A decade of next-generation sequencing in genodermatoses: the impact on gene discovery and clinical diagnostics*

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Summary

Background Discovering the genetic basis of inherited skin diseases is fundamental to improving diagnostic accuracy and genetic counselling. In the 1990s and 2000s, genetic linkage and candidate gene approaches led to the molecular characterization of several dozen genodermatoses, but over the past decade the advent of next-generation sequencing (NGS) technologies has accelerated diagnostic discovery and precision.

Objectives This review examines the application of NGS technologies from 2009 to 2019 that have (i) led to the initial discovery of gene mutations in known or new genodermatoses and (ii) identified involvement of more than one contributing pathogenic gene in individuals with complex Mendelian skin disorder phenotypes.

Methods A comprehensive review of the PubMed database and dermatology conference abstracts was undertaken between January 2009 and December 2019. The results were collated and cross-referenced with OMIM.

Results We identified 166 new disease–gene associations in inherited skin diseases discovered by NGS. Of these, 131 were previously recognized, while 35 were brand new disorders. Eighty-five were autosomal dominant (with 43 of 85 mutations occurring de novo), 78 were autosomal recessive and three were X-linked. We also identified 63 cases harbouring multiple pathogenic mutations, either involving two coexisting genodermatoses (n = 13) or an inherited skin disorder in conjunction with other organ system phenotypes (n = 50).

Conclusions NGS technologies have accelerated disease–gene discoveries in dermatology over the last decade. Moreover, the era of NGS has enabled clinicians to split complex Mendelian phenotypes into separate diseases. These genetic data improve diagnostic precision and make feasible accurate prenatal testing and better-targeted translational research.

What is already known about this topic?

- Making an accurate diagnosis of an inherited skin disease can be challenging, and genetic testing is a valuable part of patient evaluation.
- Next-generation sequencing has the potential to improve and refine how new disease genes are discovered and to demonstrate how mutations in more than one gene can be clinically significant.

What does this study add?

- Between 2009 and 2019, next-generation sequencing was used to discover 166 new inherited skin disease–gene associations, and to characterize 63 cases of multiple gene pathologies contributing to complex inherited skin disease phenotypes.
- Approximately 90% of the discoveries were made using whole-exome sequencing.
Currently, there are approximately 9000 distinct Mendelian conditions, of which more than 1000 involve skin pathology.1,2 Moreover, inherited skin diseases are often protein in their clinical manifestations, with many harbouring systemic manifestations and potentially overlapping syndromic phenotypes.3 Apart from characterized genodermatoses, patients may have clinically recognizable disorders that still lack a genetic basis, or they may have new uncharacterized diseases, or complex phenotypes that are the result of mutations in more than one gene in an individual. On top of these presentations, there may be further manipulation of the phenotype by genetic modifiers or epigenetic influences. Therefore, fundamentally, accurate diagnosis can be difficult, but it is essential to allow dermatologists to offer effective genetic counselling and optimal clinical management.4

Over the last 35 years the molecular bases of inherited skin diseases have been partially elucidated, with some initial discoveries driven by genetic linkage and candidate gene approaches.5 Genetic linkage approaches typically involved analysing large pedigrees to identify and subsequently refine genomic loci that segregated with a disease phenotype, until a specific region with several candidate genes was identified that was sufficiently small to undertake Sanger sequencing of those genes.6 In clinical genetics, this technique was first used successfully in 1986 to identify CYBB as the gene underlying chronic granulomatous disease.7,8 In dermatology, genetic linkage first contributed to delineating the genetic basis of X-linked ichthyosis, with STS identified in 1987.9,10

For autosomal recessive diseases, an alternative approach was to identify candidate genes by detecting loss of protein expression or structural differences within the skin of affected individuals, using techniques such as immunohistochemistry or transmission electron microscopy.11 Mutations in candidate genes were then confirmed by Sanger sequencing. For example, this approach led to the discovery of biallelic loss-of-function mutations in COL17A1 (encoding type XVII collagen, also known as the 180-kDa bullous pemphigoid antigen) in generalized atrophic benign epidermolysis bullosa (now known as intermediate junctional epidermolysis bullosa).12

