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ESDR 50th Anniversary Lecture summary: The past and future of rare skin disease research/therapy

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Abstract

The launch of the ESDR in 1970 coincided with genetics also entering a new era. Arriving alongside new models of DNA structure and the discovery of restriction endonucleases, the ESDR has parallel-tracked 50 years of major developments in genomics, technological innovations, and big data. Patients with rare Mendelian genetic skin diseases have witnessed the discovery of causative genes and pathogenic mutations, improved genetic counseling, and the advent of prenatal diagnosis. Translational research has also heralded early phase clinical trials of gene, cell, and protein therapies, as well as enhanced disease models, mechanism-based therapies, and impactful clinical progress.

Main article

First genetic discoveries for rare skin diseases

At the inaugural meeting of the ESDR in Amsterdam in 1970, modern-day genomic medicine still lay largely dormant. Much genetic disease research focus was on trying to identify genes for rare genodermatoses, but with limited technologies. Knowledge at the time reflected key milestones such as the elucidation of DNA structure in the 1950s (Watson and Crick, 1953), and cracking the DNA code in the 1960s (Nirenberg and Leder, 1964), with the major story of 1970 being discovery of a restriction endonuclease from *Hemophilus influenzae* (Smith and Welcox, 1970). Still to come was Sanger sequencing in the late 1970s (Sanger et al., 1977), as well as its subsequent advancement with fluorescent labeling and capillary electrophoresis in the 1990s (Luckey et al., 1990; Prober et al., 1987; Swerdlow and Gesteland, 1990; Zhang et al., 1995), which played pivotal roles in early gene identification. In fact, positional cloning approaches, localization of genetic signals followed by Sanger sequencing of genes in that locus, was the norm for gene discovery until the end of the 20th century. Before Sanger

sequencing, genomic mapping was undertaken by restriction fragment length polymorphism (RFLP) analysis (Botstein et al., 1980), involving restriction digestions followed by Southern blotting (Southern, 1975), which was then replaced by PCR-based linkage methods such as microsatellites, tandem repeats ranging between 1 and 6 bp (Dib et al., 1996). Halcyon days indeed for gene hunters.

It was not until the ESDR was approaching its 18th birthday that the first genetic breakthrough for an inherited skin disease was made: microdeletions in *STS* were shown to underlie X-linked ichthyosis (Ballabio et al., 1987; Yen et al., 1987). Then during the 1990s, a plethora of genes for major genodermatoses were identified through genetic linkage studies, including *KRT5* and *KRT14* for epidermolysis bullosa (EB) simplex (Bonifas et al., 1991; Coulombe et al., 1991; Lane et al., 1992) and *TGM1* for autosomal recessive congenital ichthyosis (ARCI; Huber et al., 1995). Linkage studies also led to *COL7A1* mutations in dystrophic EB (Christiano et al., 1993; Hilal et al., 1993; Ryynanen et al., 1992). For autosomal recessive genodermatoses, candidate gene approaches, based on loss of expression of the encoded protein, also proved successful in discovering genes for both old and new genodermatoses (McGrath et al., 1995; 1997).

New databases, next-generation sequencing and accelerated discoveries

The draft human genome sequence in 2001 (Lander et al., 2001; Venter et al., 2001) reduced many obstacles to disease-gene mapping and paved the way for a landslide of monogenic disease gene discoveries. Moreover, cataloguing of common human variation from landmark studies such as the international HapMap project (International HapMap, 2003) and development of SNP array technology, which allowed the simultaneous detection of hundreds of thousands of SNPs in the human genome (Wang et al., 1998), further assisted linkage work in gene discovery.

Nevertheless, it was the advent of next-generation sequencing (NGS) early in the 21st century (Wheeler et al., 2008) which revolutionized genomics, allowing more rapid gene-disease discovery. One form of NGS, whole exome sequencing (WES), has accounted for >90% of recent disease gene identification (Chiu et al., 2021). First successfully used to find the gene for Miller syndrome (Ng et al., 2010), WES has aided the discovery of >130 genes for established genodermatoses and >30 brand new genodermatoses (for review, see Chiu et al., 2021). In parallel, international sequencing efforts for cataloguing genetic variation across populations, such as the 1000 genomes project and gnomAD (the successor of ExAC) (Consortium, 2015; Karczewski et al.; 2020, Lek et al., 2016), has enabled researchers to confidently exclude common variants in WES data as potentially pathogenic.

In dermatology, the first successful application of WES was in 2011 with identification of the major pustular psoriasis gene, *IL36RN*, encoding the receptor antagonist of IL-36 (Onoufriadis et al., 2011), which has led to subsequent therapeutic targeting of IL-36 (Uppala et al., 2021). WES has also been used to identify >60 instances of mutations in two separate genes impacting on overall phenotype, such as concurrent acrodermatitis enteropathica (*SLC39A4*) and recessive dystrophic EB (*COL7A1*) (Vahidnezhad et al., 2020), or even two distinct recessive forms of EB (simplex and junctional) in the same individual (*EXPH5* and *COL17A1*) (Vahidnezhad et al., 2018a). Thus, NGS and WES especially, has enabled clinical scientists to redefine phenotypes by splitting clinical components into respective diseases.

