reduced rosacea risk in diabetics at an advanced disease stage. This is, to our knowledge, a previously unreported finding, but some residual confounding or chance cannot entirely be ruled out. Whether insulin enhances this effect *per se* or whether it reflects a proxy for disease severity remains unclear.

CONFLICT OF INTEREST

JJV is an employee at Galderma, France. The other authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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A Mutation-Independent Therapeutic Strategy for Dominant Dystrophic Epidermolysis Bullosa

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TO THE EDITOR

Dominant dystrophic epidermolysis bullosa (DDEB) is a rare genetic blistering skin disorder with no known cure. The disorder is caused by mutations in the *COL7A1* gene leading to weakened α1(VII) (collagen VII) homotrimers, whose function is to knit epidermal and dermal skin layers together (Parente *et al.*, 1991; McGrath *et al.*, 1993). Over 60 *COL7A1* mutations have been implicated in DDEB, making this disorder, similar to many other dominant disorders, extremely heterogenous (Nakamura *et al.*, 2004).

A gene therapy strategy for DDEB involving suppression of mutant *COL7A1* transcripts using allele-specific RNA interference (RNAi) molecules targeting a mutation site has been adopted *in vitro* (Pendaries *et al.*, 2012). This informative study demonstrated potent and specific RNAi-mediated allele-specific suppression of a *COL7A1* splice-site mutation in cells; similar levels of RNAi specificity have been obtained by others (Hickerson *et al.*, 2008; Lindahl *et al.*, 2008; Atkinson *et al.*, 2011). In contrast, for many *COL7A1* mutations and indeed other target genes, allele-specific RNAi suppression has not been achieved (de Yñigo-Mojado *et al.*, 2011; Pendaries *et al.*, 2012 and Morgan *et al.*, unpublished data).

Given the vast array of *COL7A1* mutations implicated in DDEB, the development of RNAi-mediated mutation-specific therapies targeting each mutant is not technically/economically feasible. In this study, we have addressed the substantial problem of DDEB-associated mutational heterogeneity and have tested four mutation-independent small interfering RNAs (siRNAs; si1-si4) targeting different regions of the *COL7A1*

Abbreviations: DDEB, dominant dystrophic epidermolysis bullosa; NT, nontargeting negative control RNAi; R, replacement COL7A1 gene; RNAi, RNA interference; siRNA, small interfering RNA

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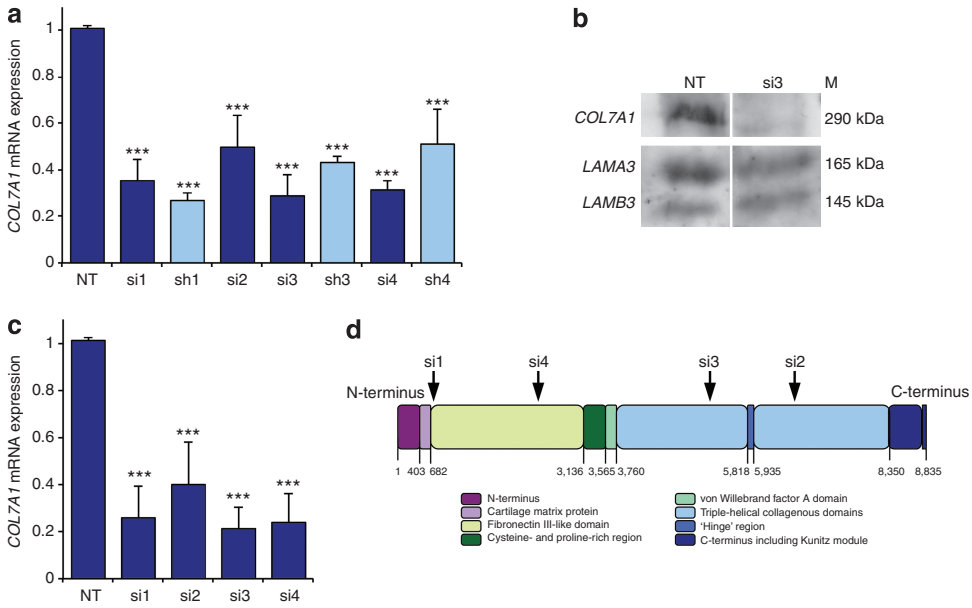