Further spurring Mendelian gene discovery was the development of dense, genome-wide linkage maps and the knowledge gleaned from the Human Genome Project during the 1990s and early 2000s.5,13 The number of discoveries increased from approximately 40 disease-associated genes prior to the identification of CYBB in 1986,5 to having over 1000 disease genes documented in the Online Mendelian Inheritance in Man (OMIM) database by early 2000.14 However, despite these successes genetic linkage and candidate gene studies were costly and laborious. Moreover, the genetic basis of many Mendelian phenotypes proved to be intractable to these approaches.4,9,15

Indeed, reports of novel gene discoveries somewhat stalled until the advent of new unbiased methods for the interrogation of the genome using next-generation sequencing (NGS) technologies, particularly whole-exome sequencing (WES).5,16,17 Unlike whole-genome sequencing (WGS), which analyses around 3·2 billion nucleotides of human DNA, WES allows the analysis of the roughly 1·5% of the human genome that encompasses most exons of all ~20 000 human genes, revealing around 25 000 variants in any individual.18 Given that roughly 85% of the reported pathogenic mutations in monogenic diseases reside within protein-coding regions, at present WES represents a useful innovation for both gene discovery and molecular diagnostics.16 Furthermore, WES offers the opportunity to interrogate diseases that were previously intractable to gene identification given their rarity, clinical and genetic heterogeneity, and paucity of multiplex families.16,19

Regarding clinically relevant testing, the first successful application of WES was performed in 2009, identifying a single candidate gene, DHODH, responsible for Miller syndrome in four affected, unrelated individuals.20,21 Since this development, there has been a steady growth in the identification of new genes, with a reported trajectory of 263 novel discoveries per year (Figure 1).22 Despite these advances, only 20% (4081 of around 20 000) of identified human protein-coding genes have an established association with one or more disease traits.23 On average, each person is found to carry approximately 250–300 loss-of-function variants in annotated genes,24 harbouring 50–100 de novo single-nucleotide mutations that may potentially cause damaging mutations to the coding sequence.25,26 It has also been noted that multiple gene mutations in the same individual are recognized to account for at least 4% of cases for which molecular testing is diagnostic.27 with a diagnostic rate that is even higher (12%) in cohorts of selected phenotypes.23,28 Therefore, genetic variation and/or multiple genetic mutations may have the potential to exhibit manifestations that can complicate or confuse clinical interpretation of the genotype–phenotype relationship, but that the new methodologies can identify and assess.

NGS has also been integral towards developing our understanding of mosaic disorders, which harbour genotype–phenotype associations that were previously difficult to assess with traditional sequencing techniques due to the somatic nature of their causative mutations. This includes the identification of AKT1 mutations as the underlying genetic basis for Proteus syndrome,29 and, more recently, mutations in RHOA underlying a novel mosaic neuroectodermal syndrome.30 For the
purposes of this review, we do not explore use of NGS for mosaic disorders, and for this information the reader is directed elsewhere.31

The main aim of this review is to document the impact of NGS on gene discovery for inherited skin diseases and dissection of complex Mendelian disorders that include skin abnormalities. We hope the tabulated data (comprehensively listed in Tables S1–S3; see Supporting Information) will provide a useful reference resource for recent history in the era of molecular diagnostics in dermatology and NGS.

**Methods**

A comprehensive review of the PubMed database was undertaken for reports in the English language from 1 January 2009 to 31 December 2019. Two separate searches were undertaken for (i) original disease gene discoveries for genodermatoses and (ii) identifying individuals with more than one genetic disease, at least one of which involved the skin as a major part of the overall phenotype. Full details of the search protocols and criteria are presented in Figures S1 and S2 (see Supporting Information).

**Results**

**Inherited skin disease gene discoveries**

We identified 166 new disease–gene associations for inherited skin diseases discovered using NGS approaches between 2009 and 2019 (Table S1; see Supporting Information). However, by amalgamating subtypes of inherited skin diseases as one entity, we identified 160 different genes associated with 122 distinct disorders due to genetic heterogeneity and pleiotropy. The annual number of published disease–gene associations for inherited skin diseases increased between 2009 and 2013 and peaked in 2015 (n = 29), although further discoveries have continued to be made thereafter (Figure 2a). Of the 166 genodermatoses that were reported, 131 (78.9%) were known disorders with a previously characterized phenotype, while 35 (21.1%) were completely novel with an uncharacterized phenotype. Eighty-five disorders (51.2%) were autosomal dominant (with 43 of 85 mutations occurring de novo), 78 (47.0%) were autosomal recessive and three (1.8%) were X-linked (Figure 2b). The nature of the inherited skin diseases was mostly developmental disorders (27.7%), followed by keratinizing (14.5%) and ectodermal disorders (13.3%) (Figure 2c).