<u>More technologic innovation for gene discovery</u>

Although WES has facilitated gene identification, it has not provided all the diagnostic answers. For example, 15-20% of patients with ARCI currently lack causative gene pathology despite extensive WES screening (Simpson et al., 2020). Thus, other techniques have emerged to address the limitations of WES and are being utilized as complementary or alternative

methodologies such as RNA-Seq, whole genome sequencing (WGS), or long read-sequencing (LRS). Indeed, transcriptome sequencing has improved genetic diagnosis in Mendelian disease, including genodermatoses, detecting variants that affect splicing which may be missed or uninterpretable by WES (Cummings et al., 2017; Gonorazky et al., 2019; Kremer et al., 2017; Lee et al., 2020; Saeidian et al., 2020; Vahidnezhad et al., 2018b; Youssefian et al., 2021). Moreover, LRS (>10kb read length) adds capability to unravel structural variation as well as identifying variants in repetitive or GC-rich regions (Logsdon et al., 2020). Recent technologic advances such as single-cell sequencing and the expansion of metagenomics have already helped elucidate the functional consequences of variants in key cell populations and may also unravel somatic mutations (Reynolds et al., 2021; Wu et al., 2018). Improving understanding of phenotype is a major goal for the years ahead and large population-based cohorts, combining phenotypic and genetic data, will be pivotal in enhancing knowledge of genotype–phenotype relationships for skin diseases (Bycroft et al., 2018).

Deriving benefits for patients

Ultimately, for rare diseases, basic research endeavors should lead to clinical benefit. One return for patients over the last 50 years has been advances in options for prenatal testing. From mid-trimester fetal skin biopsies in the 1980s for limited disorders (e.g., severe recessive forms of EB or ichthyosis) (Rodeck et al., 1980), DNA-based analyses became feasible in the 1990s, offering earlier testing in a broader range of conditions (Fassihi et al., 2006), including development and licencing of preimplantation genetic diagnosis or haplotyping (Fassihi et al., 2010). Non-invasive prenatal testing (e.g., testing fetal DNA in maternal plasma in early pregnancy) has been available for several non-dermatologic diseases for almost a decade although it is yet to have much impact for genodermatoses (Scotchman et al., 2020).

The last four decades have also witnessed major efforts to develop new therapies for genodermatoses, creating cellular and animal disease models, and piloting early phase clinical trials of gene, cell, and protein therapies (Hou et al., 2021). Amongst all rare skin diseases, the classical forms of EB have gathered most attention. Following discovery of the major EB genes in the 1990s, there was much excitement that effective gene therapies would follow rapidly. Nearly 30 years later, however, most gene therapy successes have been limited to early phase trials of *ex vivo* correction of keratinocytes or fibroblasts and application to small parts of skin (for review, see Hou et al., 2021). One spectacular exception, however, was a single case of intermediate junctional EB in whom 80% skin regeneration followed *LAMB3* correction of keratinocyte holocone stem cells (Hirsch et al., 2017), an approach that is gaining more clinical traction (Bauer et al., 2017; De Rosa et al., 2021). Fundamental to determining new therapies for EB has been the wishes and priorities of patients, who determined improving wound healing, reducing pain, and alleviating itch were principal concerns (Davila-Seijo et al., 2014). Thus, alternative (and sometimes complementary) approaches have been assessed in pursuit of systemic benefit, including bone marrow transplantation (Wagner et al., 2010) or intravenous allogeneic mesenchymal stromal cells with immunomodulatory and anti-inflammatory properties (Petrof et al., 2015). Indeed, targeting inflammation and signaling pathways implicated in disease pathobiology has led to clinical trials of the anti-hypertensive drug losartan, which has anti-TGFβ properties, in children with recessive dystrophic EB (Nyström et al., 2015). Likewise, focusing on targeting inflammatory profiles has also led to testing of anti-IL-17 systemic therapies (and other biologics) in individuals with various forms of ichthyosis (Paller, 2020). Moreover, better characterization of skin and disease pathology has led to examples of mechanism-based therapies, such as use of low dose EGFR inhibitor in Olmsted syndrome in which primary mutations in *TRPV3* lead to EGFR transactivation (Greco et al., 2020), or use of a MEK inhibitor in arteriovenous malformations resulting from *MAP2K1*

mutations, providing benefit where conventional treatment with sirolimus failed (Lekwuttikarn et al., 2019). Thus, over the next few years, we are likely to see this dual approach to therapy – some efforts focused directly on correcting the primary gene/protein pathology and other strategies targeting the disease footprint and its overall impact on the individual. For the latter, targeted therapy guided by single-cell transcriptomic analysis (Kim et al., 2021) and anecdotal use of biologics in various genodermatoses are very much part of the current therapeutic effort.

ESDR audiences have also witnessed several other novel approaches in genodermatosis therapeutics including nonsense mutation readthrough with aminoglycosides (Woodley et al., 2017), the exploitation of revertant mosaicism through skin grafting (Gostynski et al., 2014), RNA approaches to gene correction (Ablinger et al., 2021), and most recently, genomic base editing (Osborn et al., 2020), with the expectation that further innovation will follow and that the next generation of scientists and clinicians will deliver real benefits for patients with inherited diseases. For patients, the expectation is that while cures for genetic diseases remain desirable goals, research success will be measured in how much quality of life has been changed for the better. Reflecting on the last 50 years (see Figure 1), the ESDR and its global partners look forward to being part of the wave of progress in basic and translational research, and to see the lives of patients improve before too many more landmark anniversaries come around.

Conflicts of interest

None declared.

CRediT statement

Conceptualization, A.O., J.A.M.; Writing – original draft, A.O., J.A.M.; Writing – review & editing, A.O, J.A.M.

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Figure legends

Figure 1. Timeline of key discoveries in genetics and their impact in dermatology for patients with rare inherited skin diseases (with selected examples cited for translational progress, predominantly in dystrophic epidermolysis bullosa). ***Illustration assistance provided by Heather McDonald, BioSerendipity, LLC, Elkridge, MD.***

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