EB Simplex Superficialis Resulting from a Mutation in the Type VII Collagen Gene

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Abbreviations:

DEB, dystrophic epidermolysis bullosa; DDEB, dominant forms of DEB; EBS, epidermolysis bullosa simplex; EBSS, epidermolysis bullosa simplex superficialis

To the Editor:

Epidermolysis bullosa simplex (EBS) is an inherited blistering disease characterized by intraepidermal cleavage (Gedde-Dahl and Anton-Lamprecht, 1990;Fine *et al*, 1991). A very rare subset of EBS, termed "EBS superficialis" (EBSS), has been described in two families byFine *et al* (1989). Skin biopsy of these patients shows clefts of variable size just beneath the level of the stratum corneum, which can be completely separated from the rest of the epidermis in some cases. In two of the patients reported, there are also some clefts in the lower one-third of the epidermis.

Together with this unusual clinical picture, most of the patients show atrophic scarring, milia, nail dystrophy, and blistering involving the oral cavity. After the first description of EBSS in two unrelated families, no other cases have been reported, emphasizing the rareness of these findings.

Up to now, mutations in the genes *KRT5*, *KRT14*, and *PLEC1* have been described underlying different subsets of EBS. None of them, however, is a good candidate for the superficial lesions observed in EBSS. *KRT5* and *KRT14* are expressed in basal keratinocytes, whereas mutations in the *PLEC1* gene are associated with EBS together with muscular dystrophy (Smith *et al*, 1996). In the absence of a candidate gene for EBSS, we performed a genome-wide screen in one of the

pedigrees previously described (Fine *et al*, 1989). The second family was not included in the study, as only the proband showed evidence of blister formation, suggesting a sporadic mutation. Briefly, the affected individuals studied here belong to a five-generation pedigree with an autosomal dominant pattern of inheritance (**Figure 1a**). In affected family members, variably sized clefts were noted just beneath the level of the stratum corneum in each biopsy specimen (Fine *et al*, 1989). In some, clefts were subcorneal; in others, lower intraepidermal. In none of the affected individuals was sub-lamina densa cleavage noted, nor was any diminution of type VII collagen staining noted using the anti-type VII antibody LH 7:2 (Fine *et al*, 1989).

A genome-wide screen was performed using a panel of 324 microsatellite markers, with an average marker spacing of 10 cM and a semiautomated fluorescence-based genotyping system (Aita *et al*, 1999). Two-point linkage analyses were carried out using the MLINK program of the FASTLINK suite of programs (Lathrop *et al*, 1984;Cottingham *et al*, 1993;Schaffer *et al*, 1994). A disease allele frequency of 0.001 and an autosomal dominant mode of inheritance with complete penetrance were assumed. The marker allele frequencies were estimated from observed and reconstructed genotypes of founders within the pedigree. To avoid computation errors due to observed allele frequencies of 0.0, alleles for all markers were re-coded using the RECODE program (Weeks, 2000). Multipoint analyses and reconstruction of pedigrees were carried out using the SIMWALK program version 2.6 (Sobel and Lange, 1996).

The results of the initial genome-wide scan revealed three chromosomal regions with a maximum two-point LOD score greater than 1.4, on chromosomes 3 (Z_{max}= 1.62), 8 (Z_{max}= 1.80), and 10 (Z_{max}= 1.40). Haplotype and multipoint analyses of additional markers allowed us to exclude the regions on chromosomes 8 and 10, and to more decisively establish the linkage to chromosome 3.

A total of 28 additional markers were used for the fine-mapping of the EBSS locus. Maximum two-point LOD scores of 4.11 and 3.77 at $\theta = 0.0$ were obtained for markers D3S2420 and D3S3582, respectively. Multipoint linkage analysis showed a maximum LOD score of 5.96 for marker D3S2420 (**Figure 1b**). Finally, analysis of the reconstructed haplotypes confirmed the linkage results and placed the disease locus within a 2.94 cM region on chromosome 3, flanked by markers D3S3624 and D3S1289 (**Figure 1a**).