Examples illustrating the nature of these identified discoveries include the association between EGFR and a neonatal inflammatory skin and bowel disease, and the association between DSC3 and a desmosomal genodermatosis causing skin fragility and hypotrichosis. Notably, as reported in 2014, Campbell et al. conducted WES on a 12-month-old infant with hypotrichosis, food intolerance and a generalized eruption characterized by erythema, superficial scales, erosions and subsequent pustule formation (Figure 3).32 They identified a homozygous, loss-of-function p.Gly428Asp mutation in EGFR and demonstrated pathogenicity using immunofluorescence microscopy, confocal microscopy, whole-genome expression microarray analyses and Western blotting.32 Similarly Onoufriadis et al. performed WES on a 5-year-old boy exhibiting hypotrichosis with follicular papules, as well as skin fragility with blisters acrally and over traumatic sites (Figure 4).33 They identified a homozygous, loss-of-

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**Figure 1** Selected significant annual highlights in next-generation sequencing-based gene discoveries for inherited skin diseases since the first successful application of whole-exome sequencing (WES) in 2009. PLACK syndrome: peeling, leuconychia, acral keratoses, cheilitis and knuckle pads; SAM syndrome: severe skin dermatitis, multiple allergies and metabolic wasting. For the full list of gene discoveries, see Table S1 in the Supporting Information.
function p.Leu727* mutation in DSC3, and confirmed pathogenicity using immunofluorescence microscopy, electron microscopy and quantitative real-time polymerase chain reaction.33

For both of the above two gene discoveries WES was the method used, which has been the approach taken for around 90% of all skin disease gene discoveries using NGS. Comparatively fewer discoveries have been made using targeted NGS (7-8%) or WGS, either alone or in conjunction with WES (total of 6-6%) (Figure S3a; see Supporting Information).

**Figure 2** The impact of next-generation sequencing (NGS) on gene discoveries for inherited skin diseases between 2009 and 2019. (a) The number of new disease–gene associations for inherited skin diseases that were discovered using NGS approaches between 2009 and 2019. There is a steady increase in discoveries from 2009 to 2013, with a peak in 2015 (n = 29). (b) Breakdown of the inheritance patterns for the identified inherited skin diseases. There is an approximately even split between autosomal dominant (AD) and autosomal recessive (AR) inherited skin diseases (51% vs. 47% respectively). AD conditions are also almost evenly split into dominant and de novo dominant inheritance. (c) Breakdown of the disease subcategories for the identified inherited skin diseases. The ‘other’ category consists of various syndromes with skin involvement, including inherited bone-marrow-failure syndromes, rheumatic disorders and neurodegenerative disorders.

**Complex genodermatoses with multiple gene mutations**

We identified 63 individual cases of multiple gene mutations, 61 of which were diagnosed by WES (97%), with one case established through WGS and one case with a gene targeted panel, both involving NGS technologies. Male individuals comprised 54% of the cases (32 of 59), with consanguinity present in 44% of cases (24 of 54). Of the cases positive for consanguinity, 79% (19 of 24) showed dual autosomal recessive genetic mutations.
Clinical examples of two separate autosomal recessive diseases occurring simultaneously in the same individual are shown in Figures 5 and 6. WES was used in a 4-year-old Kuwaiti boy who presented with complete absence of nails and uncombable sparse hair (Figure 5). The clinical conundrum was whether this represented a variant of hair and nail ectodermal dysplasia disorder or two separate autosomal recessive ectodermal conditions. WES investigation showed autosomal recessive mutations in both RSPO4 and PADI3, reflecting a combination of congenital anonychia (RSPO4) and uncombable hair (PADI3), rather than a single ectodermal dysplasia disorder.

