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LET'S HEAR FROM A COLLEAGUE

Whole-exome sequencing in the clinical setting: Establishing a foothold for precision medicine in genodermatoses and other diseases

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ABSTRACT

The concept of "precision medicine" has been a mainstay in discourses about the future of medicine, although it was not until the completion of the Human Genome Project that genetic associations to Mendelian diseases have risen dramatically. Since genetic variations in most (85%) monogenic or oligogenic diseases reside in exons, whole-exome sequencing (WES) serves as a pivotal tool in the identification of causative variants in genodermatoses and other diseases, leading to efficient and timely diagnosis. Here, we share our current diagnosis protocol for genodermatoses using WES as a first-tier solution. Two cases are presented to demonstrate the process of identifying germline variants and one case for a somatic variant. In the first case, a germline missense mutation in *COL7A1* (exon73:c.G6127A) was identified for a patient that presented with clinical symptoms of dystrophic epidermolysis bullosa (DEB). Immunofluorescence study revealed decreased collagen VII expression in the dermal-epidermal junction. In case 2, we detected a germline missense mutation in *KRT16* (exon1:c.374A>G) in a patient with palmoplantar keratoderma (PPK) and congenital pachyonychia. Sanger sequencing and segregation analysis confirmed the variant detected in WES. For case 3, a patient with linear nevus comedonicus was found to have a somatic missense mutation in *NEK9* (exon4:c.500T>C), which was only detected in the lesional DNA sample. Thus, WES shows great potential as a diagnostic tool for monogenic or oligogenic genodermatoses. Since omics is a technology-driven tool, we expect that reaching precision medicine is ever closer.

KEYWORDS Whole-exome sequencing, genodermatoses, precision medicine

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THE ROLE OF WHOLE-EXOME SEQUENCING (WES) IN PRECISION MEDICINE

Precision medicine is an approach for disease treatment and prevention by taking into account individual variability, and unique genetic background. Precise medical treatment entails obtaining unbiased diagnosis supported by genetic and clinical information. Though the concept of precision medicine itself is not new, the association of genetic mutations to Mendelian diseases did not accelerate until the completion of Human Genome Project during the 1990s and early 2000s, as shown in the increasing trend of disease-gene associations reported in the Online Mendelian Inheritance in Man (OMIM) database.[1–3] The inclusion of next-generation sequencing (NGS) technologies, such as whole-exome sequencing (WES), in clinical workflows have further increased the reports of novel gene discoveries. Since the first documented use of NGS technology in a clinical setting in 2009, the pace of novel genotype-phenotype discoveries has gained steadily at roughly 263 per year.[4]

Compared to whole-genome sequencing (WGS), which analyzes the entire 3.2 billion nucleotides of human DNA, WES analyzes only the exonic regions representing ~1.5% of the human genome.[3,5] Since roughly 85% of reported pathogenic mutations in monogenic and oligogenic diseases resides at protein-coding (exonic) regions, WES presents as a practical and efficient tool in the molecular diagnosis of mono- and oligogenic diseases.[6] Currently, WES accounts for roughly 90% of all novel genetic discoveries in genodermatoses conducted by NGS technology.[3]

Identification of pathogenic/likely pathogenic variants which agree with the disease clinical presentation ascribes a high degree of confidence and precision to diagnosis workflows. A correct and precise diagnosis not only allows for patients to benefit from early prevention procedures to be undertaken by genetic counseling, but clinicians could also adapt therapeutic strategies for the patients based on the diagnosis and relevant pathophysiologic focus.[7–10] In this review, we demonstrate the ability of our current clinical and molecular diagnostic protocol for

