GWAS) is a hypothesis-free method for identifying single nucleotide polymorphisms (SNPs) correlating with disease risk. Dense, targeted genotyping arrays such as Immunochip can be used for both replication of GWAS loci and genetic fine-mapping of all variants in disease-associated loci, further narrowing down the association signal. Bioinformatics can then be used to both locate and functionally annotate SNPs in linkage dysequilibrium (LD) with the lead SNPs. In noncoding regions, SNPs coinciding with regulatory features such as histone modifications or transcription factor binding sites are most likely to have a functional effect. Appropriate functional experimental techniques can then be used to investigate genotype-specific protein interactions (ChIP), DNA conformation (3C), and gene expression (eQTL and reporter gene assays) in disease-relevant cell types. SNPs associated with disease that coincide with coding regions of genes require bespoke experimental confirmation dependent on both position within gene (e.g., binding domain) and function of protein (e.g., enzymatic activity). Once a causal variant affecting gene function has been identified, its effect on relevant biochemical pathways and resultant disease phenotype can be investigated.

affect regulatory mechanisms. Transcriptome studies in psoriasis have demonstrated that gene expression is often tissue or cell type specific (Filkor et al., 2013; Jabbari et al., 2012; Li et al., 2014; Suarez-Farinas et al., 2012; Tian et al., 2012). As well as protein coding genes, long noncoding RNAs have recently been shown to have substantially different expression between skin and other tissues (Tsoi et al., 2015a). With respect to the selection of relevant cell types in psoriasis, dysregulated gene transcripts in psoriatic skin are often derived from keratinocytes, fibroblasts, and immune cells, whereas GWAS candidate gene expression often derives from multiple immune cell types, particularly neutrophils (Swindell et al., 2014). Additionally, interaction analysis of psoriasis GWAS hits with cell-specific epigenetic marks of gene activity revealed T helper cells (Th1, Th2, and Th17) to be likely key cells in driving susceptibility to psoriasis (Farh et al., 2015). Research has also shown that endothelial cells, which highly express the candidate gene *CARD14,* are likely to be important (Harden et al., 2014). Stimulation is also likely to be an important factor, especially because psoriatic lesions are thought to be subjected to a range of cytokines, such as tumor necrosis factor-α, OSM IL-22, IL-17A, and IL-1α (Bernard et al., 2012; Guilloteau et al., 2010; Rabeony et al., 2014). An inflammatory milieu may be required for pathogenic mechanisms to occur.

BIOINFORMATIC APPROACHES TOWARD FUNCTIONAL ANNOTATION OF GWAS VARIANTS

Before expensive, hypothesis-driven laboratory experiments are undertaken, bioinformatics may be used to annotate disease-associated SNPs. Publically available data can be interrogated in order to (i) define the set of associated variants that may be causal, (ii) determine which of these variants is correlated with the expression of genes, and (iii) annotate the associated variants with epigenetic features that indicate which variants are present in potential gene regulatory regions.

Freely available databases such as 1000 Genomes (Altshuler et al., 2012) can be interrogated to identify SNPs in LD with the index GWAS SNP. Statistical packages may then be used to prioritize potential causative SNPs. For example, the Probabilistic Identification of Causal SNPs (PICS) algorithm combines the underlying haplotype structure and the strength of the genetic evidence in a bayesian analysis to assign probability scores for the likelihood of each SNP in LD being causal (Farh et al., 2015). The Probabilistic Annotation INTegratOR (PAINTOR) combines the genetic association data with functional annotation data to score SNPs (Kichaev et al., 2014), whereas the Combined Annotation Dependent Depletion (CADD) algorithm gathers evidence from multiple resources to assign a score as to the likelihood of any variant being deleterious (Kircher et al., 2014).

If the associated genetic variants are involved in differential regulation of gene expression, there should be a correlation between genotype and gene expression (Nicolae et al., 2010). Expression quantitative trait loci (eQTLs) can be identified in databases such as GenVAR (Yang et al., 2010), GTex (Lonsdale et al., 2013), and RegulomeDB (Boyle et al., 2012). Importantly, the lead SNP associated with disease risk must be the lead SNP correlating with expression (lead eQTL)—and not merely in strong LD—for evidence of altered gene expression to be fully informative. Ideally, the colocalization of both signals from the same SNP needs to be statistically proven (Guo et al., 2015). To date it has been unusual for GWAS association signals to coincide with eQTL signals; this may due in part to eQTLs acting in a cell- and stimulation-specific manner. For example, a recent analysis identified cell-specific *cis*-eQTL effects in monocytes and $CD4^+$ T cells for several traits, including psoriasis (Raj et al., 2014).

A wealth of bioinformatic data has been generated by international efforts such as ENCODE (Dunham et al., 2012) and NIH Roadmap Epigenomics (Bernstein et al., 2010), which annotate different cell types with epigenetic markers of genome activity. Online tools use these data to apply functional scores to SNPs based on information about their regulatory features. For example, the Ensembl Variant Effect Predictor (VEP) indicates noncoding SNP consequences and assigns scores to exonic SNPs through predictors of protein function: PolyPhen (Ramensky et al., 2002) and SIFT (Kumar et al., 2009). Another useful tool, PrediXcan, combines information from large-scale transcriptome datasets with genotype data to enable the identification of disease-associated genes in a GWAS locus (Gamazon et al., 2015).