The clinical importance of NGS was also noted in a 17-year-old girl who presented with widespread refractory erythematous and desquamative plaques, generalized alopecia and failure to thrive (Figure 6). She was clinically diagnosed with recessive dystrophic epidermolysis bullosa (RDEB), but WES revealed a homozygous mutation in COL7A1, supporting the RDEB diagnosis, and also an additional homozygous mutation in SLC39A4, which is associated with zinc deficiency in acrodermatitis enteropathica. An increased dose of zinc supplementation was given, with rapid and dramatic resolution of many of her skin manifestations.

Table S2 (see Supporting Information) demonstrates 13 cases of dual gene mutations involving solely skin pathology. There were six cases (46%) that involved genetic mutations associated with ichthyosis. The large number of cases involving inherited ichthyoses may be in part due to the significant number of genes (around 50) that have been implicated in syndromic and nonsyndromic forms of the disease. Cases with skin disorders involving dual gene mutation phenotypes were reported over a shorter and more recent time period (2014–2019) than cases involving a skin disorder jointly with other organ system gene mutations (2011–2019), which are listed in Table S3 (see Supporting Information).

Table S3 demonstrates 50 cases of multiple gene mutations involving both genodermatoses and other organ system phenotypes. The largest pool of multiple genetic mutations came from Posey et al., accounting for 17 of the reviewed cases. Their case series highlighted an incidence of 15.8% (16 of 101) for cases showing multiple gene mutations involving dermatological disease. The largest WES mutational case series was from Monies et al., comprising a total of 2217 individual cases of mutation, of which 43 (1.94%) harboured a dual or triple mutation, with three cases involving significant genodermatoses.

Our review yielded only two cases with a triple genetic mutation involving skin. The first case was diagnosed with Wiedemann–Steiner syndrome (autosomal dominant, heterozygous KMT2A mutation) with centronuclear myopathy 1 (autosomal dominant, heterozygous MYF6 mutation) and autism (X-linked Xp22.31 deletion). The second case was diagnosed with pseudoxanthoma elasticum (autosomal recessive, compound heterozygous ABCC6 mutations), homocystinuria–megaloblastic anaemia (autosomal recessive, compound heterozygous MTRR mutations) and myopathy, lactic acidosis and sideroblastic anaemia 2 (autosomal recessive, compound heterozygous YARS2 mutations). Mutations in the FLG gene had the highest frequency (five cases), followed by ARID1B (n = 3), NFI (n = 3), SLC45A2 (n = 3) and WNT10A (n = 3) mutations.

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Discussion

One of the major promises of the genomic medicine era has been to give patients with inherited diseases accurate clinical diagnoses based on individual gene and mutation data. Implicit to this is the consideration of the relationship between individual genotypes and their associated clinical phenotypes and how to dissect and document these precisely.

Of the 166 inherited skin disease genes identified, there was an approximately even split between autosomal dominant and autosomal recessive conditions (51-2% vs. 47-0%, respectively), with the autosomal dominant conditions also divided approximately equally into dominant and de novo dominant inheritance. Other studies have similarly demonstrated the relative over-representation of genes underlying autosomal recessive disorders, reflecting the more straightforward filtering of homozygous or compound heterozygous variants compared with the identification of single and clearly pathogenic heterozygous variants for autosomal dominant diseases. The high number of de novo dominant variants is also likely due to the relative ease of filtering NGS data in the setting of trio analysis (affected individual plus both unaffected parents) compared with analysis of single-case exomes or those of small pedigrees. These technical considerations probably account for the high number of de novo variants discovered in this review (Figure 2b). Moreover, our data highlight the diagnostic power and utility of NGS, as de novo variants were previously less tractable to discovery using traditional gene discovery methods.

This review also assessed the literature to identify 63 examples in which mutations in more than one gene contributed to the clinical features of a genodermatosis, either just in the skin or beyond the skin. Several of these examples directly improve diagnostic accuracy, by showing that the phenotype of a suspected syndrome is actually caused by the co-occurrence of multiple genetic disorders with mutations in distinct genes. The occurrence of dual, or multiple, molecular diagnoses in an individual has only begun to emerge in the last 10 years with the availability of NGS technologies such as WES. Multiple genotypes can present in an individual as blended phenotypes that may result from overlapping or

Figure 4 Autosomal recessive loss-of-function mutations in DSC3 resulting in (a) hypotrichosis with follicular papules of the scalp and (b) skin fragility with blisters of the extremities and sites of trauma. Clinical images adapted with permission from Onoufradis et al.

