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Crossing Barriers; Restoring Barriers? Filaggrin Protein Replacement Takes a Bow

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In this issue of the Journal, Stout and colleagues report a novel and creative approach to replacement of genetically determined absence or deficiency of epidermal proteins. While these early data are certainly interesting, further validation work is required to determine the utility of this approach in genodermatoses.

Journal of Investigative Dermatology (2014) **134**, 313–314. doi:10.1038/jid.2013.506

One in ten Europeans carries at least one filaggrin (*FLG*) loss-of-function (LOF) mutation (Irvine and McLean, 2006). These common variants are the strongest genetic risk yet identified for atopic dermatitis (AD), with an overall odds ratio for AD of ~4 (Rodriguez *et al.*, 2009). In addition to the risk of AD posed by LOF mutations, variation in *FLG* repeat numbers (10, 11, or 12 repeats; intragenic copy-number variation) also confers significant risk of AD (Brown *et al.*, 2012). Furthermore, in addition to these genetically deter-

mined deficiencies in *FLG* production, *FLG* expression is downregulated secondarily in AD due to the disease *per se* (Kezic *et al.*, 2011), as recently reviewed (McAleer and Irvine, 2013). In addition to a role as an AD risk gene, *FLG* LOFs are modifiers of disease within AD. Individuals with AD who carry a *FLG* LOF mutation are more likely to develop asthma, to have more severe and more persistent disease, to have allergic sensitizations, and to develop eczema herpeticum (McAleer and Irvine, 2013).

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Clinical Implications

- Loss-of-function (LOF) mutations in filaggrin (FLG) are common and greatly increase the risk of developing atopic dermatitis.
- LOF *FLG* mutations also predispose to asthma, more severe and persistent dermatitis, allergic sensitization, and eczema herpeticum, making protein replacement an attractive treatment option.
- In the flaky mouse model, a targeted recombinant partial filaggrin protein, applied topically, improves stratum corneum phenotype, suggesting this may be a useful therapeutic approach. A caveat is that the flaky tail mouse also harbors a mutation in *TMEM79*, which greatly impairs barrier function and may facilitate easier transfer of the targeted protein.

Filaggrin replacement therapies therefore have wide potential applicability in management of AD. The *FLG* gene encodes a proprotein that has no known function when expressed in the granular layer. Dephosphorylation of the proprotein, in concert with derepression of a protease cascade, leads to rapid liberation of functionally active filaggrin monomers in an elaborately controlled process (Sandilands *et al.*, 2009). Theoretical gene-based approaches to enhance or replace *FLG* deficiency might include read-through drugs that focus on the mutant allele or drugs that increase *FLG* expression by driving the promoter of the 'healthy' allele or alleles. Direct protein replacement is another potential approach, but profilaggrin is an exceptionally large molecule, making replacement difficult. Obstacles common to all topical approaches to replacement therapies are delivery across the stratum corneum barrier and the need to target the replacement gene or protein accurately to the intracellular cytoplasmic space in the relevant, differentiation-specific epidermal compartments.

In this issue of the *JID*, Stout, *et al.* (2014) report a novel and potentially exciting approach to *FLG* replacement. They have considered and attempted to solve many of the obstacles mentioned above. To reduce the size of the replacement product, they produced, using a commercially available bacterial expression system, a single murine *Flg* repeat, with flanking sequences to allow processing. To achieve intracellular

delivery, they linked this repeat sequence covalently to a cell-penetrating peptide derived from the HIV TAT protein. They report a progressive series of complementary experiments using cell culture, skin equivalents, and the flaky tail mouse. Their results show that the recombinant protein is delivered, dependent on the linked RMR motif, to the keratinocyte cytoplasmic space. In human skin equivalents and in the flaky tail mouse, the protein is delivered throughout the epidermis, as it is not restricted to differentiation-specific compartments. This lack of specificity may reflect the importance of the full profilaggrin protein, including the untranslated 5'- and 3'-domains, in protein localization. Morphological aspects of the flaky tail stratum corneum phenotype were apparently restored. Analysis of post-translational processing was restricted to proof of production, presumably by proteolytic cleavage of the protein construct, of a single filaggrin repeat. The unintended effects of *FLG* expression, including possible immunogenicity, in atypical epidermal compartments, including the basal layer are unknown. Further analysis of the complex post-translational modification of *FLG* was not performed, and this will be important to determine the likely therapeutic effects of protein replacement.

An engineered peptide with one filaggrin repeat benefits skin in a mouse model of AD.

These proof-of-principle results are certainly interesting, but will require further work, first to determine safety, and then dosing and efficacy, including dosing interval, and ultimately duration of effect in human subjects. Although the flaky tail mice do not appear to mount an immunological response to the fusion protein, humans may respond differently. If such a therapy were to overcome or avoid these issues and become available for human use, it might first be used in those with active AD or problematic ichthyosis vulgaris. It might also have a preventative role for those who carry *FLG*-null alleles. To that end, it will be important to identify disease modifiers and predictors of disease in individuals with *FLG* mutations because only 40% will develop AD. Finally, this novel delivery system may well have broader applicability for patients with one of the many, now genetically well understood, genodermatoses currently waiting for gene or protein replacement therapies.

CONFLICT OF INTEREST

The author states no conflict of interest.

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