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IN THIS ISSUE…

Glycine Substitution Mutations in the *COL7A1* Gene: Implications for Inheritance of Dystrophic Epidermolysis Bullosa – Dominant vs. Recessive

Phenotypic Variability of Epidermolysis Bullosa. Epidermolysis bullosa (EB) is a clinically heterogeneous group of disorders characterized by blistering and erosions of the skin and mucous membranes, associated with variable additional clinical manifestations in the nails, teeth and eyes, as well as the gastrointestinal and vesicourinary tract (1, 2). The most severe forms of the disease are associated with early demise of the affected individuals, and in some patients, mutilating scarring of the hands and feet and aggressively metastasizing squamous cell carcinomas develop. EB is divided into distinct subcategories primarily based on the level of blister formation within the skin, as documented by transmission electron microscopy or immune-epitope mapping. As many as 14 genes are currently known to harbor mutations in different variants of EB, and the spatial expression of these genes within the skin, the types and combinations of mutations and their consequences at mRNA and protein levels, when superimposed on an individual patient's genetic background and exposure to environmental trauma, all contribute to the tremendous phenotypic variability noted in this group of disorders (3).

Molecular Basis of the Dystrophic Forms of EB. Inheritance of dystrophic EB (DEB) can be either autosomal dominant (DDEB) or autosomal recessive (RDEB). Nevertheless, patients with all variants of DEB demonstrate ultrastructurally a sub-lamina densa plane of tissue separation. Moreover, the fragility of skin can be explained by abnormalities in anchoring fibrils, critical attachment complexes that extend from the lamina densa to the underlying dermis and secure the stable association of the dermal-epidermal junction (Fig. 1). Anchoring fibrils in patients with DEB have been shown to be morphologically altered, reduced in number or completely absent. Patients with DEB, both dominant and recessive, harbor mutations in the *COL7A1* gene which encodes type VII collagen protein, the predominant, if not exclusive, component of the anchoring fibrils (4). *COL7A1* emerged initially as a candidate gene for mutations when immunofluorescence staining of the skin of patients with the most severe forms of RDEB demonstrated lack of type VII collagen epitopes (5).

Cloning of human type VII collagen gene and the corresponding cDNA provided the opportunity to assess the hypothesis that type VII collagen serves as the candidate gene/protein system for this group of blistering disorders. Specifically, genetic linkage studies utilizing informative polymorphic markers in