Figure 1. RNA interference (RNAi) suppression of *COL7A1*. Relative *COL7A1* mRNA levels in HaCaT cells transfected with small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) (**a**) and siRNA-transfected normal human dermal fibroblast (NHDF) cells (**c**) compared with nontargeting negative control RNAi (NT)-treated cells. Error bars represent SD. siRNA3 (si3) suppressed *COL7A1* by 71 ± 8.7% and 79 ± 8.5% in HaCaTs and NHDFs, respectively, and by 57 ± 2.4% in shRNA format (sh3). (**b**) Western blot of collagen VII protein levels in medium isolated from si3 or NT-transfected HaCaT cells. Laminin 332 was used as a loading control. Collagen VII and laminin 332 α3 (*LAMA3*) and β3 (*LAMB3*) chains are indicated. Collagen VII protein was visibly reduced in si3-treated cells compared with NT-treated cells. (**d**) Collagen VII domain organization. Arrows represent the *COL7A1* targets for si1-4. ***$P<0.001$. M, molecular weight.

gene (Figure 1d). A single potent mutation-independent suppressor in conjunction with a replacement gene designed to be refractory to suppression represents a therapy for DDEB, which corrects the primary genetic defect, but in a mutation-independent manner.

The two elements of this dual-component therapy for DDEB were evaluated as follows: si1-si4 targeting positions 733–753, 6,356–6,376, 5,384–5,404, and 2,902–2,922 of the *COL7A1* sequence (NM_000094), respectively, and a nontargeting control siRNA (NT) were tested (Supplementary Materials and Methods online). In 24-well plates, 5 nM si1-si4 and control NT were transfected into 1×10^5 keratinocytes, HaCaTs (Boukamp *et al.*, 1988). RNA was isolated 24 hours after transfection (Allen *et al.*, 2008). *COL7A1* RNA levels were suppressed significantly by si1-si4 by 65 ± 9%, 50 ± 13.5%, 71 ± 8.7%, and 68 ± 3.7%, respectively ($n=4$; $P<0.001$; Figure 1a). Short hairpin RNA equivalents of si1, si3, and si4 (sh1, sh3, and sh4) were generated (Brummelkamp *et al.*, 2002), and after transfection into immortalized HaCaT cells significant suppression of *COL7A1* transcript levels of 73 ± 3.4%, 57 ± 2.4%, and 49 ± 15%, respectively, was demonstrated ($n=3$; $P<0.001$; Figure 1a).

Notably, suppression of *COL7A1* transcript was also confirmed in primary cells, normal human dermal fibroblast primary cells, and significant suppression levels of 75 ± 13.7%, 60 ± 17.7%, 79 ± 8.5%, and 77 ± 12% were demonstrated with si1-si4, respectively ($n=3$; $P<0.001$; Figure 1c). Similarly, in HaCaT cells, suppression at the protein level by the most efficient siRNA, si3, was clearly visible by western blotting 48 hours after RNAi transfection (Figure 1b).

The most potent RNAi molecule, si3, was investigated further for this mutation-independent suppression and replacement therapeutic strategy for DDEB and a replacement *COL7A1* gene (R) was generated with altered degenerate nucleotides at the si3 target site (Figure 2a). Alterations in codon usage was minimized when designing R by using conservative codon changes, and thus expression levels would be optimal. As the transcript expressed from R contains six-nucleotide mismatches (Figure 2a in red) at the si3 target site, it was expected that, although R encodes wild-type Collagen VII protein, RNAi suppression by si3 should be minimal or eliminated.

Indeed, when 0.6 μg R and 1.25 nM of si3 or NT were co-transfected in a six-well plate into HEK293 cells, R transcript was not suppressed significantly by si3 (Supplementary Materials and Methods online). In contrast, wild-type *COL7A1* RNA was suppressed by 77 ± 11% by si3 compared with the non-targeting RNAi (NT) ($n=3$; $P<0.001$), and no expression of wild-type *COL7A1* RNA was observed in untransfected cells (Figure 2b and c). Similarly, at the protein level, replacement Collagen VII protein was not suppressed by si3, as evaluated by western blotting, whereas the wild-type protein was noticeably reduced (Figure 2b and c). Hence, we have clearly demonstrated that R RNA is resistant to suppression by si3, whereas wild-type *COL7A1* is potently suppressed by ~80%.

