

GENETICS OF STRUCTURAL SKIN DISORDERS

Next-Generation Diagnostics for Genodermatoses

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MOLECULAR DIAGNOSTICS AND THE ROAD TRAVELED

For the last three decades, finding genes responsible for inherited diseases relied mostly on genetic linkage studies and candidate gene screening. Often slow, labor-intensive, and unfulfilled, this approach of the late 20th Century nevertheless led to the identification of a number of inherited disease genes. These data, combined with the development of PCR-based methods for amplification of genomic DNA and mutation detection, led to more accurate diagnoses, better genetic counseling, and translational benefits for patients such as new prenatal diagnostic tests, as well as a platform to develop new disease mechanism-based models and therapies (see Uitto, 2012). The most prevalent genetic variants causative for specific common disorders (e.g., p.Phe508del in the *CFTR* gene in cystic fibrosis, and p.Gly6Val in the *HBB* gene in sickle cell anemia) were deemed clinically and economically suitable to format into focused diagnostic products. In contrast, for the genodermatoses, the relative rarity of these skin diseases and the lack of recurrent mutations meant that molecular progress has not led to similar developments. Notably, DNA diagnostics for inherited skin diseases have remained mostly confined to a few academic and hospital laboratories. Recently, however, gene hunting and mutation detection has moved into an era in which next-generation sequencing technologies recast not only the scientific discovery process but also the logic of detecting such inherited markers in the clinic.

EPIDERMOLYSIS BULLOSA AND THE DIAGNOSTIC JOURNEY

This journey of molecular discovery in inherited skin diseases is exemplified by the disease epidermolysis bullosa (EB) (see Bruckner-Tuderman and Has, 2012). Transmission electron microscopy in the 1960s was able to establish three distinct levels of blister formation, thus providing diagnostic categories of EB into simplex, junctional, and dystrophic (Pearson, 1962). In the 1980s, the introduction of antigen mapping (Hintner *et al.*, 1981) and immunofluorescence microscopy using new anti-basement membrane zone antibodies (Fine *et al.*, 1984) was able to speed up diagnostic subtyping—most recessive types of EB could be rapidly diagnosed within 2–3 days based on the skin biopsy immunoreactivity (or lack thereof) to specific probes. In the 1990s, the genes for the major forms of EB were cloned and a plethora of pathogenic mutations emerged (for review, see Varki *et al.*, 2006, 2007). Genetic characterization proved particularly useful in making accurate diagnoses in mild cases of EB in which the skin biopsy findings were too subtle to discriminate between subtypes. Currently, mutations in 14 different genes have been implicated in the pathogenesis of the various types of EB.

MUTATION DETECTION AND THE MOLECULAR ROUTE MAP

But there have been limitations to current molecular diagnostics: delineating a single DNA mutation within the gene encoding a single skin protein does not give you all the answers, either biological or clinical. Disparity

in genotype–phenotype correlations, mosaic patterns of disease, inter- and intra-familial variability, and differing prognoses and disease behaviors are all familiar to dermatologists and clinical geneticists. Since the discovery of the structure of the DNA double helix in 1953 (Watson and Crick, 1953), there have been several notable milestones in the development of DNA sequencing. From Sanger's chain termination sequencing introduced in the late 1970s through its advancement by way of fluorescent labeling and capillary electrophoresis in the 1990s, to the recent introduction of massive parallel sequencing, advances in DNA sequencing have accelerated and clinical translation has beckoned (Pettersson *et al.*, 2009). Next-generation sequencing methodologies have reduced the cost of characterization of the 6 billion nucleotides composing a diploid genome to less than \$2,000 (April 2012 costs). Affordability of high-resolution epigenetic assessment has kept pace, with interrogation of ~80% of genomic CpGs for less than \$2,000 and whole-genome localization of histone marks for as little as ~\$500 (Cheng and Cho, 2012). The plummeting cost of diagnostics, however, poses paradoxical difficulties—whereas clinicians once grappled with potential diagnoses missed by limited tests, the future is likely to overwhelm with large numbers of genetic variants of uncertain significance.

CLINICAL DIAGNOSTICS AND FORKS IN THE ROAD

This generally inverse relationship between informativeness and actionability

of a clinical test is a crucial prism to examine the evolution of genetic testing. The simple, consistent conformational changes induced by sickle cell anemia variants have enabled cheap chromatography-based tests, often less than \$50 in cost, to persist as first-line screening to the current day (Nalbandian *et al.*, 1975). Similarly, testing for hexosaminidase A levels adopted broadly in the 1960s remains the most common means of testing for Tay-Sachs disease. However, the cost of DNA sequencing in that interval has dropped to ~\$150–200 for specific genetic variants. Today, a similar sort of sum (perhaps ~\$500) can purchase sequencing of the entire protein-coding region of a genome (about 60–70 million nucleotides). Consequently, the greatest competition for the traditional Tay-Sachs test may not be a Tay-Sachs test, but one screening dozens to hundreds of metabolic genes carrying mutations in Ashkenazi Jews (Srinivasan *et al.*, 2010). The burden of interpreting some of these extremely rare variants, with unpredictable clinical penetrance, falls increasingly on the treating subspecialist.

CREATING A DIAGNOSTIC PATH FOR GENODERMATOSES

With regard to genodermatoses, however, there are some specific challenges. In contrast to classic quantitative traits, such as metabolic disease or blood pressure, the more specific and discrete morphological phenotypes typifying many dermatoses (e.g., epidermal blistering diseases) have revealed surprisingly diverse genes converging on a common phenotype. Today, we understand that a truly comprehensive genetic diagnostic for an unclassified ichthyosis must survey dozens of genes regulating cholesterol metabolism, immunoglobulin regulation, cytoarchitectural components, and determinants of terminal keratinocyte differentiation, each candidate defect linked to a distinct spectrum of systemic manifestations (Oji *et al.*, 2010). The morpho-spatial nature of genodermatoses also adds the conundrum of mosaic disease (Cho, 2010).

The visible lesions defining the skin disease may represent clonal mutations (as in epidermal nevi), and any genetic testing must therefore sample affected tissue directly in these disorders. In the absence of available commercial therapeutics for many of these conditions, let alone genetic counselors familiar with the clinical implications of rare genodermatoses, the impetus to advance such testing has often come from patients rather than clinicians or pharmaceutical companies.

EXOME SEQUENCING AND THE WAY AHEAD

Exome sequencing represents a significant milestone in the development of next-generation diagnostics for inherited skin diseases (Ng *et al.*, 2009). Already, this approach has proved insightful in revealing the molecular pathology of more than a dozen previously uncharacterized genodermatoses (for update see Cheng and Cho, 2012). The 2012 cost of exome sequencing in a case of suspected EB is similar to that required for the skin biopsy analysis (basement membrane immunohistochemistry and transmission electron microscopy) but a key issue in terms of the clinical interface is test turnaround time. For exome sequencing, the time needed to undertake data analysis and filtering represents the major hurdle to maximizing clinical utility—sometimes deciphering the bioinformatics over a few weeks is suboptimal, at least to clinicians (and patients). Nevertheless, within the next year or so, sequencing a genome in a day will be both feasible and affordable. Combined with practical bioinformatic approaches to enable rapid targeted interrogation of a specific disease gene (or panel of genes), next-generation sequencing is perhaps destined to change the way that inherited skin diseases such as EB are diagnosed. With that goal, another milestone in the diagnosis of genodermatoses will then have been reached.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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