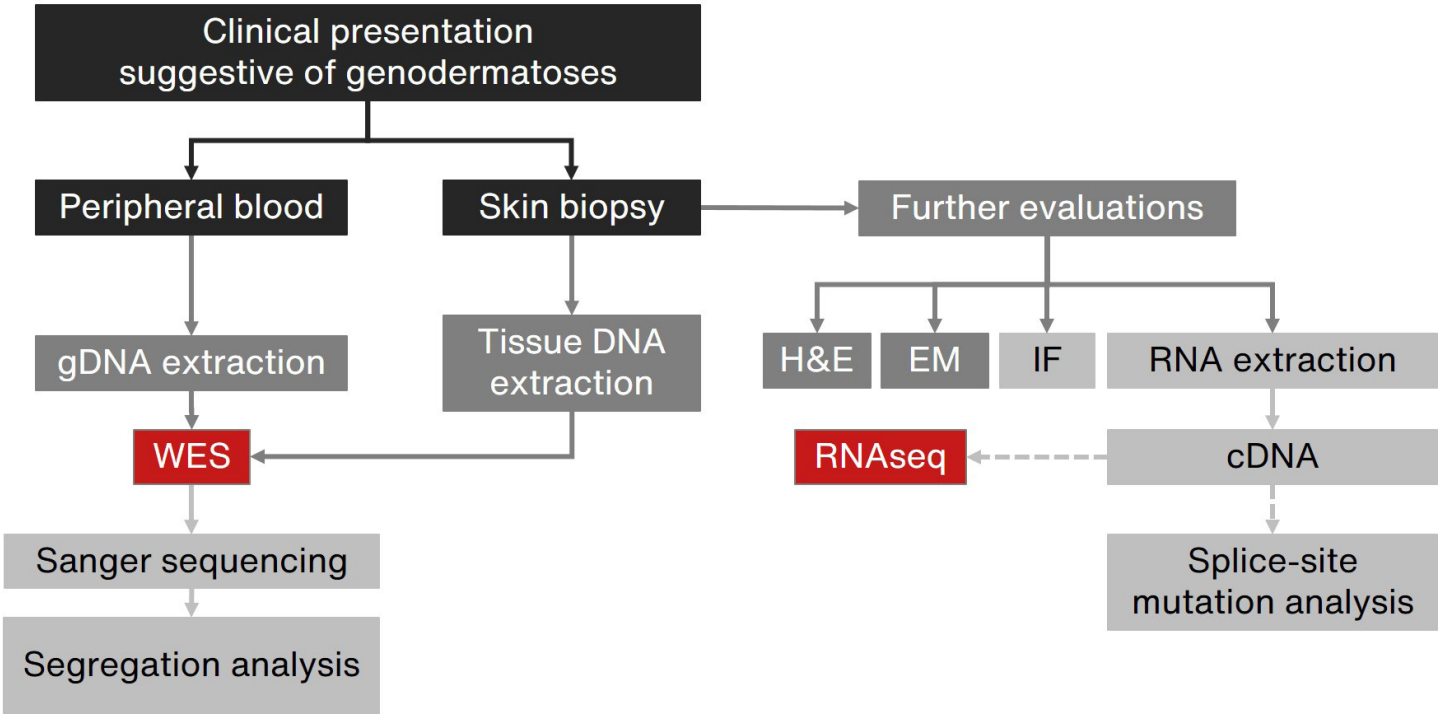

Figure 1. Schematic description of the diagnostic protocol for genodermatoses. gDNA, genomic DNA; WES, whole exome sequencing; H&E, hematoxylin and eosin; EM, electron microscopy; IF, immunofluorescence; cDNA, complementary DNA.

genodermatoses, using WES as either the first-tier solution or in conjunction with other clinical data.

The main purpose of this review is to demonstrate the practical implementation of WES as the focal point for molecular diagnosis in clinical practice. To this end, we would share our experience of two cases with germline mutations and one case with a somatic mutation as examples. Readers interested in a summary of historical WES applications in genodermatoses are directed elsewhere.[3,11,12]

PROCESS OF WES-BASED GENETIC DIAGNOSIS WORKFLOW

Patients harboring the clinical phenotypes with pedigrees suggestive of genodermatoses would be advised to undergo genetic investigations (Figure 1). DNA would be extracted from blood or biopsy skin tissue depending on the clinical presentation suggesting a germline or somatic mutation, respectively. Library preparation and WES of the extracted DNA would be done. For verifying the candidate variants identified via WES, PCR-based Sanger sequencing would also be done.