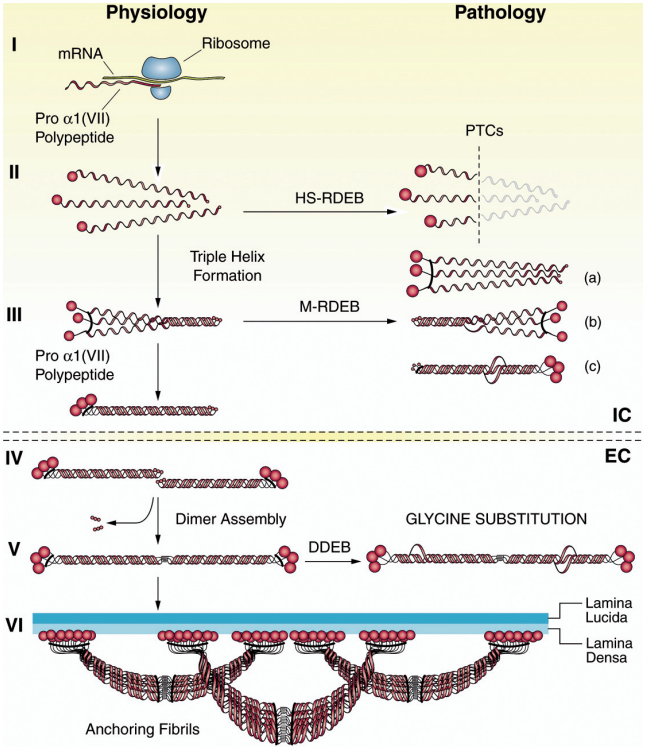

Fig. 1. Synthesis of type VII collagen and assembly of anchoring fibrils in normal skin (left side of the figure) and their perturbations in dystrophic epidermolysis bullosa as a result of mutations in *COL7A1* (right side of the figure). (I) Type VII collagen polypeptides, proα1(VII) chains, are synthesized on the ribosomes. (II) In the intracellular milieu (IC), three polypeptides associate at their carboxy-terminal ends, and (III) fold into a characteristic triple-helical conformation. (IV, V) After secretion into the extracellular space (EC) a small piece of the carboxy-terminus of each molecule is proteolytically removed, and two of the molecules assemble in tail-to-tail orientation. (VI) A large number of dimer molecules laterally assemble to form anchoring fibrils which form U-shaped structures, with their amino-terminal ends attached to the lamina densa, thus stabilizing the association of the epidermal basement membrane to the underlying dermis. Mutations in *COL7A1* can result in premature termination codons (PTCs) which manifest with severe Hallopeau-Siemens (HS) type of RDEB when present in both alleles. Missense mutations can interfere with the chain assembly (a), triple helix formation (b), or stability of the triple helix (c), resulting in mild (mitis, M) type non-HS-RDEB. Glycine substitution mutations in the collagenous domain can destabilize the triple helix and can result through dominant-negative interference in dominantly inherited DEB (DDEB). (Adapted from reference 6, with permission).

the *COL7A1* gene were consistent with the notion that most, if not all, cases with DEB are the result of mutations in this gene (see 4). Subsequent development of mutation detection strategies has allowed examination of a large number of cases with DEB, and well over 300 distinct mutations in the *COL7A1* gene have now been disclosed in both DDEB and RDEB (6, 7). The types of mutations range from premature termination codon (PTC) causing loss-of-function mutations to more subtle missense mutations. This information has facilitated genotype/phenotype correlations, improved

genetic counseling for families regarding recurrence of the disease in the same and subsequent generations, and made feasible DNA-based prenatal testing, when indicated (3). One critical and conflicting issue, however, that has impaired the accuracy of some DNA-based diagnostics and prognostics for DEB, has arisen in the precise nature of some of the *COL7A1* mutations identified. Specifically, dilemmas have occurred when the pathogenic mutation appears to be a glycine substitution located within the type VII collagen triple helix. Notably, glycine substitution mutations have been reported in both DDEB and RDEB.

Inheritance of DEB – Dominant vs. Recessive. DEB is typically divided either into dominantly inherited or recessively inherited forms, the assumption being that a single dominant mutation in one allele of *COL7A1* leads to phenotypic manifestations, while in recessively inherited forms of the disease mutations need to be present in both alleles. The consequences of these mutations at the phenotypic level are then thought to reflect the types of mutations and their positions along the type VII collagen. In particular, glycine substitution mutations are thought, in general, to be dominant ones resulting in dominant-negative interference of the anchoring fibril assembly, while a characteristic recessive mutation is a PTC in both alleles which results in synthesis of a truncated, non-functional polypeptide or complete absence of the protein through PTC-mediated mRNA decay (Fig. 1). However, more than 50 pathogenic glycine substitutions have been reported in RDEB (6, 7). When inherited *in trans* with a wild-type *COL7A1* allele, these mutations are typically silent, but when they are homozygous or inherited with a mutant other *COL7A1* allele, they lead to RDEB. Recessive glycine substitutions in type VII collagen are thought to impair protein synthesis, intracellular and extracellular protein transport or secretion, assembly into anchoring fibrils, or a combination thereof. At present the literature contains a similar number of pathogenic dominant or recessive glycine substitution mutations in type VII collagen. Remarkably, a small number of specific glycine substitutions appear to result in both DDEB and RDEB, a topic which is further explored in *an article in this issue by Almaani et al. (8).*

These investigators report their findings that identical glycine substitution mutations in type VII collagen may underlie both dominant and recessive forms of DEB. Specifically, the authors have identified four glycine substitution mutations in different families with diverse ethnic backgrounds and which have varying clinical consequences.

