comparing *A* and *B* (Salanti *et al.*, 2008a). The average of CUs for all possible comparisons in a network yields the C-score. A larger C-score implies a greater degree of co-occurrence, either predilection or aversion, for particular head-to-head comparisons. C-scores are interpreted with statistical significance, with $P<0.05$ considered significant. The three trial networks studied by Maruani *et al.* (2014) demonstrated no significant co-occurrence.

## Use in dermatology

Another example of the application of network geometry in the field of dermatology is Kim *et al.*'s (2014) investigation of treatment modalities for basal cell carcinoma (BCC). Using a study design similar to that used by Maruani *et al.* (2014), three classes of interventions for BCC were compared: surgical, destructive, and topical. Despite a plethora of treatments for BCC, there was a substantial lack of head-to-head trials, with almost exclusive use of placebos or comparison of one agent against itself (e.g., at a different dose). The geometric pattern resembles those of chronic lower extremity wounds in the paper by Maruani *et al.* (2014), with multiple lines emanating from a common comparator. Destructive and surgical treatments were rarely compared with topical treatments.

## Other applications

One of the foundational applications of network theory was Leonard Euler's 1736 proof regarding the seven bridges of Konigsberg (modern day Prussia; Pryor and Sleigh, 2011). Euler proved the non-existence of what is now referred to as a Euler path, a continuous path passing to each node through each edge once and only once. More importantly, Euler's proof set an example of solving a real-world problem by distilling it into an abstract topological graph. Network theory has innumerable applications in the modern day. In the basic sciences, network theory is used to model biological systems, such as transcription factor circuitry and various metabolic pathways (Barabási *et al.*, 2011). It has also been used widely in social sciences to model social interactions between groups and individuals. The World Wide Web is the largest example of network topology, with over one billion nodes representing webpages and edges representing hyperlinks or URLs (Albert and Barabasi, 2002).

## Conclusion

Network theory is a powerful tool that provides visual representations of complex structures. Maruani *et al.* (2014) have applied this modality brilliantly to dermatological treatments in an effort to reduce research gaps identified by the CER initiative. In an ideal world case, head-to-head comparisons would be performed when a multitude of healthcare interventions exist for a particular disease. A strong evidence base best informs treatment decisions for greatest patient benefit. Network geometry is an emerging model to accomplish this method investigation and analysis.

CONFLICT OF INTEREST

RD is an employee of the US Department of Veterans Affairs and is supported by grants from the CDC and NIH, and also serves as the chairman for the Colorado Skin Cancer Task Force. The US Department of Veterans Affairs, CDC, and NIH had no role in the design and execution of the study. Any opinions expressed herein do not necessarily reflect the opinions of the US Department of Veterans Affairs. CK states no conflict of interest.

## REFERENCES

Albert R, Barabasi AL (2002) Statistical mechanics of complex networks. *Rev Mod Phys* 74: 47–54

Barabási AL, Gulbahce N, Loscalzo J (2011) Network medicine: a network-based approach to human disease. *Nat Rev Genet* 12:56–68

Kim DD, Tang JY, Ioannidis JP (2014) Network geometry shows evidence sequestration for medical vs. surgical practices: treatments for basal cell carcinoma. *J Clin Epidemiol* 67:391–400

Maruani A, Samimi M, Lorette G *et al.* (2014) Comparative effectiveness of topical drugs in dermatologic priority diseases: geometry of randomized trial networks. *J Invest Dermatol* 135:76–83

Pryor KO, Sleigh J (2011) The seven bridges of Konigsberg. *Anesthesiology* 114:739–40

Salanti G, Higgins JP, Ades AE *et al.* (2008a) Evaluation of networks of randomized trials. *Stat Methods Med Res* 17:279–301

Salanti G, Kavvoura FK, Ioannidis JPA (2008b) Exploring the geometry of treatment networks. *Ann Intern Med* 148:544–53

*See related article on pg 151*

# Diagnosis by Numbers: Defining Skin Disease Pathogenesis through Collated Gene Signatures

Amr Salam[1] and John A. McGrath[1]

**Disease gene expression profiles can be utilized as biomarkers for diagnostic, prognostic, and targeted therapeutic purposes, although individual data sets may be of limited generic value. To develop broader clinical relevance from disease gene signatures, Inkeles *et al.* demonstrate how mining publically available microarray data from a range of skin disorders can elucidate disease pathways, generate a multi-disease classifier, and identify potential therapeutic targets. This integrative molecular classification and functional analysis offers a new approach to understanding disease pathogenesis, with significant implications for diagnostics and the development of personalized medicine.**

*Journal of Investigative Dermatology* (2015) **135,** 17–19; doi:10.1038/jid.2014.389

DNA microarrays work on the principle that complementary strands of nucleic acid bind together, and the use of arrays has proven invaluable through the ability to examine expression of thousands of genes simultaneously. It is now almost