DDEB is a debilitating, untreatable condition for which development of gene therapies has been hampered by

a

Target	CCG	AAT	GGT	GCT	GCA	GGC	AAA
Amino acid	P	N	G	A	A	G	K
Possible codons	CCG 0.18 CCA 0.16 CCT 0.21 CCC 0.46	AAT 0.27 AAC 0.73	GGT 0.16 GGG 0.45 GGA 0.25 GGC 0.14	GCT 0.21 GCG 0.22 GCA 0.13 GCC 0.45	GCA 0.13 GCG 0.22 GCT 0.21 GCC 0.45	GGC 0.14 GGG 0.45 GGA 0.25 GGT 0.16	AAA 0.64 AAG 0.36
R	CCC	AAC	GGC	GCC	GCC	GGG	AAA

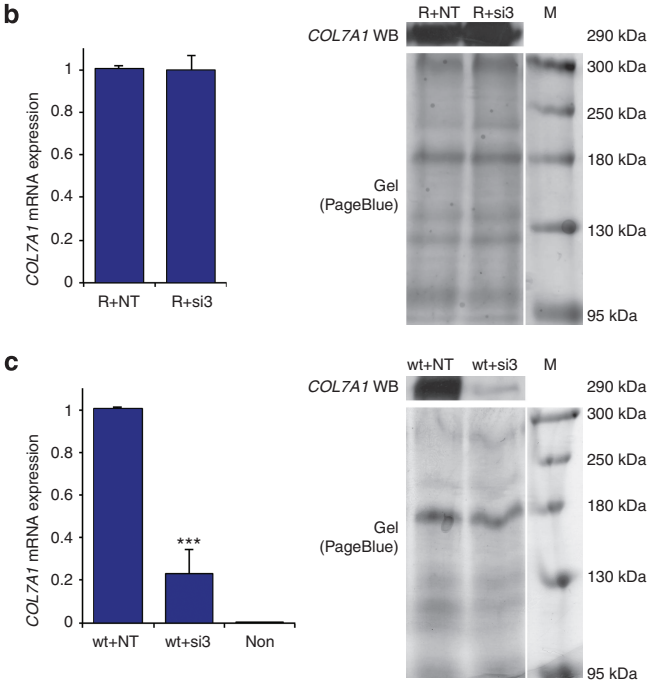

Figure 2. Replacement *COL7A1* gene resistance to small interfering RNA 3 (siRNA 3) suppression. (**a**) Replacement (R) *COL7A1* for si3 with six nucleotide changes (red) over the si3 target site. Codons used most frequently in *COL7A1* were selected (numbers indicate relative codon usage frequencies). (**b**) HEK293 cells were co-transfected with R and either si3 or non-targeting (NT) siRNA. No significant differences were found in cells treated with R and si3 compared with those with R and NT at the RNA or protein levels. A PageBlue-stained gel was used as a loading control; protein ladder standard sizes are indicated. Co-transfections of wild-type (wt) *COL7A1* with si3 resulted in 77 ± 11% RNA suppression and a visible reduction in collagen VII protein. Untransfected cells did not express *COL7A1* (**c**). Error bars represent SD. ***$P<0.001$. *COL7A1* WB, *COL7A1* western blot; M, molecular weight.