Skin biopsy specimens of the patients may be sent for further histopathological evaluation. Hematoxylin and eosin (H&E), immunofluorescence (IF), or electron-microscopy (EM) would be performed. For transcriptome analysis, RNA could also be extracted from skin biopsy specimen for RNA sequencing analysis (Figure 1).

ILLUSTRATIVE CASES FOR THE CAPABILITY OF WES

CASE 1: GERMLINE *COL7A1* MISSENSE MUTATION IN EPIDERMOLYSIS BULLOSA

For germline mutations detected via WES, we outline the diagnostic process in a patient with dystrophic epidermolysis bullosa (DEB). The proband, a 30-year-old Taiwanese female, had erythematous papules and plaques with bullae over bilateral pretibial and elbow region (Figure 2A). Dystrophic changes on teeth and all 20 nails were also noted. Her mother also exhibited similar clinical features, which initially suggested an autosomal dominant inheritance (Figure 2B). The patient was clinically diagnosed with DEB and underwent further genetic evaluation.

Both autosomal dominant and recessive forms of DEB are caused by mutations in *COL7A1*, which encodes collagen VII – a major component of anchoring fibrils.[13,14] Through WES, the proband was found to harbor a previously reported missense mutation in *COL7A1* (NM_000094:exon73:c.G6127A:p.G2043R), leading to a glycine to arginine substitution (Figure 2C).[15] Segregation analysis via PCR and Sanger sequencing revealed that the mother of the proband also harbored the same mutation (Fig-

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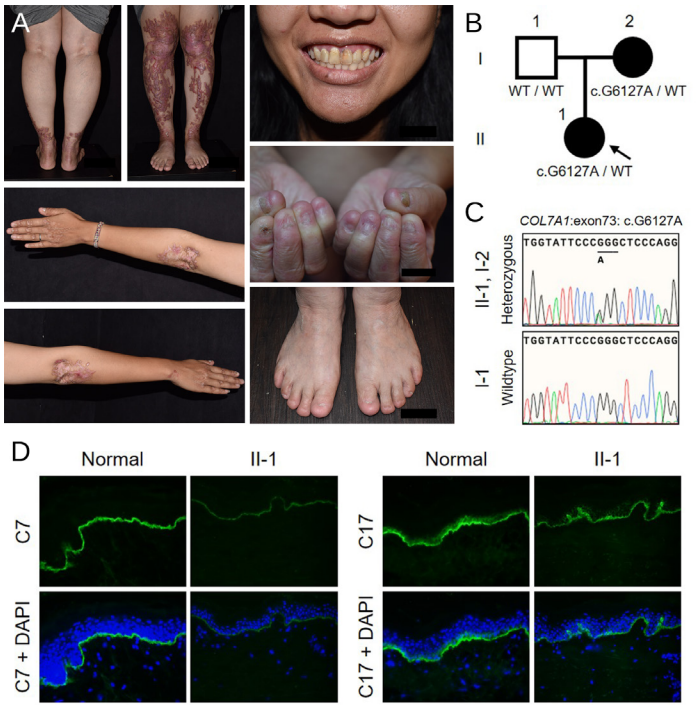

Figure 2. Genetic diagnosis and further IF evaluation of a patient with DEB. **A.** Erythematous papules and plaques on bilateral pretibial and elbow area of the patient (Left). Dystrophic changes in teeth and all 20 nails (Right). **B.** Pedigree summarizing the result of segregation analysis. **C.** Segregation analysis revealing *COL7A1* (NM_000094:exon73:c.G6127A:p.G2043R) mutation in both the proband and her mother. **D.** IF staining shows decreased collagen VII (C7) and normal collagen XVII (C17) expression in the skin specimen of the patient.

ure 2C). Immunofluorescence (IF) revealed decreased collagen VII expression relative to the healthy control, although collagen XVII expression was unperturbed (Figure 2D).