[1]*St John's Institute of Dermatology, King's College London, Guy's Hospital, London, UK*

*Correspondence: John A. McGrath, St John's Institute of Dermatology, King's College London, Guy's Hospital, London SE1 9RT, UK. E-mail: john.mcgrath@kcl.ac.uk*

## Clinical Implications

- An improved understanding of the pathogenesis of several skin diseases can be obtained from integration of multiple publically available gene expression data sets.
- Comparing gene signatures of multiple diseases from multiple sources is valid using normalized data tools such as Frozen Robust Multi-array Average.
- Gene signature data from multi-disease classifiers have the potential to improve diagnostics for skin disease and to identify optimal therapeutic targets for stratified or personalized medicine.

20 years since the development of microarray methods to measure differential gene expression (Schena *et al.*, 1995; Lockhart et al., 1996), and the technology has advanced enormously thereafter, with the creation of RNA-sequence in 2008 (Wang *et al.*, 2009), and more recently single cell transcriptomic analysis (Shalek *et al.*, 2014), as well as new methodology that can even localize specific transcripts within individual cells (Lee *et al.*, 2014). Initial concerns in the 1990s about multiple different platforms, probe design, and internal controls and standards have gradually eased with improved concordance of data and widespread laboratory experience (Kawasaki 2005). Moreover, gene expression profiling technology has afforded the opportunity to study disease-specific genes in detail and to discover pivotal new data concerning disease processes and pathways. Nevertheless, most human studies, certainly within dermatology, have tended to compare a single disease with appropriate controls, or perhaps two different skin diseases. Such approaches may generate comparative insights but are not ideal in identifying data germane to understanding pathogenic mechanisms that may be disease specific or shared with other disorders.

Some studies in investigative dermatology have tried to address this shortfall. For example, Kamsteeg *et al.* (2010) used real-time quantitative PCR, immunohistochemistry, and bioinformatics to study gene expression from the epidermis in various forms of eczema (atopic dermatitis, allergic contact dermatitis, and irritant contact dermatitis), in comparison with psoriasis and normal skin. The investigators found that by analyzing the expression levels of seven genes, the type of inflammatory response could be reliably predicted with a multi-disease classifier. However, in reality, the differential diagnosis of such lesions could include a broader range of other inflammatory dermatoses, as well as some infective and neoplastic conditions, thus limiting clinical precision. This type of study also has a high error rate and the impact of disease severity may further skew interpretation and restrict clinical application.

> Inkeles *et al.* (2014) demonstrate that gene expression profiles from a single disease, despite being derived from independent data sets, nearly always cluster together into groups with related pathogenesis, findings that may improve clinical diagnosis of skin disorders.

### Generation of multi-disease classifiers to accurately diagnose skin diseases

In this issue of the Journal, Inkeles *et al.* (2014) present a somewhat different methodology to assess gene signatures in the context of skin disease diagnostics. The concept was not to generate new data but to mine the plethora of existing data that can be accessed readily online. The NCBI Gene Expression Omnibus (GEO) is a rich source of gene expression data that can be easily and openly viewed (http://www.ncbi.nlm.nih.gov/geo/). Most journals, including the *Journal of Investigative Dermatology*, require authors of new work to deposit gene expression data in a repository such as GEO, thus making the data available to all. The approach taken in this new study was to collect and examine publically available gene expression data from 311 skin biopsy specimens from 16 inflammatory, infectious, and neoplastic disorders and to use the data to construct a disease classifier. Their study normalized samples to a standard reference set of microarrays and thereby eliminated the need for control samples in each data set. The authors designed a random forest classifier that, based on the expression of a limited number of genes, could accurately predict disease diagnosis with 92% sensitivity and over 99% specificity. For validation, the classifier was further assessed using 194 independent samples: sensitivity and specificity remained unchanged. Inkeles *et al.* (2014) also provide select examples into the clinical application of such a classifier. In one case, biopsies taken from a patient ambiguously diagnosed with atopic dermatitis were later diagnosed as psoriasis by the classifier. The patient subsequently went on to develop characteristic inflammatory psoriatic plaques confirming the classifier label and modifying the original clinical diagnosis.

### Multi-disease classifiers provide insight into disease pathogenesis

Inkeles *et al.* (2014) demonstrate that gene expression profiles from the same diseases, despite being derived from independent data sets, nearly always cluster together into groups with related pathogenesis, findings that may improve clinical diagnosis of skin disorders. Using this model, the same finding was also true for normal skin samples from different batches. For further analysis in the disease cohorts, T helper type 1 versus T helper type 2 cytokine profiles were interrogated. The authors examined IFN gene expression profiles, but as these are only weakly detected on microarrays data were derived from Type I and Type-II-specific-induced transcriptional

profiles of peripheral blood mononuclear cells to determine IFN signatures. Across the 16 different skin conditions there was an inverse correlation between IFN-β and IFN-γ, notably with particular diseases mapping to reproducible ratios—and thus offering molecular signposts to disease diagnostics. Collectively, the data provide valuable insight into the molecular processes that underlie various diseases and furthermore infer that similar processes underpin diseases from the same disease classifier.