A 7-year-old girl was diagnosed with bullous disease of the newborn, and was shown to be heterozygous for the p.Gly1483Asp missense mutation. In this case the frequency of blistering decreased with age and completely disappeared by the age of 4 months. Another, 16-month-old child was found to be homozygous for this mutation and had more extensive disease. Importantly, however, neither one of the latter patient's parents, who were heterozygous carriers of this mutation, had any clinical abnormalities.

Another glycine substitution mutation, p.Gly1770Ser, was found in homozygous state in 3 children with generalized skin fragility and oral erosions, consistent with severe RDEB. The parents of two of these patients with the same mutation in one allele only, showed features of nail dystrophy or pretibial DEB, suggesting that this mutation can also act as a dominant one.

Another mutation, p.Gly2213Arg, was found to be heterozygous in a 45 year-old woman with the diagnosis of EB pruriginosa, while an unrelated 25-year-old man was compound heterozygote for this mutation together with a frame shift mutation (c.4918delG), resulting in severe generalized blistering consistent with RDEB. Interestingly, however, neither of his parents, who were heterozygous carriers of the p.Gly2213Arg mutation, had any clinical abnormalities.

Finally, a 49-year-old man was shown to be heterozygous for the p.Gly2369Ser mutation associated with EB pruriginosa. However, this same mutation, when homozygous in an unrelated 6-year-old girl, conferred generalized skin fragility consistent with severe RDEB. On the other hand, her parents, both shown to be heterozygous carriers of this mutation, had no clinical abnormalities consistent with DEB.

These studies clearly suggest that some glycine substitution mutations in *COL7A1* can serve both as a dominant and recessive mutation. Considering the phenotypic manifestations of the reported individuals, it appears that, in general, these glycine substitution mutations cause a milder, dominantly inherited disease when heterozygous, while homozygous mutations or compound heterozygous mutations result in more severe phenotype. These conclusions also raise a few questions of the interpretation. For example, the patient with bullous disease of the newborn and p.Gly1483Asp mutation had clinical presentation apparently only during the first few months of life, yet adult heterozygous carriers of the same mutation were reported to be clinically unaffected. Could it be that these individuals had an earlier postnatal blistering tendency which was unreported? Could this variability in phenotype reflect incomplete penetrance due to currently unrecognized genetic, epigenetic or environmental modulatory factor? Should some of these mutations, such as p.Gly1770Ser, perhaps be called semi-dominant since apparently all individuals, whether heterozygous or homozygous for this mutation, showed some phenotypic abnormalities, which were generally more severe in homozygous individuals?

Clinical implications. The practical implications of these findings relate to the utility of *COL7A1* mutations for genetic counseling of the risk of recurrence

in families with known mutations (9). For example, should heterozygous carriers of a mutation, such as p.Gly1483Asp, which in some cases shows clinical manifestations while in others not, be counseled for being at risk for which DEB phenotype, if any? This question is also directly relevant to prenatal testing that is now in routine use for the most severe forms of EB (10, 11). These and similar questions could be answered by examination of a larger cohort of families with evidence of both dominant and recessive mode of inheritance as a result of the same mutation.

In addition to focusing on the glycine substitution mutations that may be accompanied with both dominant and recessive forms of DEB, the authors report 30 previously unreported glycine substitution mutations that apparently are associated exclusively with either dominant or recessive forms of DEB. These are important additions to the still growing *COL7A1* mutation database which serves as a valuable resource for evaluation and management of patients with dystrophic forms of EB, with implications for genetic counseling of families at risk of recurrence. In addition, to tabulating in the paper all known "mixed" glycine substitution mutations that can cause both dominant and recessive forms of DEB, in the Supplementary material to the article, the authors have listed all currently known/published glycine substitution mutations in type VII collagen, dividing these into dominant or recessive. This information provides a useful resource for all clinicians and molecular laboratories involved in classifying DEB and improving diagnostic precision for this group of mechanobullous diseases.

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