signaling and decreased calcium signal spreading in wounded monolayers. Also consistent with the results in *Casr*-knockout mouse KCs (Tu et al., 2007), aged KCs appear to have variably diminished intracellular calcium stores, with a lower percentage of aged cells showing increased intracellular calcium on treatment with thapsigargin, which releases calcium stores from the ER and Golgi (Celli et al., 2021). As with calcium-knockout/knockdown KCs (Tu et al., 2019, 2007), expression of the calcium organellar storage partner of CaSR, phospholipase Cγ1, is also decreased, as are E-cadherin levels. In addition, monolayers of aged KCs show delayed scratch wound closure relative to those of young KCs (Celli et al., 2021). By extrapolation to the in vivo situation, this result suggests that the reduced CaSR levels and impaired calcium signaling in aging skin may underlie the delayed skin wound healing observed in older individuals.

More importantly, there may be a way to correct this aging-related abnormality in calcium signaling. Celli et al. (2021) also showed that increasing the activation of the residual CaSR using the calcimimetic drug, NPS-R568, results in the rescue of the impaired calcium signaling in aged KCs (to levels similar to that in young KCs) as well as in partial restoration of the reduced E-cadherin levels. Interestingly, NPS-R568 has also been found to accelerate skin wound healing in young wild-type mice in vivo (Tu et al., 2019), further suggesting its potential utility as a therapy to enhance healing.

Together, these data, summarized in Table 1, underscore the importance of CaSR in regulating epidermal function (Figure 1). Furthermore, the results presented by Celli et al. (2021) suggest the possibility of developing NPS-R568 or other calcimimetic drugs, which are currently used for the treatment of primary hyperparathyroidism (Goltzman and Hendy, 2015), to improve skin wound healing in elderly individuals. Because skin can be treated topically with minimal systemic exposure, any possible actions of these medications on serum calcium or bone homeostasis could likely be circumvented by topical application, thus potentially allowing correction of skin disorders, such as impaired skin wound healing, with minimal side effects.

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Making Lemonade: Putting the Wisdom of the Genome to Work in Atopic Dermatitis

Zhaolin Zhang[1] and James T. Elder[1,2]

Getting from a GWAS hit to an actionable gene remains a challenge in complex disease genetics. In a new article of the *Journal of Investigative Dermatology*, Sobczyk et al. (2021) use a wide variety of genomic data to generate a prioritization algorithm to tackle this problem in atopic dermatitis, calling on the wisdom of the genome to generate promising results.

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The past decade has yielded great progress in the genetic analysis of complex genetic disorders, in which multiple genes and environmental factors interact to determine risk. Prominent among these are the immune-mediated inflammatory diseases (IMIDs), several of which involve the skin, including atopic

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Clinical Implications

- Most genetic signals identified by GWAS are regulatory in nature and often reside in regions of high linkage disequilibrium, making the identification of causal genes a challenge.
- To meet this challenge, Sobczyk et al. (2021) compiled over 100 molecular resources relevant to atopic dermatitis (AD) and developed a scoring system for prioritization of candidate genes across 25 AD-associated genetic regions, yielding clear top candidates for multiple AD loci but also several other regions in which genes with similarly high scores were closely spaced and functionally related.
- Indirect validation using functional enrichment and interaction tools revealed strong enrichment for cytokine-mediated signaling pathways and Jak–signal transducer and activator of transcription signaling.
- Clustering of functionally related genes likely reflects the higher-order structure of the genome in addition to gene duplication events.

dermatitis (AD), acne, alopecia areata, psoriasis, lupus, and vitiligo. While providing new insights into disease pathogenesis, GWASs of AD, psoriasis, and other IMIDs have uncovered several challenges. In addition to the modest ORs associated with most IMID susceptibility loci, most of these genetic signals occur in putative regulatory regions (Farh et al., 2015). Although the discovery that many genetic variants exert their effects via gene regulation is very exciting, the most elegant feature of genetic analysis—the ability to work with genomic DNA from blood or other sources—is replaced by a more complex scenario that requires analysis of multiple molecular readouts (e.g., mRNA, protein, DNA methylation) in disease-relevant cell types, preferably studied in their normal physiologic contexts. Besides uncertainty as to which cell types are disease relevant, these signals may emanate from only a small fraction of the cell types present in diseased tissue, and the pathogenic cell types are often hard to access experimentally in humans. Moreover, the existence of linkage disequilibrium (LD)—the nonrandom segregation of closely spaced genetic markers because of our common evolutionary history—complicates the identification of the best genetic markers and candidate genes within a genetic signal generated by GWAS. Adding further complexity, each disease-associated region can contain multiple genetic signals independent of LD (Mahajan et al., 2018), and a given genetic signal may influence the expression of multiple coordinately regulated genes by both cis- and trans-mechanisms (Võsa et al., 2018[1]). Clearly, we cannot naively interpret localization of interesting genes to the vicinities of association signals as proof that these genes exert causal roles. Indeed, overcoming this critical barrier to progress is the key to unlocking the treasure chest of genetic signals revealed by GWAS, requiring strategic retooling of available resources.