Figure 5 A 4-year-old Kuwaiti boy with congenital anonychia and uncombable hair syndrome due to separate autosomal recessive mutations in both RSPO4 and PADI3. Clinical features of (a) complete absence of nails and (b) somewhat sparse, wavy scalp hair that is difficult to comb. Clinical images adapted with permission from Hsu et al.
distinct clinical features, developing contemporaneously or even sequentially over time.\textsuperscript{23,40} In the cases presented in this review, NGS allowed the opportunity to redefine the extended phenotypic spectrum by splitting blended phenotypes into their respective diseases. From a clinical standpoint, the recognition of multiple molecular diagnoses can have important implications for genetic counselling, allowing more precise management and estimates of familial recurrence risk.\textsuperscript{23}

An example of the use of WES for the splitting of a blended phenotype is evidenced in a report of two Canadian siblings, born to consanguineous parents, with clinical features of ocular and skin hypopigmentation, congenital neutropenia, immune dysregulation and Crohn disease.\textsuperscript{41} The cluster of signs was thought to represent an extended syndromic presentation of oculocutaneous albinism with phenotypic expansion. WES was used to identify disease-causing autosomal recessive gene mutations in \textit{SLC45A2} (oculocutaneous albinism type 4) and also in \textit{G6PC3} (associated with congenital neutropenia) in both siblings, thereby allowing for closer screening for cardiac abnormalities, infectious complications and the increased risk of clonal disorders of haematopoiesis.\textsuperscript{42}

The clinical importance of using WES precision diagnostics was also seen in a case of coinheritance of cutis laxa and nephrotic syndrome.\textsuperscript{43} A 1-year-old child had clear dermatological manifestations of cutis laxa with wrinkled skin, prominent veins and arthrogryposis, but they also had significant renal and urinary tract abnormalities that are not typically associated with cutis laxa, namely bilateral hydrourerets, persistent hydronephrosis and nephrotic syndrome. The finding of \textit{PYCR1} mutations explained the cutis laxa features, but the additional discovery of \textit{PLCE1} mutations provided genetic accountability for the renal pathology.

These cases also highlight the significance of consanguinity, which carries an increased risk to progeny of autosomal recessive disorders and congenital anomalies.\textsuperscript{44,45} As evidenced in this review, consanguinity was present in almost half of all cases with multiple disease-associated gene pathologies, with 79.2% displaying autosomal recessive genetic mutations.

WES has become increasingly important for the diagnosis of multiple genetic mutations with overlapping phenotypic skin features, in patients just presenting to dermatologists. A case presenting with a complex epidermolysis bullosa (EB) phenotype that contained skin biopsy features of both EB simplex and junctional EB underwent NGS.\textsuperscript{46} The patient’s DNA harboured biallelic mutations in both \textit{EXPH5} and \textit{COL17A1}, indicating that the phenotype was a manifestation of two distinct forms of EB in the one individual.

These cases demonstrate not only the need for precision diagnostics in clinical practice, but also the need to perform comprehensive or reanalysis of NGS data. It was noted by Liu et al. that upon reanalysis of WES samples from 2011 and 2012, there was an increased molecular diagnostic yield of samples from 24.8% to 46.8% and 25.2% to 36.7%, respectively.\textsuperscript{47} This study was performed through a manual reanalysis of the data, comparing the original data from 2011 and 2012 against updated, novel pathogenic mutations as of December 2017 or through a semiautomated computer-based algorithm with a diagnostic sensitivity of 92.9% compared with manual reanalysis. The majority of new molecular diagnoses were from newly discovered disease genes, as well as upgraded variant classifications in known disease genes. Interestingly, they found that the number of patients with multiple molecular diagnoses almost doubled from 25 to 48 of the 2250 cases after reanalysis. It has also been noted that the use of WES increases the diagnostic sensitivity of mutation detection compared with other techniques.\textsuperscript{48} This shows the contribution of continued genomic analysis to unravelling the genetic basis of clinically blended phenotypes.\textsuperscript{38} Furthermore, our review noted two cases of

\begin{figure}[h]
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\caption{A 17-year-old Iranian girl with acrodermatitis enteropathica and epidermolysis bullosa dystrophica due to separate autosomal recessive mutations in both \textit{COL7A1} and \textit{SLC39A4}. (a) Clinical presentation of generalized blisters with erosions and refractory erythematous and desquamative plaques, that (b) also involved the hands. Clinical images adapted with permission from Vahidnezhad et al.\textsuperscript{35}}
\end{figure}
triple genetic diagnoses, underscoring the reductionist power of NGS in making accurate diagnoses.18

