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INSIGHTS

A "second hit" impacts disease severity in a dominantly inherited genetic skin disorder

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In this issue of *JEM*, Bergson et al. (https://doi.org/10.1084/jem.20240827) identified variants in *HMCN1* that co-segregate with and account for variations in disease severity in individuals with a diagnosis of epidermolysis bullosa simplex (EBS) resulting from pathogenic variants in *KRT14*. The authors show that hemicentin-1 binds keratin 14 at the protein level and that silencing *HMCN1* expression disrupts the organization of K14-containing filaments in epidermal keratinocytes and their attachment to the extracellular matrix. These findings address the clinical heterogeneity observed in EBS, a rare genetic skin disorder, with general implications for all genodermatoses.

Epidermolysis bullosa simplex (EBS) was the first genetic skin blistering disorder shown to be caused by pathogenic variants in keratin genes (Bonifas et al., 1991; Coulombe et al., 1991a). Clinically, EBS is a heterogeneous condition (Coulombe et al., 2009; Sprecher, 2010). Severe forms of EBS entail generalized blistering of the skin that is manifested at birth, may involve internal stratified epithelia (e.g., oral mucosa), and may even prove lethal to newborns. In contrast, milder forms of EBS are typified by blistering localized to skin sites experiencing a higher amount of physical stress, e.g., hand and feet, with more extensive trauma required to expose the inherently fragile character of the epidermis. Pathological findings reveal that new blisters exhibit lysis or rupture of keratinocytes within the basal layer of the epidermis in EBS, whether mild or severe (Coulombe et al., 2009). At a genetic level, EBS is viewed as the equivalent of a classic monogenic disorder, with most cases (>85%) linked to mutations in either *KRT5* or *KRT14* along with very high penetrance of the pathogenic variants occurring in these two keratin loci (Coulombe et al., 2009; Szeverenyi et al., 2008). Incidentally, the *KRT5* and *KRT14* genes code for the keratin proteins that give rise to, through copolymerization, the robust intermediate filament (IF) cytoskeleton that typifies basal layer keratinocytes in the epidermis and related stratified epithelia.

Keratins comprise a relatively large family of IF cytoskeletal proteins and are most prominently expressed in epithelial cells. In surface epithelia such as skin, keratins are among the most abundant proteins in keratinocytes (Feng et al., 2013) where they form a cross-linked IF network that extends across the entire cell, from the surface of the nucleus to cell–cell and cell–matrix adhesion complexes located at the periphery (Coulombe et al., 2009). Cross-linked keratin IF networks exhibit mechanical properties that are unparalleled among cytoskeletal elements (Ma et al., 2001), enabling keratinocytes to effectively withstand frictional stress and other forms of trauma (Coulombe et al., 1991b; Ramms et al., 2013). Deficiencies or defects in keratin proteins result in epithelial fragility and underlie epithelial disorders which, like EBS, are individually rare and, in the vast majority of cases, caused by dominantly acting missense variants (Coulombe et al., 2009; Szeverenyi et al., 2008).

Remarkably, the full clinical spectrum of classic EBS phenotypes in the clinic, ranging from mild to severe, can be elicited by altering the open reading frame of a single gene, namely, *KRT14*, in both human (Coulombe et al., 2009) and mouse

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(Coulombe et al., 1991b). Classic forms of EBS represent an example of a genetic skin disorder for which the correlations between genotype and phenotype have been deemed "satisfying," certainly when compared to other keratinopathies (see below). Accordingly, missense variants that alter the evolutionarily conserved segments of the central α-helical rod domain, a region crucial for keratin proteins to form 10-nm filaments, are associated with higher sensitivity to trauma and severe skin blistering across multiple body sites. Conversely, missense variants that alter less

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well-conserved residues located in other regions of these keratin proteins are associated with lesser sensitivity to trauma, with blistering restricted to body areas experiencing the most frictional stress, e.g., hands and feet (Coulombe et al., 2009; Sprecher, 2010). While these relationships hold true throughout much of the clinical spectrum of EBS, they do not fully succeed in accounting for the complex relationship between the genotype and the phenotype in this disorder.