### Multi-disease classifiers, disease classification, and the development of personalized medicine

The core ethos underpinning the concept of personalized medicine is ''right patient, right drug, right dose, at the right time''. The integrative molecular classification, as well as the functional analysis, outlined in the study by Inkeles *et al.* (2014), offers a valuable tool in bringing personalized medicine closer to the clinical frontline. Although it is tempting to jump ahead and speculate about how multi-disease classifiers could be used to identify novel biomarkers and therapeutic targets for skin diseases, the current emphasis has to be on improving understanding of disease pathogenesis, implementing best clinical practice, and reflecting on whether current knowledge on disease classification and pathophysiology is matched by the multi-disease classifier data. Dermatology, and medicine in general, is littered with inappropriate (or frankly wrong) disease subgroupings. For example, 150 years ago epidermolysis bullosa and urticaria were thought to be similar diseases, and even in current practice, we still categorize different keratin genodermatoses as either mechanobullous diseases or forms of ichthyosis, despite the well characterized shared pathogenic overlap of keratin intermediate filament mutations and keratinocyte cytolysis. For inflammatory dermatoses, the dermatological literature contains numerous reports of imprecise entities such as ''psoriasiform eczema'' or ''eczematoid-lichenoid eruption'', but the data mining approach advocated by Inkeles *et al.* (2014) has the potential to help resolve such issues, or at least offer fresh insight into discrete or shared disease pathogeneses. However, perhaps stories of common disease pathogenesis only tend to become more relevant when therapies are introduced or challenged. The multi-disease classifier data presented by Inkeles *et al.* (2014) offer both a reality check and a glimpse into how personal diagnostics and therapeutics might develop. The study also highlights the considerable resources on gene expression data that are freely accessible in the public domain, and the value of thinking beyond the traditional disease-control experiment. There remains a rich seam of widely available skin disease gene signature data still available to mine, with significant potential benefits in store for the future classification and improved treatment of diseases in dermatology.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES

Inkeles M, Scumpia P, Swindell W *et al.* (2014) Comparison of molecular signatures from multiple skin diseases identifies mechanisms of immunopathogenesis. *J Invest Dermatol* 135:157–9

Kamsteeg M, Jansen PA, van Vlijmen-Willems IM *et al.* (2010) Molecular diagnostics of psoriasis, atopic dermatitis, allergic contact dermatitis and irritant contact dermatitis. *Br J Dermatol* 162:568–78

Kawasaki ES (2005) The end of the microarray Tower of Babel: will universal standards lead the way? *J Biomol Tech* 17:200–6

Lee JH, Daugharthy ER, Scheiman J *et al.* (2014) Highly multiplexed subcellular RNA sequencing *in situ*. *Science* 343:1360–3

Lockhard DJ, Dong H, Byrne MC *et al.* (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol* 14:1675–80

Schena M, Shalon D, Davis RW *et al.* (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:368–71

Shalek AK, Satija R, Shuga J *et al.* (2014) Single-cell RNA-seq reveals dynamic paracrine control of cellular variation. *Nature* 510:363–9

Wang Z, Gerstein M, Snyder M (2009) RNA-seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10:57–63

*See related article on pg 289*

# Healing Refractory Venous Ulcers: New Treatments Offer Hope

Robert S. Kirsner[1], Katherine L. Baquerizo Nole[1], Joshua D. Fox[1] and Sophia N. Liu[1]

**Non-healing wounds are associated with an inflammatory and proteolytic wound environment, and recent therapeutic strategies have been focused on reversing these changes. Connexins, as members of gap junctions, are important in intercellular signaling and wound repair. Connexin 43 (Cx43) downregulation is associated with normal wound healing, and it has been found to be upregulated in non-healing venous leg ulcers (VLUs). Ghatnekar *et al.* (2014) report findings of a small phase II trial performed in Indian patients with chronic VLUs, reporting that ACT1, a mimetic peptide of Cx43, accelerates healing in the treatment group. Despite standard care with compression therapy and adjuvant therapy for refractory wounds, at present in clinical practice a significant number of patients remain unhealed. The potential for ACT1 exists to help heal refractory VLUs, but it faces additional regulatory hurdles.**

*Journal of Investigative Dermatology* (2015) **135,** 19–23. doi:10.1038/jid.2014.444

[1]*Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, USA*

*Correspondence: Robert S. Kirsner, Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, 1600 NW 10th Avenue, RMSB, Room 2023-A, Miami, Florida 33136, USA. E-mail: RKirsner@med.miami.edu*