In addition to the splitting of blended phenotypes into respective diseases, NGS may also allow the consolidation of traditionally distinct Mendelian conditions into single disease entities with variable presentations along the same phenotypic spectrum, upon identification of mutations in shared genes. Examples identified from this review include isolated nephropathies and Jeune and Sensenbrenner syndromes, which all harbour mutations in WDR19,49 as well as Zimmermann–Laband and Temple–Baraitser syndromes, which both harbour mutations in KCNHI (Table S1; see Supporting Information).50,51

The continual refinement of phenotypic classifications through the genetic data generated by NGS significantly impacts on the accepted nosology of inherited skin diseases. Determining whether newly discovered mutations underly a phenotypic variant of an existing disease vs. a distinct, novel condition can be challenging, and textbook definitions may quickly become outdated. OMIM (https://omim.org) is an online database of human genes and genetic phenotypes that helps address some of these challenges. It is regularly updated and acts as a central source of information regarding the nomenclature, clinical features and discovery of inherited diseases. Indeed, OMIM groups clinically similar phenotypes resulting from different gene mutations together in a Phenotypic Series.1 Nevertheless, given the plethora of ongoing discoveries and reports, there remains a need for real-time updating and availability of genotypes and phenotypes to assist clinical practice.

The examples of multiple gene pathologies in our review represent some of the low-hanging fruit in terms of refining multiple gene–phenotype correlation. The path of NGS technologies is still relatively new and requires ongoing efforts to close some of the shortfalls such as increasing accuracy rates, decreasing interpretation inaccuracy and expanding international databases for reliable genotypic and genomic information.19,52 Moreover, genetic results are not a rigid predictor of what will happen in the future and currently do not provide a linear insight into the complex relationship between genes and environmental factors. Furthermore, consideration must also be given to the copy-number variant or common mutations in certain genes such as FLG, which can lead to skin dryness, inflammation or ichthyosis and thereby modify other skin diseases.53 Full-covereage genomic studies assisted by other systems such as the methylome, transcriptome and proteome, and functional studies will help in this respect.

Our review also demonstrates how the use of NGS approaches has changed over the past decade. WES emerged as the preferred approach over targeted NGS from 2011 (Figure S3b; see Supporting Information), partly driven by reduced costs but also because of its ability to generate gene discovery datasets. However, having already passed the current peak of gene discoveries (predominantly WES based) for both Mendelian and inherited skin diseases by the end of 2015 (Figure 2a; and Figure S3b; see Supporting Information).5 WES has been transitioning from its primary role in gene discovery to one of use in clinical diagnostics, known as clinical exome sequencing (CES).54,55 In CES, the analysis may be restricted to approximately 5000 genes that are known to be involved in the pathobiology of Mendelian human diseases, known as the ‘Mendeliome’, therefore eliminating the possibility of identifying new candidate genes in the remaining ~15 000.56,57

Nowadays, CES is mostly used to examine the whole exome and is typically used for disorders with complex phenotypes and extreme heterogeneity, when multiple unrelated disorders are suspected, or when a distinctive phenotype is absent – including the aforementioned clinical cases with blended phenotypes.55,58 CES diagnostic rates have ranged between 25% and 46%, potentially three times higher than traditional genetic tests.27,47,54,55,59 The appeal of CES is compounded by its capacity to screen for genetic mutations and variants without bias or reliance on subjective clinical assessments.58 Hence, CES is gradually transitioning into an economically competitive, frontline, diagnostic test, such as in the UK, where it can be routinely ordered.60,61

Despite the successes of NGS in gene discovery for rare Mendelian skin diseases, to date NGS has only provided limited new insights into complex skin disease pathobiology. In fact, WES was applied in association studies for psoriasis62,63 and in central centrifugal cicatricial alopecia64 based on the notion that variants contributing to the trait are more likely to be located in protein-coding regions. It is noteworthy that imputation reference panels are also generated with WGS, which can be applied in genome-wide association studies for complex traits, including skin diseases such as psoriasis, atopic dermatitis and acne.65 Population-based cohorts include the UK Biobank,66 which combines the phenotypic and genetic data of 500 000 individuals and has already released WES data for 50 000 individuals to the global research community. Going forward, an initiative to sequence the whole genomes of all 500 000 participants from UK Biobank is underway, which will enhance our understanding of genetic variation and its functional consequences on health and disease including those of dermatological interest.

