Letters

OBSERVATION

Revertant Mosaic Skin Punch Grafting in Recessive Dystrophic Epidermolysis Bullosa

In recessive dystrophic epidermolysis bullosa (RDEB), a severe skin fragility disorder caused by loss-of-function alterations in the *COL7A1* gene, chronic open wounds often lead to infections, pain, and risk of squamous cell carcinoma.[1] Revertant mosaicism (RM), a spontaneous correction of pathogenic alterations in somatic cells, represents tissue for autologous gene therapy.[2] We report a case of repair of a large, chronic open wound in a patient with RDEB through autologous punch grafting of revertant skin.

Report of a Case | A 30-year-old woman with severe generalized RDEB, confirmed to harbor compound heterozygous alterations (c.2992 + 2T>G and c.8621-30A>G) at splice sites in *COL7A1* through a blood cell genotyping (**Figure 1**A), presented an unhealed 20 × 14-cm^2 ulcer that had persisted for the past 3 years (Figure 1B). A patch of healthy skin (7.5 × 4 cm^2) was found on her left forearm (Figure 1C), representing possible RM. We obtained normal skin from a healthy donor, affected skin from the patient, and RM-possible skin from the patient's forearm. RNA transcripts from separated epidermis and dermis from each skin sample were extracted, and complementary DNA within approximately 8.3 kilobase pairs of the *COL7A1* coding sequence were synthesized and subjected to long-read sequencing using the MinION platform (Oxford Nanopore Technologies). In the epidermis from the patient's affected skin, RNA transcripts with premature stop codons near c.2992 (c.2922_2923ins83) and c.8621 (c.8620_8621ins29) were dominant, and wild-type reads were rare (1.1%). In contrast, the epidermis from the potentially revertant area exhibited high rates of wild-type transcripts (35.0%), indicating the occurrence of RM. In the dermis from revertant skin, the wild-type reads were rare (1.9%), consistent with *COL7A1* RM predominantly occurring in keratinocytes.[3]

Using skin from the RM region of the forearm, 40 to 50 two-mm punch grafts were harvested per session, with the donor site healing completely within 2 weeks (**Figure 2**A). These 2-mm punch grafts were transplanted onto the patient's chronic ulcer on the back over 8 sessions. Reepithelialization occurred within 2 to 6 weeks, in both the grafted area and surrounding region. The areas of reepithelialization exceeded the grafted area by up to 360% (Figure 2B). Immunofluorescence and transmission electron microscopy of the grafted area revealed type VII collagen and anchoring fibrils, as would be expected in healthy skin. During a 15-month follow-up, the grafted area remained intact, and the large chronic ulcer progressively healed (Figure 2C). The patient experienced pain relief and improved daily activities.

Discussion | This case demonstrates the effectiveness of using punch grafting of RM skin to treat large, chronic open wounds in a patient with RDEB. Compared to using RM cells to culture epithelial autografts,[4] we achieved sufficient coverage of the affected area to improve the patient's quality of life. Additionally, while culture epithelial autografts require 2 to 3 weeks in culture before grafting, RM punch grafting enables immediate, simple, and cost-effective treatment. The rapid healing of the donor site permits repeated collection of tissue for grafts. Furthermore, inclusion of both the dermis and epidermis likely maintains mechanical stability, aiding the engraftment and growth of the revertant keratinocytes.

Punch graft transplant of revertant skin achieved sustained reepithelialization, treating a large, chronic open wound (approximately 20 × 14 cm^2) through serial transplants from a single, small revertant patch (approximately 7.5 × 4 cm^2). These findings suggest that RM keratinocytes with functional type VII collagen have a growth advantage over the surrounding altered keratinocytes in vivo. This report highlights the occurrence of RM in keratinocytes in RDEB and emphasizes the efficacy of punch grafting of revertant skin for treating large, chronic RDEB wounds. Precise gene correction of non-RM affected skin in patients with RDEB could be an effective future treatment strategy.[5]