In a new article of the *Journal of Investigative Dermatology*, Sobczyk et al. (2021) have made an important step toward this goal. They developed a bioinformatics pipeline to systematically prioritize candidate causal genes at 25 AD loci that emerged from their earlier multiancestry GWAS of AD (Paternoster et al., 2015). Their pipeline (Figure 1) utilizes over 100 molecular resources relevant to AD, including RNA, protein, and DNA methylation quantitative trait locus (QTL) datasets derived from skin or other immune-relevant tissues, as well as other, less skin-specific datasets for regulatory variant prediction, including promoter-enhancer interactions, expression studies, and variant fine mapping. The authors weighted the prioritization algorithm to emphasize the most robust datasets and de-emphasize those in which there was a high a priori likelihood of false positive results (such as individual expression QTL signals, which are abundant throughout the genome). Although the choice of weighting schemes reflects the judgment of the authors, their rationale is based in well-founded assumptions that are not specific to AD, including the use of statistical methods such as transcriptome-wide association studies and colocalization studies that formally compare the association patterns in QTL studies and GWASs. Their results are reinforced by the striking differences in aggregate scores between individual genes and their near neighbors (for example, 1q21.3-*IL6R*, 10q21.2-*ADO*, 11p13-*PRR5L*, 5p13.2-*IL7R*, 11q24.3-*ETS1*, 2q37.1-*INPP5D*, 12q15-*MDM1*, and 14q32.32-*TRAF3*). Indirect validation studies using functional enrichment and interaction tools revealed strong enrichment for cytokine-mediated signaling pathways and Jak–signal transducer and activator of transcription signaling, consistent with the clinical efficacy of biologicals targeting IL-4 receptor-α, blocking IL-4 and IL-13 intracellular signaling.

Despite these successes, some ambiguities remain. The pipeline of Sobczyk et al. (2021) produced examples of similarly high-scoring genes that are closely spaced and functionally related, including *IL18R1*, *IL18RAP*, and *IL1R1* in the IL-1 like gene cluster on chr 2q12.1 and *IL2RA* versus *IL15RA* on chr 10p15.1. Recently, *IL2RA* has been independently implicated by CRISPR-Cas mutagenesis experiments demonstrating an effect of the T-allele at rs61839660 on *IL2RA* gene expression (Simeonov et al., 2017). In other cases, the structural and functional relatedness of closely spaced, high-scoring gene candidates was less clear, including *LRRC32* and *EMSY* on Chr 11q13.5; *KIF3A*, *PDLIM4*, *SLC22A4*, and *IRF1* in the 5q31.1 cytokine gene cluster; and *STMN3*, *LIME1*, and *ARFRP1* on 20q13.33. Supporting the candidacy of *KIF3A*, the derived (nonancestral) alleles at two implicated SNPs near *KIF3A* are CpG dinucleotides that can become methylated, reducing *KIF3A* expression when present, decreasing barrier function, and increasing risk for allergic skin responses (Stevens et al., 2020).

Taking a longer view, all of these findings seem to relate to the wisdom of

[1] Võsa U, Claringbould A, Westra HJ, Bonder MJ, Deelen P, Zeng B, et al. Unraveling the polygenic architecture of complex traits using blood eQTL metaanalysis. bioRxiv 2018.