Although WES remains a powerful gene discovery tool, it still has some limitations. Firstly, WES is not suited to detecting multiple types of genetic variants, including noncoding variants, insertion–deletion variants (indels), structural variants, copy-number variants and homologous regions of the genome. This deficit is due to technical limitations intrinsic to how the exons are captured from DNA samples and processed when conducting WES.15,67,68 WES may also miss critical exons in disease-associated genes as it is estimated to cover only around 80–85% of the exome sufficiently.58 Indeed, several studies have failed to identify mutations via WES due to this incomplete coverage.68–74 For genetically heterogeneous conditions with well-defined disease-associated genes, NGS-based targeted gene panels have been used as a more reliable alternative to the relatively shotgun-based approach of WES. Although it cannot be used as broadly, this targeted approach demonstrates better coverage of candidate genes, has faster turnaround times, and generates fewer incidental findings than WES.52,58

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Many gene panels exist for a wide range of conditions, including inherited skin diseases. For example, targeted gene panels designed for cases of autosomal recessive congenital ichthyosis (ARCI) resulted in the identification of six distinct, homozygous novel mutations in CERS3 and new phenotypic clues for mutations in six different genes associated with ARCI, including an anteriorly overlapped ear at birth as a new potential sign for those with ALOXI12B mutations.

Other techniques are emerging that also address the shortcomings of WES and are being utilized as complementary approaches (RNA-sequencing for transcriptomics, deep learning) or even potential successors (WGS, long-read sequencing). WGS is an alternative NGS technique to WES that has the capacity to generate a more reliable and richer dataset, which lowers the rates of false positives, allows detection of a broader range of variants and, ultimately, yields greater diagnostic power for identifying disease-causing mutations. It is currently less commonly used due to higher costs, an ongoing clinical and research focus on the exome, and difficulties interpreting the significance of noncoding variants.

The application of transcriptome sequencing as a distinct but complementary technique to NGS addresses some of these limitations. In fact, transcriptome sequencing has been applied successfully to the genetic diagnosis of Mendelian diseases, by detecting variants that affect splicing and are missed or uninterpretable by WES and WGS. This methodology has also been applied to epidermolysis bullosa, whereby homozygous splice-site mutations in CD151, KRT5 and COL17A1 have been shown to result in exon skipping or aberrant splicing. As the cost of sequencing falls while our interpretation of genomic data improves, uptake of WGS may overtake WES in its application due to the richer dataset produced. Already, the recent completion of the UK-based 100,000 Genomes Project in December 2018 heralds another milestone driving WGS into the clinical sphere. The project is pioneering an evolution of the genomics workforce and associated bioinformatics infrastructure within the UK National Health Service, and is poised to revolutionize our approach to genetic diseases.

In conclusion, these developing techniques can all be used synergistically to facilitate our transition from simple genome sequencing to functional genomic exploration, to enhance our clinical diagnostic capabilities and to accelerate our progress towards personalized genomics.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Figure S1 Flowchart of the literature search undertaken to identify gene discoveries for inherited skin diseases using next-generation sequencing approaches between 2009 and 2019.

Figure S2 Flowchart demonstrating the literature search undertaken for the review of genodermatoses cases involving multiple gene mutations identified via next-generation sequencing approaches between 2009–2019.

Figure S3 Breakdown of the next-generation sequencing approaches used to discover new disease–gene associations for inherited skin diseases between 2009 and 2019.

Table S1 New disease–gene associations for inherited skin diseases that were discovered using next-generation sequencing approaches between 2009 and 2019.

Table S2 Summary of multiple gene mutations involving skin phenotypes identified from next-generation sequencing.

Table S3 Summary of multiple gene mutations involving genodermatoses and other organ system phenotypes identified via next-generation sequencing technologies.