In a study reported in this issue of *JEM*, Bergson et al. (2025) tackled the conundrum of clinical heterogeneity by performing exome sequencing on 195 individuals from 90 families with an EBS clinical diagnosis along with confirmed genetic evidence of EBS-causing pathogenic variants. Meticulous filtering of the resulting massive dataset revealed three variants in the *HMCN1* locus, which encodes hemicentin-1, a large modular protein that belongs to the fibulin family of extracellular matrix (ECM) proteins (Welcker et al., 2021). The authors found that the three *HMCN1* variants identified co-segregate with the disease phenotype in affected individuals within four families with known pathogenic variants in *KRT14* and, critically, are associated with a significantly more severe EBS presentation. Importantly, assessment of the patient cohort included in the study and of an additional >8,000 healthy subjects strongly suggest that the *HMCN1* variants are not intrinsically pathogenic. Bergson et al. (2025) used a multimodal strategy combining computational, modeling, genetic, microscopy-based, and 3D cell culture methods to assess the expression and distribution of wild-type hemicentin-1 in healthy skin and, importantly, test whether the three *HMCN1* variants they identified disrupt the properties of hemicentin-1. These efforts revealed that hemicentin-1 localizes primarily to basal keratinocytes in the healthy human epidermis, where it is proximal to and physically interacts with K14. The *HMCN1* variants alter evolutionarily conserved residues within Ig subdomains of hemicentin-1 and are predicted to disrupt their structure. The authors show that knockdown of *HMCN1* expression in HaCaT (human) keratinocytes, achieved via siRNA, disrupts the organization of K14-containing filaments and lowers the steady-state levels of the K14 protein. Finally, the authors show the occurrence of fragility in the basement membrane zone of 3D epidermal equivalents generated from normal human keratinocytes and fibroblasts in which *HMCN1* expression has been downregulated by siRNA-mediated knockdown. Altogether, the study by Bergson et al. (2025) is innovative and rigorous, and yields convincing evidence that *HMCN1* can act as a modifier locus with a sizable impact on disease severity in EBS. These findings usher in a novel and far-reaching perspective from which to understand phenotype–genotype correlations in EBS and other genodermatoses (see below).

In retrospect, the discovery reported by Bergson et al. (2025) aligns well with our understanding of the pathophysiology and genetic underpinnings of EBS. Indeed, *HMCN1* encodes a protein that regulates the formation of the basal lamina and organization of hemidesmosomes. The latter are membrane-spanning adhesion complexes found in basally located keratinocytes in the epidermis and related stratified epithelia, where they mediate keratinocyte attachment to the basal lamina on the extracellular side, and attachment to K5-K14 filaments on the intracellular side. Coincidentally, a subset of EBS cases have been shown to result from pathogenic variants in genes whose protein product helps anchor keratinocytes to the ECM in stratified epithelia, e.g., *ITGB4* (coding for integrin β4; Jonkman et al., 2002) and *COL17A* (BP180/collagen XVII; Huber et al., 2002), or in *PLEC*, a gene whose protein product (plectin) connects keratin IFs to hemidesmosomes (Smith et al., 1996). Otherwise, there is a precedent for a nonpathogenic variant in *KRT5* (E170K) being able to significantly modulate the severity of EBS presentation caused by a pathogenic variant in the same gene (E418K; Yasukawa et al., 2002). By shedding light on a role for *HMCN1*, the study by Bergson et al. (2025) calls attention to the complexity of the protein-based networks that underlie mechanical resilience, the degree to which the entities making up these networks are interdependent, and the opportunity to uncover them for what they are thanks to the application of omics approaches.

The study by Bergson et al. (2025) raises, as expected, several issues that are relevant not only to EBS but also to other keratin-based genetic disorders and genodermatoses in general. At a cellular level, an open issue relates to how hemicentin-1, K14, and possibly its assembly partner K5 interact at biochemical and functional levels to account for the mechanical resilience of and, possibly, other properties of keratinocytes residing in the basal layer of the epidermis. Another open issue of great interest relates to how *HMCN1* variants that are intrinsically nonpathogenic affect the properties of the basement membrane zone and the mechanical resilience of basal layer keratinocytes. At a clinical level, whether *HMCN1* variants alter the clinical presentation of EBS beyond the severity of skin lesions, and otherwise impact the clinical presentation of EBS cases involving pathogenic variants in *KRT5*, *ITGB4*, *COL17A*, or *PLEC*, represents additional issues of high interest. Besides, whether *HMCN1*, related genes, or mechanisms play a role in the amelioration of EBS symptoms with age, a defining clinical attribute of this disorder (Coulombe et al., 2009; Sprecher, 2010), is a possibility worth thinking about. At a broader level, the application of whole-exome and whole-genome sequencing (now a more realistic proposition in terms of costs and analysis; see Valencia et al. [2015]), as done here by Bergson et al. (2025), is bound to significantly advance our understanding of the complex pathophysiology underlying several diseases and open up new avenues for diagnosis, genetic counseling, and therapeutic management. Finally, there are keratin-based genetic disorders, e.g., pachyonychia congenita (Samuelov et al., 2020; Zieman and Coulombe, 2020), for which the dissonance between the genotype and the phenotype is considerably more pronounced than what is seen clinically for EBS. The concepts and approaches deployed in the study by Bergson et al. (2025) provide an inspiring blueprint to reinvestigate pachyonychia congenita and other genodermatoses with a new perspective.

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