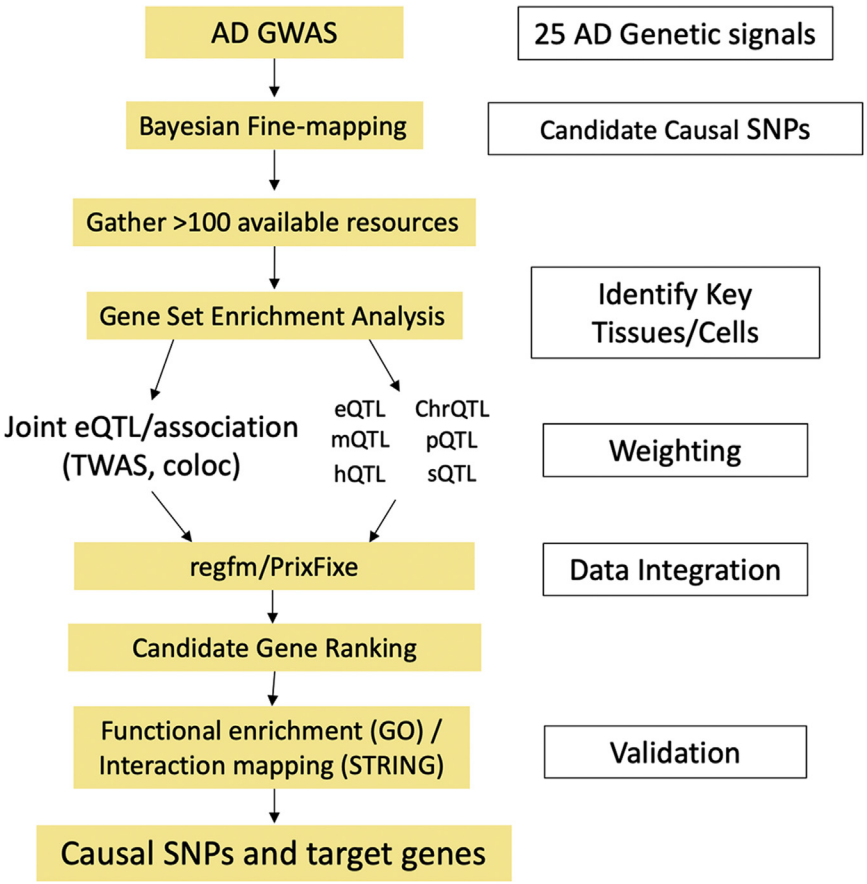

Figure 1. Gene prioritization workflow. AD, atopic dermatitis; chrQTL, chromatin accessibility quantitative trait locus; eQTL, expression quantitative trait locus; GO, Gene Ontology; hQTL, histone quantitative trait locus; mQTL, DNA methylation quantitative trait locus; pQTL, protein quantitative trait locus; sQTL, splicing quantitative trait locus; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; TWAS, transcriptome-wide association study.

the genome, which favors spatial colocalization of functionally related genes. Prime examples of this wisdom include the major histocompatibility complex on chr 6p21.3, the epidermal differentiation complex on chr 1q21.3, the α- and β-globin gene clusters, and the 2q12.1 (IL-1-like) and 5q31.1 (T helper type 2–related) cytokine gene clusters. It is attractive to speculate that the evolutionary reasons for such clustering extend beyond simple gene duplication to encompass important features of three-dimensional chromatin organization. These include the formation of topologically associating domains (Delaneau et al., 2019) and even higher orders of chromatin structure, including A and B compartments of active versus inactive chromatin and chromosome territories within the nucleus (Szabo et al., 2019). These higher-order levels of genomic organization do not appear to be broadly conserved across phyla and likely depend on physical properties of chromatin that are not yet fully understood (Szabo et al., 2019). In mammals, these features of higher-order chromatin organization function to bring together promoters and enhancers in different combinations, which in turn depend on the cellular context via the coordinated expression of transcription factors, chromatin remodeling proteins, and chromatin loop anchors, notably the CTCF/cohesion complex. Indeed, DNA methylation has been shown to influence the anchoring function of CTCF across various cell lineages, via two specific positions in the CTCF binding site (Wang et al., 2012), and grand canyons with markedly reduced DNA methylation are found in stem cells and early progenitor cells (Zhang et al., 2020). Thus, inclusion of DNA methylation datasets appears to have been a wise choice for prioritizing AD loci.