significant mutational heterogeneity and the nature of the target tissue. Some mutation-specific gene therapeutics have been able to discriminate between mutant and wild-type alleles and provide a potential nucleotide-based therapy that does not require a replacement gene, as the mutant allele is specifically silenced and expression of the wild-type allele is maintained (Pendaries *et al.*, 2012; Hünefeld *et al.*, 2013). However, for many mutations, allele-specific RNAi remains elusive. To circumvent technical difficulties with designing allele-specific suppressors together with the cost burden of developing gene therapies for every *COL7A1* mutation, a suppression and replacement gene therapy (Millington-Ward *et al.*, 1997) has been developed for DDEB, which corrects the primary defect but is independent of mutation. In principle, one therapy could treat all DDEB patients irrespective of their underlying *COL7A1* mutation. If therapies are to attract the investment required to proceed to Phase I/II clinical trial, developing widely applicable therapies for rare orphan diseases such as DDEB is crucial; exploiting fundamental features of the genome, such as codon redundancy, as adopted in the current study in principle will facilitate this. Furthermore, the inclusion of both elements of this dual-component therapeutic in a single vector would further expedite progression toward clinical trial. In terms of efficacy, there is previous evidence in cells that modulation of the ratio of mutant:wild-type collagen VII (from 50:50 to 10:90) improves the thermal stability of collagen trimers, suggesting that in principle achieving levels of suppression of the mutant such as that in the current study (70–80%) together with potent replacement should provide beneficial effects (Fritsch *et al.*, 2009). However, additional challenges for DDEB include a requirement for novel delivery systems to enable dissemination of the therapeutic thoughout the body and longevity of therapeutic effects. In this regard, viral and nonviral vectors and local versus systemic administration of therapies are being considered but require further development (Has and Kiritsi, 2013; Hünefeld *et al.*, 2013).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Molecular Profiling to Diagnose a Case of Atypical Dermatomyositis

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TO THE EDITOR

Dermatomyositis (DM) is a rare systemic autoimmune disease characterized by inflammation of the skin and muscle. Skin rash in DM may precede the onset of muscle disease by months to years, and in some cases overt muscle disease may remain mild or never occur. DM skin disease manifests in varied ways including facial edema, pink to violaceous patches on the eyelids (heliotrope sign), violaceous scaly plaques with atrophy on the shoulders and hips, violaceous atrophic scaling papules on the interphalangeal joints (Gottron's papules), and prominent nailfold telangiectasias. However, these findings are variably present, and DM skin disease can share clinical features with cutaneous lupus, overlap connective tissue disease syndromes, UV light–induced reactions, and psoriasis (Sontheimer, 2002), often creating diagnostic difficulty at early presentation.

Psoriasis is an inflammatory skin disease most commonly characterized by well-demarcated plaques with micaceous scales. Histopathologically, psoriasis displays epidermal acanthosis, confluent parakeratosis, subcorneal neutrophils, and thinning of the suprapapillary plate. In contrast, DM is characterized by an interface dermatitis with a superficial perivascular infiltrate, and dermal mucin deposition. However, DM patients often present with erythematous scaly plaques in the scalp or on extremities, which can be clinically confused with psoriasis. In addition, many skin biopsies of DM can actually show minimal to no interface dermatitis, and have evidence of neutrophilic inflammation (Ito *et al.*, 1995; Caproni *et al.*, 2004), perivascular dermatitis, and even epidermal hyperplasia not dissimilar to that seen in psoriasis.

Molecular profiling has widely been used to aid the diagnosis of cancer patients since the publication of landmark work by Golub *et al.* (1999). It has also been applied in autoimmune diseases such as systemic lupus erythematosus for similar purposes (Chaussabel *et al.*, 2008). In this report, we compare the molecular features of DM and psoriasis and apply molecular profiling to aid in the diagnosis of an ambiguous case of DM.

Our index case, a 32-year-old woman, presented in June 2005 with the 3-month onset of well-demarcated violaceous erythematous, scaling plaques on her elbows, extensor forearms, dorsal hands, lateral thighs, knees, and lower legs, concerning for either psoriasis or Gottron's sign of DM (Figure 1a–c). Diffuse erythema was also present on her scalp and periungual areas, but she did not display psoriatic nail changes, geographic tongue, or heliotrope sign. Antinuclear antibodies were positive (titer 1:2,560) with a homogenous pattern. Other antibodies including anti-Jo1, SSA, SSB, anti-dsDNA, and anti-centromere were negative. Given her clinical ambiguity, a biopsy of one of these psoriasis-like lesions on the knee was performed. The biopsy demonstrated regular epidermal acanthosis, parakeratosis, mild, superficial perivascular infiltrate, and dilated papillary dermal vessels consistent with psoriasis (Figure 1d). No interface dermatitis was detected. However, a colloidal iron stain revealed abundant mucin throughout the dermis (Figure 1e), which is unusual in psoriasis and more consistent with DM. Given the inconclusive results from traditional histopathology, genome-wide expression analysis was applied to determine

Abbreviation: DM, dermatomyositis

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