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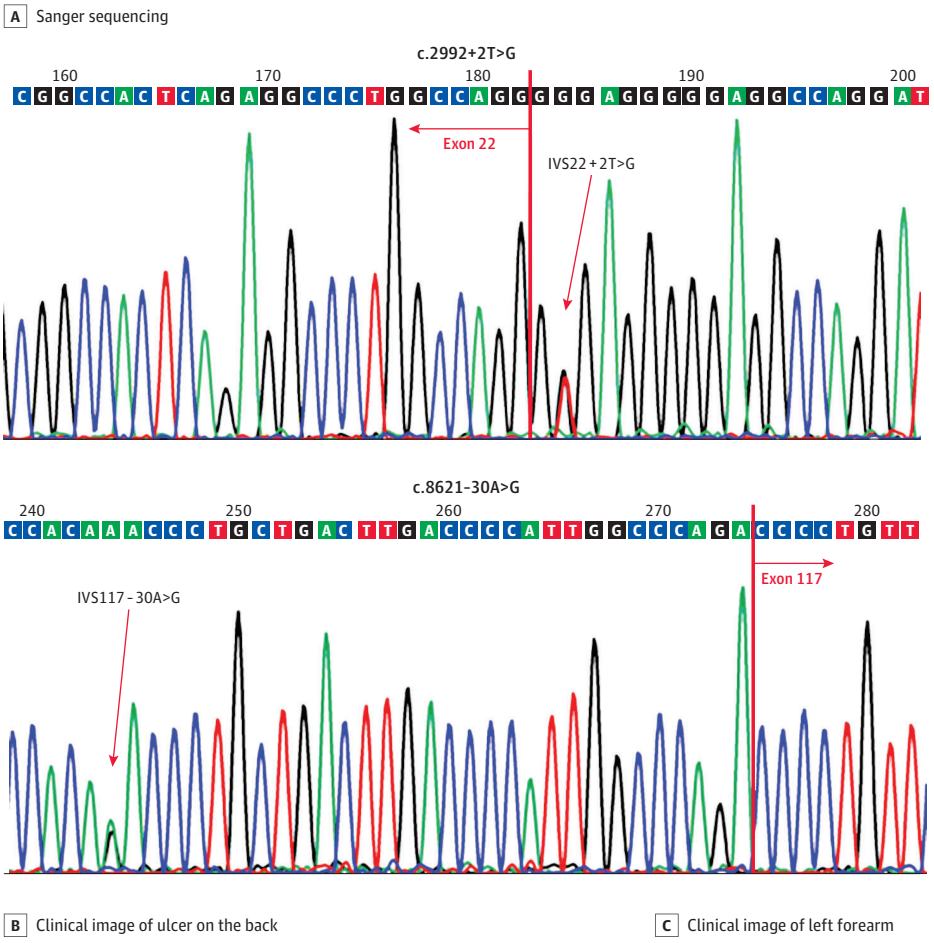
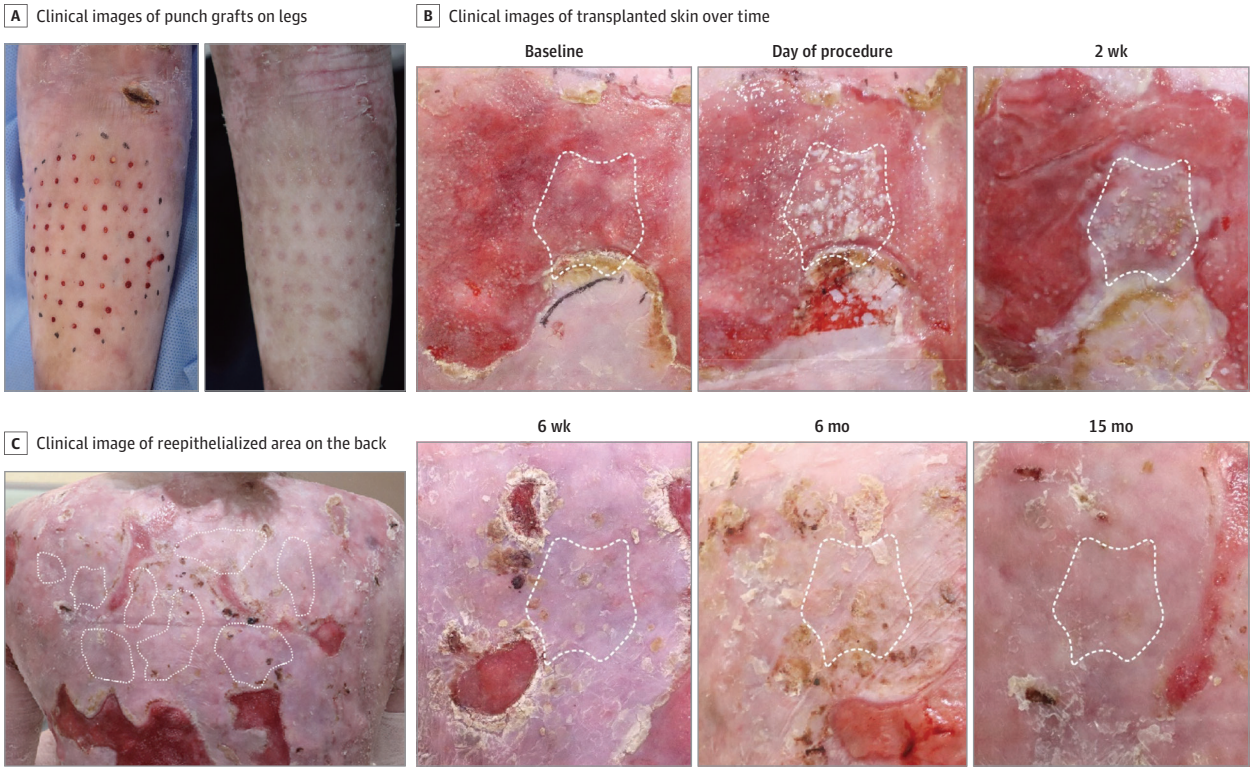
Figure 1. Detection of Revertant Mosaicism of *COL7A1* RNA Transcripts in a Patient With Recessive Dystrophic Epidermolysis Bullosa (RDEB) and a Large, Chronic Open Wound



A, Sanger sequencing showed a compound heterozygous *COL7A1* alteration in the patient (c.2922 + 2T>G and c.8621-30A>G [red arrows]). B, A persistent 20 × 14-cm^2 ulcer, which was unhealed for 3 years, on the back of the patient with severe generalized RDEB. C, A patch of revertant skin on the left forearm of the patient, lacking blistering (white dashed line).

Figure 2. Punch Graft of Revertant Skin Onto a Chronic Wound in a Patient With Recessive Dystrophic Epidermolysis Bullosa and Assessment of Long-Lasting Reversion Through Histological Analysis



A, Harvesting of 40 to 50 punch grafts, each 2 mm in size, from the revertant skin (left), and rapid healing of the donor site within 2 weeks after each harvesting (right). B, Engraftment and reepithelialization of the transplanted revertant skin. The transplanted punch biopsy specimens were accepted in the grafted area without any major complications, and complete reepithelialization occurred within a period of 2 to 6 weeks. The grafted area is outlined by white dashed lines. C, The locations of the 8 punch-grafted areas are indicated by white dotted lines. Both the grafted area and reepithelialized area remained intact throughout the 15-month follow-up period.

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