According to the different maps derived from the Human Genome Project (Human Genome Project Working Draft;National Center for Biotechnology Information;Ensembl Genome Server;GeneMap'99), the region flanked by markers D3S3624 and D3S1289 spans approximately 10 Mb of genomic DNA on 3p21. Coincidentally, the *COL7A1* gene, in which mutations are responsible for DEB (Uitto *et al*, 1999;Fine *et al* 2000), lies within this genetic interval. Type VII

collagen is the main constituent of the anchoring fibrils and at the microscopic level, DEB is characterized by skin cleavage beneath the lamina densa. The morphologic defect is a reduced number or complete absence of the anchoring fibrils, in the dominant and recessive forms of DEB, respectively (Tidman and Eady, 1985). In DEB skin, blisters appear just beneath an intact lamina densa. In contrast, the family we studied and reported by Fine *et al*(1989) showed blister formation just beneath the stratum corneum. On the basis of the differences in phenotype between these two subtypes of EB, the *COL7A1*gene was not considered to be a candidate gene for EBSS. Nevertheless, to unequivocally rule out this possibility, we performed heteroduplex analysis and direct sequencing of the coding region.

Quite unexpectedly, we identified a heterozygous transition in exon 73, 6100G→A, leading to the amino acid change G2034R (**Table I**). Exon 73 codes for a 67-amino-acid collagenous polypeptide sequence preceded by the 39-amino-acid noncollagenous segment (Christiano *et al*, 1994a). The substitution of a glycine residue within the collagenous domain of the molecule, characterized by the repeating Gly-X-Y sequence, is the major class of pathogenetic mutations in the dominant forms of DEB (DDEB) (Christiano *et al*, 1994b,1995;Burgeson *et al*, 1995). Moreover, mutations involving the glycine residue at position 2034 have been previously reported (Kon *et al*, 1997;Hammami-Hauasli *et al*, 1998;Rouan *et al*, 1998;Mecklenbeck *et al*, 1999) (**Table I**). The very same amino acid substitution has been described in three families with different forms of DDEB: the "Cockayne-Touraine" variant of DDEB (Kon *et al*, 1997), DDEB "generalisata" (Hammami-Hauasli *et al*, 1998;Mecklenbeck *et al*, 1999), and an unspecified subtype of DEB (Mecklenbeck *et al*, 1999). In addition, a different amino acid substitution affecting the same residue, G2034W, has also been reported.Rouan *et al* (1998) identified this second amino acid change in a family with a mild form of DDEB, mainly involving the hands, feet, and mouth. In another study,Mecklenbeck *et al* (1999) described the same mutation, G2034W, in two families with the so-called DDEB "localisata" (**Table I**). In these families, a clear DDEB phenotype has been reported, and these types of glycine substitution mutations are the most prevalent in DDEB.

In light of our results, we believe that the clinical phenotype in the EBSS kindred studied here actually represents a case of DDEB, rather than a unique subset of EBS. The molecular data suggest that the subcorneal cleavage observed in different members of this kindred would likely not be pathogenic or contribute to the disease process. As the proband from the second family reported in the original work (Fine *et al*, 1989) was not available for this study, these findings do not fully exclude the possibility that rare forms of EBS having superficial skin cleavage may also exist. Although it is true that the family studied here does indeed have several clinical findings that are commonly associated with DEB, data from the National EB Registry have also shown that at least

10%-25% of all EBS patients have one or more of these "dystrophic" features as well, making the diagnosis based on clinical phenotypes sometimes imprecise. Genetic studies such as the one presented here become an invaluable tool to clarify the true molecular basis of a disease like EBSS, where the clinical features cannot be used to unequivocally classify a particular phenotype. Collectively, these findings allow us to reclassify a previously uncharacterized form of EB as another clinical variant of DDEB.

