GENETICS OF STRUCTURAL SKIN DISORDERS

Heritable Filaggrin Disorders: The Paradigm of Atopic Dermatitis

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THE PROFILAGGRIN/FILAGGRIN PROTEIN

Mutations in the filaggrin gene (*FLG*) are one of the most common pathogenic single-gene defects identified to date. *FLG* encodes an important epidermal protein abundantly expressed in the outer layers of the epidermis (Sandilands *et al.*, 2009). Approximately 10% of individuals of European ancestry are heterozygous carriers of a loss-of-function mutation in *FLG* resulting in a 50% reduction in expressed protein (Irvine and McLean, 2006).

As a protein, filaggrin is an unusual beast in biology. It is expressed as a giant, inactive, polymeric protein, profilaggrin, which is a major constituent of keratohyalin granules. At the interface between the outermost granular layer keratinocytes and the stratum corneum, this proprotein is cleaved into several identical filaggrin subunits. It has been postulated that these monomers condense the cytoskeleton and contribute to squame formation. In the cornified layers *per se*, filaggrin undergoes further posttranslational modifications and is ultimately proteolyzed into a hygroscopic pool of amino acids and chemical derivatives thereof, termed natural moisturizing factor (NMF). Thus, filaggrin has a central role in the biogenesis and subsequent hydration of the stratum corneum (Irvine *et al.*, 2011).

DISEASE ASSOCIATIONS

The identification of an association of filaggrin with atopic dermatitis (AD) was a slow burning one. Shortly after the identification of this protein in the late 1970s (Dale, 1977), its role in keratin filament aggregation was rapidly established (Dale *et al.*, 1978). Some 7 years later, the first evidence that filaggrin was of potential causal interest in ichthyosis vulgaris (IV) emerged (Sybert *et al.*, 1985). It was to take a further two decades to fully disclose the molecular genetics of IV. That delay was due, in large part, to the technical difficulty of sequencing this highly repetitive gene and also due to the confusing inheritance of IV, which it transpired, which was best explained by semi-dominant Mendelian inheritance, rather than by traditionally understood dominant or recessive inheritance (Smith *et al.*, 2006).

Once Smith *et al.* (2006) had cracked the technical aspects of sequencing *FLG* in carefully chosen clinical pedigrees, the first two recurrent loss-of-function mutations (R501X and 2282del4) were identified. Identification of further recurrent mutations swiftly followed (Sandilands *et al.*, 2007). Surprisingly, these mutations proved to be prevalent in European populations, with ~9% of these populations carrying at least one *FLG* mutation (Irvine *et al.*, 2011). An ethnospecific mutation profile was seen with population-specific mutation spectra reported in well-studied populations such as the Singapore Chinese (Chen *et al.*, 2011). In this population, multiple very low-frequency mutations collectively contribute to the overall total, compared with the Irish population where five recurrent mutations constitute most of the total mutations (Chen *et al.*, 2011).

Once the molecular genetics had been established, rapid genotyping of large patient collections was possible and the association with AD was established initially in small collections of Irish children with AD and in Scottish children with AD and asthma (Palmer *et al.*, 2006). These findings have since been replicated many times in varying disease collections and population cohorts; the association is one of the strongest between any gene and a complex disease (Rodriguez *et al.*, 2009). Further analysis of additional disease and population collections revealed that AD associated with *FLG* mutations is, in general, more severe, more persistent, more 'atopic', more likely to be associated with asthma, and more likely to have herpes infections (Irvine *et al.*, 2011). Overall, collections of AD show a prevalence of *FLG* mutations of between 20 and 50% (Irvine, 2007). In addition, *FLG* mutations are associated with peanut allergy (Brown *et al.*, 2011). The totality of the contribution of filaggrin to human disease is not limited to low prevalence loss-of-function mutations, as more prevalent intragenic copy number mutations also contribute substantially to risk, with the total filaggrin repeat number (the filaggrin index) significantly predicting AD risk with a lower odds ratio but a higher frequency and therefore a significant overall contribution to disease in addition to the lower frequency null alleles (Brown *et al.*, 2012).

THE ROLE OF FILAGGRIN IN EPIDERMAL BARRIER FUNCTION

The filaggrin story placed a primary epidermal barrier defect firmly in the forefront of AD pathogenesis and has led to multiple avenues of inquiry to elucidate early pathogenic pathways in AD. The *flaky tail* mouse, a double mutant for murine *Flg* and the matted gene (*ma*), shows a primary epidermal barrier defect compounded massively by allergic inflammation (Fallon *et al.*, 2009) findings consistent with *ex vivo* studies that show evidence for modulation of filaggrin expression by the Th2 cytokines, IL-13 and IL-4 (Howell *et al.*, 2007). Furthermore, filaggrin may have pleiotropic effects on multiple pathways relevant to the pathogenesis of AD including on the growth of *Staphylocccus aureus* (Miajlovic *et al.*, 2010), skin pH (Jungersted *et al.*, 2010), lipid lamellae secretion and stratum corneum structure (Gruber *et al.*, 2011), and the expression of pro-inflammatory cytokines (Kezic *et al.*, 2012). *FLG*-null alleles lead directly to decreased stratum corneum NMF (Kezic *et al.*, 2008); careful assessment of these levels demonstrates three separate subpopulations of AD based on *FLG* genotype, viz $FLG^{+/+}$, $FLG^{+/-}$, and $FLG^{-/-}$ (O'Regan *et al.*, 2010).

THERAPEUTIC TARGETS

Filaggrin expression is downregulated in all cases of moderate-to-severe AD (Kezic *et al.*, 2011), making it an appropriate therapeutic target for AD in the broadest sense, rather than being confined to AD associated with *FLG*-null alleles. The filaggrin index effect, where a modest 20% increase in filaggrin copy number leads to a 40% reduction in AD susceptibility (Brown *et al.*, 2012), is highly suggestive that filaggrin upregulation therapies will be of potential wide utility in AD. Comparatively little is known about the regulation of filaggrin gene expression, although this is now an area of active interest by academia and pharma alike. The fact that filaggrin expression has been shown to be regulated by environmental humidity (Katagiri *et al.*, 2003) indicates that there are signaling pathways in the skin that actively control filaggrin expression and skin barrier homeostasis in general. These are tractable problems that will lead to specific therapeutic approaches once these pathways are fully understood and targeted pharmacologically.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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