CASE 2: GERMLINE *KRT16* MISSENSE MUTATION IN PALMOPLANTAR KERATODERMA

Another example of germline mutation detection via WES is presented in a patient with palmoplantar keratoderma (PPK). Thick, hyperkeratotic, compact keratin plaques on the bilateral soles have been reported by the patient since childhood (5 years old) (Figure 3A). The patient also showed hypertrophic dystrophy of the toenails. Minimal fingernail changes were noted. The patient's family history showed that the mother of the proband also had a similar phenotype (Figure 3B).

PPK has been previously reported to follow an autosomal dominant inheritance pattern. Mutated genes related to focal PPK include *KRT16*, *KRT6c*, *DSG1*, and *TRPV3*.[16] Results of genetic diagnosis using WES revealed a heterozygous *KRT16* mutation (NM_005557:exon1:c.374A>G:p.N125S) in both the proband and the proband's mother (Figure 3B, 3C). The same mutation was reported previously for a patient with focal non-epidermolytic PPK and pachyonychia congenita.[17]

CASE 3: SOMATIC *NEK9* MISSENSE MUTATION IN LINEAR NEVUS COMEDONICUS

WES is also equally capable of detecting somatic mutations. Nevus comedonicus (NC) is a severe, localized form of acne with linear configuration along the Blaschko lines. This suggests that NC is a mosaic disorder resulting from a somatic mutation during embryonic development.[18,19] We show the capacity of WES to detect low-frequency somatic mutations in a 14-year-old Filipino diagnosed with unilateral NC (Figure 4A). Identification of the mosaic mutational pattern was carried out by obtaining DNA from the patient's peripheral blood and lesional skin. WES of the DNA extracted from the skin biopsy revealed a frequency of 14% for the candidate variant *NEK9*:exon4:c.500T>C:p.I167T (Figure 4B).[19] We then targeted this region by PCR and Sanger sequencing. Visualization of the sequencing chromatograms revealed that the *NEK9*:exon4:c.500T>C mutation can only be detected in the lesional skin DNA, confirming the candidate *NEK9* variant as a somatic mutation (Figure 4C). This mutation has been previously shown as a gain-of-function mutation resulting in increased phosphorylation at Thr210, which is necessary for NEK9 activation.[19]

STRENGTH OF WES IN GENETIC DIAGNOSIS

Since the introduction of WES into clinical practice in 2009, WES has played a primary role in identifying monogenic and oligogenic genodermatoses. In the diagnostic protocol described, pa-

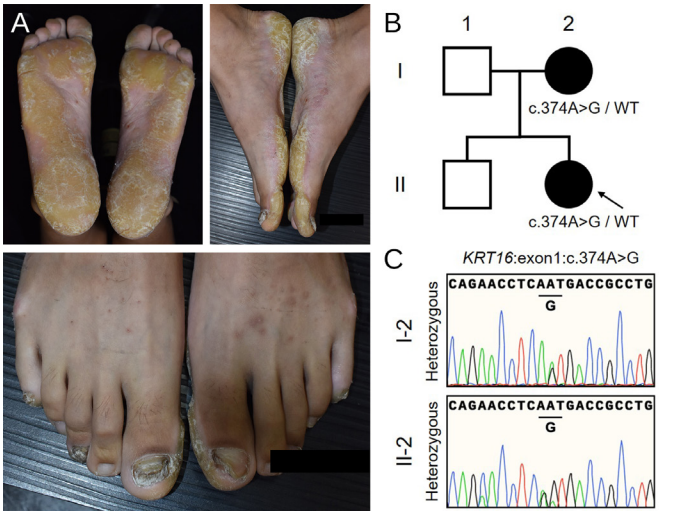

Figure 3. Genetic diagnosis of a patient with focal PPK and pachyonychia congenita. **A.** The proband shows compact hyperkeratotic keratin plaques on bilateral soles and hypertrophic dystrophy of the toenails. **B.** Pedigree and summary of segregation analysis. **C.** Results of Sanger sequencing reveal heterozygous *KRT16*:exon1:c.374A>G mutation in both the proband and her mother.

