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Epidermolysis Bullosa Simplex: Recurrent and *De Novo* Mutations in the KRT5 and KRT14 Genes, Phenotype/Genotype Correlations, and Implications for Genetic Counseling and Prenatal Diagnosis

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Epidermolysis bullosa simplex (EBS) is a mechano-bullous disorder characterized by intraepidermal blistering within the basal keratinocytes as a result of trauma to the skin. As part of the DNA diagnostics program, our laboratory has analyzed a cohort of 57 patients with the initial referral diagnosis of EBS. Among these patients, 18 were found to harbor heterozygous mutations in the keratin 5 or keratin 14 genes, KRT5 and KRT14, respectively, whereas in 14 cases, the disease was associated with mutations in both alleles of the plectin gene. Among the keratin mutations, 12 were distinct and six were novel, and in most cases there was no family history of a blistering disease. Prenatal diagnosis of eight pregnancies with keratin gene mutations, at risk for EBS either because one of the parents was affected (three cases) or history of a previously affected child as a result of a *de novo* mutation (five cases), predicted two fetuses being affected and six being normal. No recurrence of the *de novo* mutations in these pregnancies was disclosed. Collectively, the data suggest that a significant number of cases diagnosed as EBS are due to plectin mutations, and many cases result from *de novo* mutations in KRT5 and KRT14 genes. These findings have implications for genetic counseling and prenatal diagnosis for EBS.

Key words: epidermolysis bullosa/bullous diseases/prenatal diagnosis
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Epidermolysis bullosa (EB), a group of heritable mechanobullous disorders, displays a spectrum of severity of skin manifestations (Fine *et al*, 1999). EB is traditionally divided into three broad categories based on the level of tissue separation within the cutaneous basement membrane zone (BMZ), as determined by diagnostic transmission electron microscopy and/or immunoepitope mapping (Fine *et al*, 2000). One of them is EB simplex (EBS), which is characterized by tissue separation within the basal layer of epidermis due to fragility of basal keratinocytes. In junctional forms of EB tissue separation takes place within the dermo-epidermal basement membrane, primarily at the level of lamina lucida, whereas in dystrophic forms the blistering occurs below the lamina densa within the upper papillary dermis at the level of anchoring fibrils (Uitto and Richard, 2004). The variability in the phenotypic presentation of EB is now known to reflect the presence of mutations in ten different genes (Pulkkinen *et al*, 2002; Uitto and Richard, 2004). The compartmentalized expression of the affected genes within the cutaneous BMZ and in extracutaneous tissues, as well as the types and positions of the mutations and their consequences at the mRNA and protein levels, explain the tremendous variability in EB phenotype (Uitto and Pulkkinen, 2001).

Abbreviations: BMZ, basement membrane zone; EBS, epidermolysis bullosa simplex

EBS is the most common subtype of EB, and epidemiological data emanating from the US National Epidermolysis Bullosa Registry have suggested that EBS accounts for at least half of all cases of EB (Fine *et al*, 1999). Furthermore, the relative incidence of EBS may be much higher than that since a proportion of affected patients, most notably those with mild blistering, do not come to the attention of physicians. Traditionally, EBS has been divided into subcategories reflecting the clinical severity and the types and distribution of the lesions. EBS Dowling–Meara, which presents with generalized herpetiform groups of vesicles or bullae, can be associated with early infant mortality (Dowling and Meara, 1954). EBS Köbner manifests with generalized blistering (Köbner, 1886), whereas EBS Weber–Cockayne presents with localized blistering primarily on the hands and feet (Cockayne, 1938). A rare subtype of EBS is associated with mottled pigmentation (Fischer and Gedde-Dahl, 1979). These four types of EBS are inherited in most cases in an autosomal-dominant fashion. An autosomal-recessive form of EB associated with late-onset muscular dystrophy (EBS-MD) has been classified as EBS because the blistering is intracellular within the basal keratinocytes. Similarly, a rare autosomal-dominant variant of EBS, the so-called Ogna type, harbors mutations in the plectin gene (Koss-Harness *et al*, 2002). It should be noted, however, that blistering in EBS-MD and EBS-Ogna is low at the level of hemidesmosomes at the apical pole of keratinocytes (see Uitto *et al*, 1996; Pfendner *et al*, 2005).

In the classic forms of EBS, the basal cells disintegrate as a result of shearing trauma to the skin, and the cell fragility is due to defective intermediate keratin filament network resulting from mutations in the basal keratin genes, KRT5 and KRT14 (Irvine and McLean, 1999; Cassidy *et al*, 2002; Rugg and Leigh, 2004). The majority of the cases show autosomal-dominant inheritance, although rare autosomal-recessive cases have been described (Hovnanian *et al*, 1993; Batta *et al*, 2000). In addition, a number of cases are sporadic with no family history of blistering diseases.