The spatially colocated gene sets nominated by the prioritization algorithm of Sobczyk et al. (2021) clearly occupy a smaller fraction of genomic space than do the large gene clusters exemplified above. Nevertheless, based on the wisdom of the genome principle, we can expect to find more and more examples of disease-associated genetic signals in which genes not created by simple duplication may prove to work together in particular functional contexts. If, as we now appreciate, most disease-associated variants are regulatory, it will not be surprising to learn that more than one structurally unrelated gene may be responsible for mediating the effect of certain genetic signals, not only in AD but in many other IMIDs as well. With the rapid recent advances in gene expression, DNA methylation, and epigenetic profiling, including the use of single cells and spatially defined sequencing from tissue sections, we can expect that the months and years to come will see extensive use of all of these tools in taking the lemons of genetic complexity identified by GWAS to create a tasty lemonade of functional genomic insights for AD and other IMIDs.

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Treating Psoriasis with a Light Touch

Makoto Sugaya[1]

Although several anticytokine antibodies and inhibitors are available for the treatment of psoriasis, more effective or better-tolerated alternatives would be of interest. In their article in the *Journal of Investigative Dermatology*, Michiels et al. (2021) propose a new strategy that targets an alternative activation pathway of the IL-22 receptor to attenuate murine psoriasis-like skin inflammation without affecting IL-22–dependent barrier defense in the gut.

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Psoriasis is a common chronic skin disease that is often associated with arthritis, cardiovascular diseases, and metabolic syndrome. The negative impact of the disease on patients' QOL can be immense. As a consequence of advances in research techniques, we now understand some aspects of psoriasis at the single-cell level (Liu et al., 2021). Although several anticytokine antibodies and inhibitors are available for the treatment of psoriasis, improved alternatives would be welcome. In their new article in the *Journal of Investigative Dermatology*, Michiels et al. (2021) propose a new strategy to treat imiquimod (IMQ)-induced psoriasis-like dermatitis in mice by targeting the noncanonical activation of signal transducers and activators of transcription (STAT) 3 that is downstream of IL-22 receptor activation in the skin without affecting IL-22–dependent barrier defense in the gut.

> "The suggestion that avoiding over-activation of signaling pathways through cytokine receptors with a 'light touch' to treat inflammatory diseases without losing beneficial effects that result from low level tonic cytokine signaling is exciting and will require further study."

Organ-specific function of cytokines

Some cytokines are ubiquitously expressed, whereas others are selectively expressed in different organs or cells. The functions and actions of most cytokines are shared between different cells in different organs. For example, TNF-α, IL-1β, and IL-6 are proinflammatory cytokines that activate many different kinds of cells, including immune cells, endothelial cells, and epithelial cells in various organs. In contrast, the expression of certain cytokines, including chemokines, is tissue specific. For example, CCL17 and CCL27 are chemokines that are predominantly expressed in the skin, and they are essential for the migration of skin-homing T cells. A variety of antibodies against cytokines are now available for clinical use, and we should consider the possibility of tissue-specific effects when using these biological agents.

Psoriasis is a common inflammatory skin disorder that involves cross-talk between epidermal keratinocytes (KCs), dermal vascular endothelial cells, dendritic cells, and T helper (Th) 17 cells. The key cytokines in psoriasis include TNF-α, IL-23, and IL-17. TNF-α has been reported to be involved in various diseases, and antibodies against this cytokine are effective for different inflammatory diseases such as psoriasis, rheumatoid arthritis, ulcerative colitis, and pyoderma gangrenosum. When utilizing anti–TNF-α antibodies in patients with psoriasis, systemic infections and development of malignancies are potentially severe side effects, whereas exacerbation of inflammatory diseases in other organs is very rare. In contrast, antibodies against IL-17A have the potential to initiate or exacerbate inflammatory bowel diseases, even though they rapidly clear psoriatic skin lesions (Fauny et al., 2020). These adverse events occur despite the fact that IL-17A levels within the lamina propria of patients with Crohn's disease are elevated (Fujino et al., 2003) and that it has been suggested that this cytokine could be a therapeutic target in patients with inflammatory bowel disease. A possible explanation is that although the IL-23–IL-17A axis is clearly involved in psoriasis (Li and Brown, 2017), anti–IL-23 antibodies moderately reduce Th17-mediated autoimmunity, whereas more effective neutralization of IL-17A adversely affects gut homeostasis and intestinal wall integrity (Whibley and Gaffen, 2015).

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