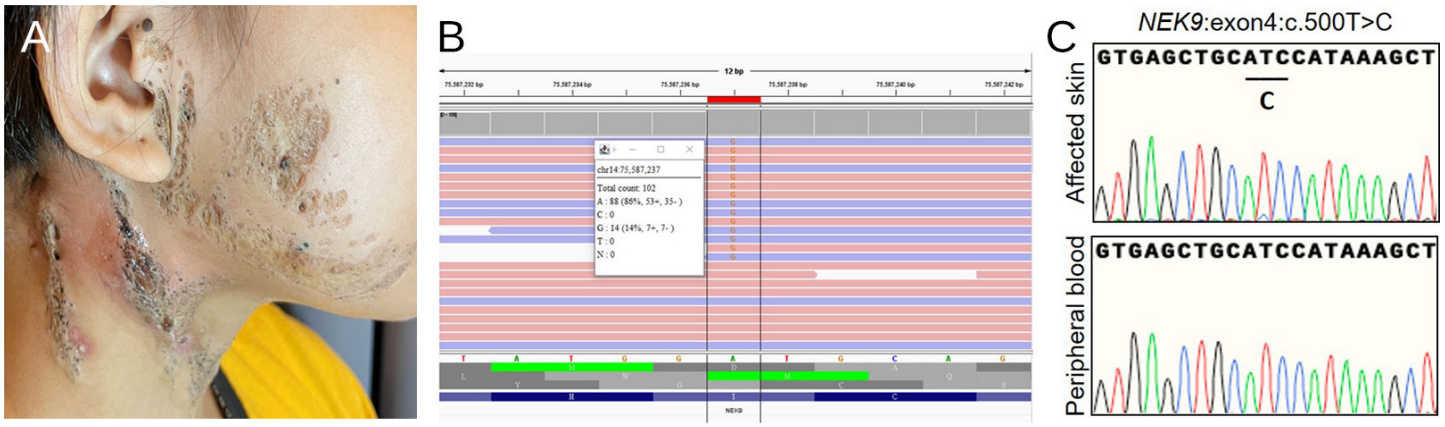

Figure 4. WES using lesional DNA reveals somatic NEK9 mutation in a unilateral nevus comedonicus patient. **A.** Linear nevus comedonicus on the right neck and right cheek of the patient. **B.** IGV visualization of the WES-detected *NEK9*:exon4:c.500T>C:p.I167T mutation. **C.** Sanger sequencing of DNA from the affected lesion specimen (upper) and peripheral blood (lower) reveals a somatic mutation.

tients with phenotype(s) suggestive of genodermatoses would be asked for consent to be enrolled in the genetic diagnosis workflow using WES. Genetic diagnosis could then be confirmed by histopathological evaluation, PCR/qRT-PCR, immunofluorescence, and immunohistochemistry. In cases where unique inflammatory signatures or pathways are involved, RNA sequencing is employed to identify dysregulated genes and pathways which can thus be used to identify possible treatments.

To date, WES represents an efficient technique that could be practically implemented into clinical practice. Furthermore, WES has become a powerful tool in diagnosing complex genodermatoses involving more than one gene. This is evidenced in a previous reported case showing histologic characteristics of both epidermolysis bullosa (EB) simplex and junctional EB.[20] WES study revealed homozygous mutations in *EXPH5* and *COL17A1* – two different, unlinked EB-associated genes.

LIMITATIONS OF WES

Despite the capability and efficiency of WES in genetic diagnosis, there are still limitations. Since WES can only target protein-coding exonic regions, detection of non-coding variants is usually unsatisfactory. For compensation, WGS could be used to target the entire genome although costs for such assays is higher than WES.[21] Copy number variations (CNV), structural variants, and homologous regions of the genome, which are highly repetitive elements, remains challenging for NGS platforms due to its reliance to the shotgun short-read approach. Instead, long-read sequencing, also known as third-generation sequencing, which generates read lengths of over 10,000 bp could be used to improve CNV detection and structural analysis.[22]

CONCLUSION

In summary, WES has shown to be a reliable method in the molecular diagnosis of monogenic and oligogenic genodermatoses. Future development and universalization of various sequencing technologies would allow for increased robustness and accuracy of variant detection, which brings us closer to achieving personalized precision medicine.[9]

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