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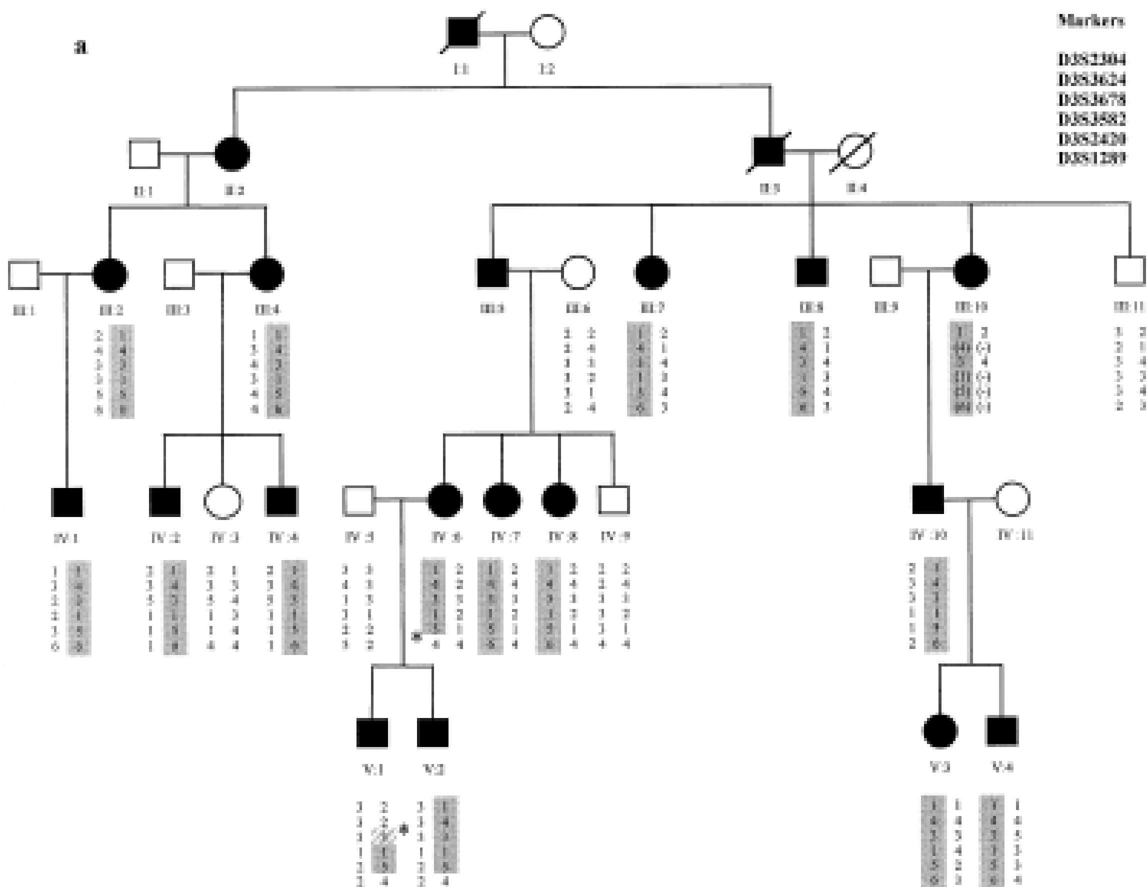
Acknowledgments

We thank the family for their participation in this study and Dr. Peter Cserhalmi-Friedman, Columbia University, for helpful comments and discussion. The authors gratefully acknowledge NIH USPHS grants R01 AR43602, R01 AR44924, and K02 AR02047 (to A.M.C.), K01-HG00055-01 (to D.G.), and HG00008 (to J.O.), the National Epidermolysis Bullosa Registry (to M.S.W. and J.D.F.), the Skin Disease Research Center of Columbia University P30 AR44345 (to J.O. and A.M.C.), and the Dermatology Foundation.

Figures



b

Lod Score

Map Position (cM)

Haplotype and multipoint analysis in the pedigree with EBSS, (a) Pedigree with EBSS and haplotypes for microsatellite markers on chromosome 3. The disease-associated haplotype is shaded. Asterisks indicate the key recombination events. Non-informative marker in cross-hatched. Genotypes in parenthesis have been inferred; (b) multipoint LOD scores for family with EBSS. Markers used are listed on the X-axis. The map positions were computed by SIMWALK 2.6. The

order and distance between the markers were deduced from the genetic map a the Center for Medical Genetics, Marshfield Medical Researcho Foundation (Broman et al, 1998).

Tables

Mutation	**Type of EB**	**Inheritance**	**Reference**
G2034R	EBSS	AD	This study
	DEB-CT[a]	AD	Kon *et al* (1997)
	DEB-generalisata	AD	Hammami-Hauasli *et al* (1998);Mecklenbeck *et al* (1999)
	DEB[b]	AD	Mecklenbeck *et al* (1999)
G2034W	DEB-mild	AD	Rouan *et al* (1998)
	DEB-localisata	AD	Mecklenbeck *et al* (1999)
	DEB-localisata	AD	Mecklenbeck *et al* (1999)

[a] DDEB-CT: Cockayne-Touraine variant of DDEB.
[b] The subtype of EB has not been specified.