The DebRA Molecular Diagnostics Laboratory, located at Jefferson Medical College, Philadelphia, Pennsylvania, has provided DNA-based diagnostic services to the global EB community since 1996. As of today, we have analyzed over 1000 families with different forms of EB, most with severe recessive dystrophic or lethal junctional variants. Among the families studied, there were 57 individuals referred to us with the diagnosis of EBS. DNA analysis revealed specific mutations either in KRT5 or KRT14 in 18 families (see below). Careful examination of the remaining 39 cases reveals that in 16 cases, the referral diagnosis of EBS was not correct or was not consistent with electron microscopic or immunofluorescence findings, whereas 14 cases were found to harbor mutations in PLEC1 (see Pfendner *et al*, 2005). These observations reinforce the importance of good histopathological, immunofluorescence, and ultrastructural data prior to molecular genetic screening. In the remaining nine cases, the electron microscopic and/or immunohistochemical data supported the diagnosis of EBS, but no mutations in the KRT5, KRT14, or plectin genes could be identified in spite of extensive sequencing of the exons and flanking introns. In the latter cases, it is possible that mutations in these three candidate genes may exist in the regions not examined by us for mutations, including the promoter regions and intronic sequences beyond 100–200 bp away from the intron–exon junctions.

DNA was isolated from peripheral blood specimens, or in case of the prenatal testing, from chorionic villus samples, after a written informed consent was obtained from the patients or their guardians. The studies were approved by the Institutional Review Board of Thomas Jefferson University, and they adhere to the Declaration of Helsinki Principles. PCR amplification of exons and flanking intronic sequences of KRT5 and KRT14 (Whittock *et al*, 2000; Wood *et al*, 2003), followed by direct dideoxide nucleotide sequencing, of the probands' DNA resulted in identification of heterozygous keratin 5 or keratin 14 gene mutations in 18 families (Table I). Twelve distinct mutations were disclosed, six of them previously unpublished. In seven cases, the mutations resided in KRT5 and in eleven cases in KRT14, indicating that mutations in either gene can result in EBS at an approximately equal frequency. In 11 cases, DNA was available from both parents, and in only three cases the corresponding mutation was present in the peripheral blood DNA of one of the parents (Table I). In seven families in which DNA samples were not available from the parents, there was no family history of a blistering skin disease, and specifically, the parents were clinically unaffected. Based on this information, it appears that a large number of cases in this cohort (15 of 18) may represent *de novo* mutations in KRT5 or KRT14. It is likely, however, that more severe cases and in particular those who have no known family history are overrepresented in this cohort, as these cases were referred to for genetic evaluation at birth or shortly thereafter to clarify the mode of inheritance.

Examination of the mutation database indicated that, with the exception of one case (no. 16), the mutations were missense substitutions primarily within the helix initiation or termination peptides. A recurrent R125C mutation was encountered in three families, and the same arginine residue was also mutated twice to histidine (R125H). The previously reported mutation N123S was disclosed in four families. Careful examination of the clinical features, as reported by the referring physicians and summarized in Table I, revealed that the recurrent mutation N123S appears to be associated with severe generalized blistering with oral mucous membrane involvement (EBS Dowling–Meara). In one case (no. 1), the severity of the airway involvement necessitated tracheotomy. One case of EBS with palmoplantar keratoderma and mottled pigmentation harbored the P25L mutation in KRT5 (no. 13), confirming previously reported association of this particular mutation with the mottled pigmentation phenotype (Uttam *et al*, 1996; Nobuhara, 2003). Finally, a single amino acid deletion (dell183) (no. 16), a *de novo* event, resulted in generalized blistering but without scarring (EBS Köbner).

EBS has been considered to be a relatively mild disease; however, our observations clearly indicate that EBS can be severe. The more severe phenotype appears to be associated with certain mutations, such as N123S of KRT14. This mutation resides within the 1A domain of the keratin molecule and is predicted to severely perturb the intermediate filament network.

These observations clearly impact the genetic counseling of the families regarding the recurrence risk of EBS to clinically normal parents with a previously affected child with EBS. The extent of the risk for recurrence in each family is variable, but estimates from other heritable diseases suggest a range from 0.02% to 14% depending on the disorder (Byers *et al*, 1988; Bakker *et al*, 1989; Green *et al*, 1999; Mettler and Fraser, 2000). The *a priori* risk can be determined from a general formula, in which there is only one affected sibling in a family of otherwise untyped normal siblings as between 1.6% and 4.8% (van der Meulen *et al*, 1995). For genetic counseling purposes, we quote an EBS recurrence risk in a family of one affected offspring as between 2% and 5%, consistent with the calculated value. In case of EB, the risk of recurrence is real, as has been documented by *de novo* cases with dystrophic or junctional EB (Cserhalmi-Friedman *et al*, 2001, 2002).

As indicated above, the severity of EBS is highly variable, but at one end of the spectrum, the disease can result, although rarely, in premature demise of the affected individuals during the early postnatal period. In such cases, prenatal diagnosis has been requested by the parents in subsequent pregnancies (Rugg *et al*, 2000; Pfendner *et al*, 2003). We have performed DNA-based prenatal diagnosis in eight EBS families with established keratin mutations (Table II). In three cases (S1, S7, and S8), one of the parents was affected with EBS, whereas in the remaining five cases the parents were clinically normal but they had a previously affected child with a *de novo* keratin mutation. Among the