EXPERIMENTAL APPROACHES TOWARD FUNCTIONAL ANNOTATION OF GWAS VARIANTS

Experimental approaches can be used to characterize the effect of putative causal variants on gene expression, deduce the mechanism by which this occurs, and link this to the disease phenotype. As an example, a noncoding SNP associated with myocardial infarction was recently shown to alter *SORT1* cell-specific (liver) expression via creation of a transcription factor binding site, ultimately leading to altered levels of low-density lipoprotein cholesterol (Musunuru et al., 2010). Causal genetic mechanisms such as this can be deduced using targeted experimental techniques; several of which are described below.

DNA interactions

Noncoding regulatory elements have been shown to interact with distant genes through DNA looping in a cell-type specific manner (Dryden et al., 2014; Mifsud et al., 2015; Tolhuis et al., 2002). To test if a specific DNA interaction exists, chromosome conformation capture (3C) can be utilized (Dekker et al., 2002). 3C is a powerful hypothesis-driven method that works best over relatively small regions of DNA (10 kb to 1 Mb) (Naumova et al., 2012). In order to capture the interactions, the DNA is first cross-linked within the cell environment, followed by digestion with a restriction enzyme creating small fragments. These fragments undergo intramolecular ligation, followed by reversal of the original cross-links. The product containing the interacting DNA is detected using quantitative PCR. The method has recently been developed into hypothesis-free Hi-C, which utilizes ligation of labeled nucleotides coupled with high-throughput sequencing to identify all genomic interactions at relatively low resolution (Belton et al., 2012; Lieberman-Aiden et al., 2009). A further derivative of Hi-C, so-called capture Hi-C, gains resolution by enriching target loci with RNA baits (Dryden et al., 2014; Jager et al., 2015; Mifsud et al., 2015) and is an ideal technique for interrogating target genes at psoriasis-associated loci.

Protein interactions

Variants in regulatory regions such as enhancers and gene promoters are likely to interfere with transcription factor or histone binding (McVicker et al., 2013), with a subsequent effect on gene expression. Therefore, a complementary approach to studying DNA-DNA interactions is to study DNA-protein interactions at GWAS risk loci, using an in vivo technique known as chromatin immunoprecipitation (ChIP) (Christova, 2013). ChIP involves formaldehyde cross-linking of DNA and its bound proteins in a living cell, followed by chromatin fragmentation, immunoprecipitation with an antibody specific for the protein of interest, reversal of cross-links, and identification of the DNA by quantitative PCR (ChIP-qPCR) or sequencing (ChIP-Seq). When the method is used in cells of different genetic backgrounds, experimental evidence can be gained as to whether a putative causal risk allele at a particular SNP affects the level of protein binding to DNA and is, therefore, functional.

Gene expression

Within appropriate cell types, the effect of regulatory regions on subsequent gene expression can be examined using reporter gene assays. In this technique, the regulatory region containing the disease-associated variant is cloned into a vector containing a reporter gene such as luciferase. In a relevant cell type, expression of the luciferase gene can be inferred from the amount of luciferase enzyme activity. In the near future, it is likely that such techniques will be combined with targeted genome editing in order to identify how individual SNP alleles affect gene expression. DNA currently can be altered using novel CRISPR/Cas9 genome-editing systems (Cong et al., 2013), as was recently demonstrated in human primary T cells (Schumann et al., 2015). CRISPR/Cas9 systems are likely to become standard tools for evaluating the effect of altering single SNPs on gene expression, as they can better reflect the in vivo changes that confer disease risk. From experiments such as this, novel disease pathways can be predicted by referral to RNA-seq databases, thereby identifying genes and noncoding RNAs that are coexpressed with the gene in question.

An example of an autoimmune disease locus where the causal mechanism has been successfully identified is at *TNFAIP3* in systemic lupus erythematosus (Wang et al., 2013). Independent genetic variants in and around *TNFAIP3* are associated with multiple traits, including psoriasis (Nair et al., 2009), rheumatoid arthritis (Thomson et al., 2007) and systemic lupus erythematosus (Han et al., 2009). In systemic lupus erythematosus, the causal variant was localized to a pair of tandem polymorphic dinucleotides (TT>A) in an enhancer region 42 kb downstream of the *TNFAIP3* promoter (Adrianto et al., 2011). A functional study using several techniques including luciferase reporter assays, 3C, and ChIP showed that TT>A interacts with *TNFAIP3* through DNA looping, hence bringing the transcription factor NF-κB into close proximity with the *TNFAIP3* promoter (Wang et al., 2013). The systemic lupus erythematosus risk variant of TT>A was found to have reduced ability to bind NF-κB, which led to aberrant expression of *TNFAIP3*. A similar process could be used to elucidate the casual variant in psoriasis.

CONCLUDING REMARKS

To fully exploit the robust GWAS data already generated and to better understand the genetic susceptibility to psoriasis, one of the post-GWAS challenges is the identification and functional annotation of causal variants in known risk loci and the genes they regulate. Incorporating

GWAS data and functional experiments can describe biological pathways that lead to disease, providing targets for novel therapy development; diagnostic or prognostic biomarkers, or biomarkers to target the right treatments to the right patients.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2015.11.025.

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