Plectin Gene Mutations Can Cause Epidermolysis Bullosa with Pyloric Atresia

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Epidermolysis bullosa with pyloric atresia (EB-PA), manifesting with neonatal blistering and gastric anomalies, is known to be caused by mutations in the hemidesmosomal genes *ITGA6* and *ITGB4*, which encode the α6 and β4 integrin polypeptides, respectively. As part of our molecular diagnostics program, we have now encountered four families with EB-PA in which no mutations could be identified in these two genes. Instead, PCR amplification followed by heteroduplex scanning and/or direct nucleotide sequencing revealed homozygous mutations in the plectin gene (*PLEC1*), encoding another hemidesmosomal protein previously linked to EB with muscular dystrophy. Our findings provide evidence for additional molecular heterogeneity in EB, and emphasize the importance of screening EB-PA patients not only for α6β4 integrin but also for plectin deficiency.

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Epidermolysis bullosa (EB) is a phenotypically diverse group of heritable mechanobullous disorders characterized by blistering and erosions of the skin and mucous membranes (Fine *et al*, 2000). Ten different genes expressed within the cutaneous basement membrane zone are now known to harbor mutations that underlie different forms of EB (Pulkkinen and Uitto, 1999; Uitto and Richard, 2004). Adding to the phenotypic complexity of EB is the fact that several well-characterized variants are associated with extracutaneous manifestations with considerable morbidity and mortality (Uitto *et al*, 1997; Fine *et al*, 2000). One of these variants, EB with pyloric atresia (EB-PA; OMIM #226730), manifests with neonatal blistering associated with PA, a combination that can lead to early postnatal demise of the affected individuals. EB-PA has been shown to result in most families from mutations in the genes encoding the subunit polypeptides of α6β4 integrin, *ITGA6* and *ITGB4*, respectively (Pulkkinen and Uitto, 1998; Pulkkinen *et al*, 1998). Another variant, EB with muscular dystrophy (EB-MD; OMIM #226670), is characterized by neonatal blistering accompanied by proximal muscle weakness that can develop during childhood (early onset) or in the third or fourth decade of life (late onset). EB-MD is caused by mutations in the gene encoding plectin, *PLEC1* (GeneBank U53204), which is expressed not only in the hemidesmosomes but also in the sarcolemma and the Z-lines of the skeletal muscle (Uitto *et al*, 1996).

As part of the diagnostic services to the global EB patient community provided by the DebRA Molecular Diagnostics Laboratory, which was established at Jefferson Medical College in 1996, we have analyzed approximately 1000 families with different forms of EB, including 35 families with EB-PA. A total of 56 distinct mutations in the EB-PA families have been identified in the *ITGB4* gene (see Pulkkinen *et al*, 1998; Nakano *et al*, 2001) and four of them in the *ITGA6* gene (Pulkkinen *et al*, 1997; Ruzzi *et al*, 1997). In this report, we describe four cases with EB and PA and neonatal lethality in which analysis of the *ITGB4* and *ITGA6* genes, including direct sequencing of exons and flanking intronic sequences, yielded no pathogenetic mutations. Subsequent mutation analysis of *PLEC1*, however, identified homozygous mutations in each case.

The proband in each family was a newborn with clinical findings of blistering and PA, and they died from complications of the disease shortly after birth. Information on the families as well as clinical and diagnostic features of the proband are included in Fig 1 and Table I. These studies were approved by the Institutional Review Board of Thomas Jefferson University, and they adhere to Declaration of Helsinki principles. A written informed consent was obtained from the patients or their guardians. PCR amplification of 33 exons of *PLEC1*, followed by heteroduplex scanning and/or direct dideoxynucleotide sequencing of the probands' and/or parents' DNA resulted in identification of homozygous mutations in each family (Fig 2). The parents were found to be heterozygous carriers of the corresponding mutations, consistent with consanguinity in each family (see Fig 1). Two of the mutations, Q305X and Q3029X (cases 2 and 3, respectively), were nonsense mutations resulting from C-to-T transitions and reflecting hypermutability of putative 5-methylcytosine within exons 10 and 33, respectively. One of the mutations (case 1) was an out-of-frame deletion, 1563del4, predicting a premature stop-codon 30 bp downstream from the site of deletion within exon 15. Finally,

Abbreviations: EB, epidermolysis bullosa; EB-MD, EB with muscular dystrophy; EB-PA, EB with pyloric atresia

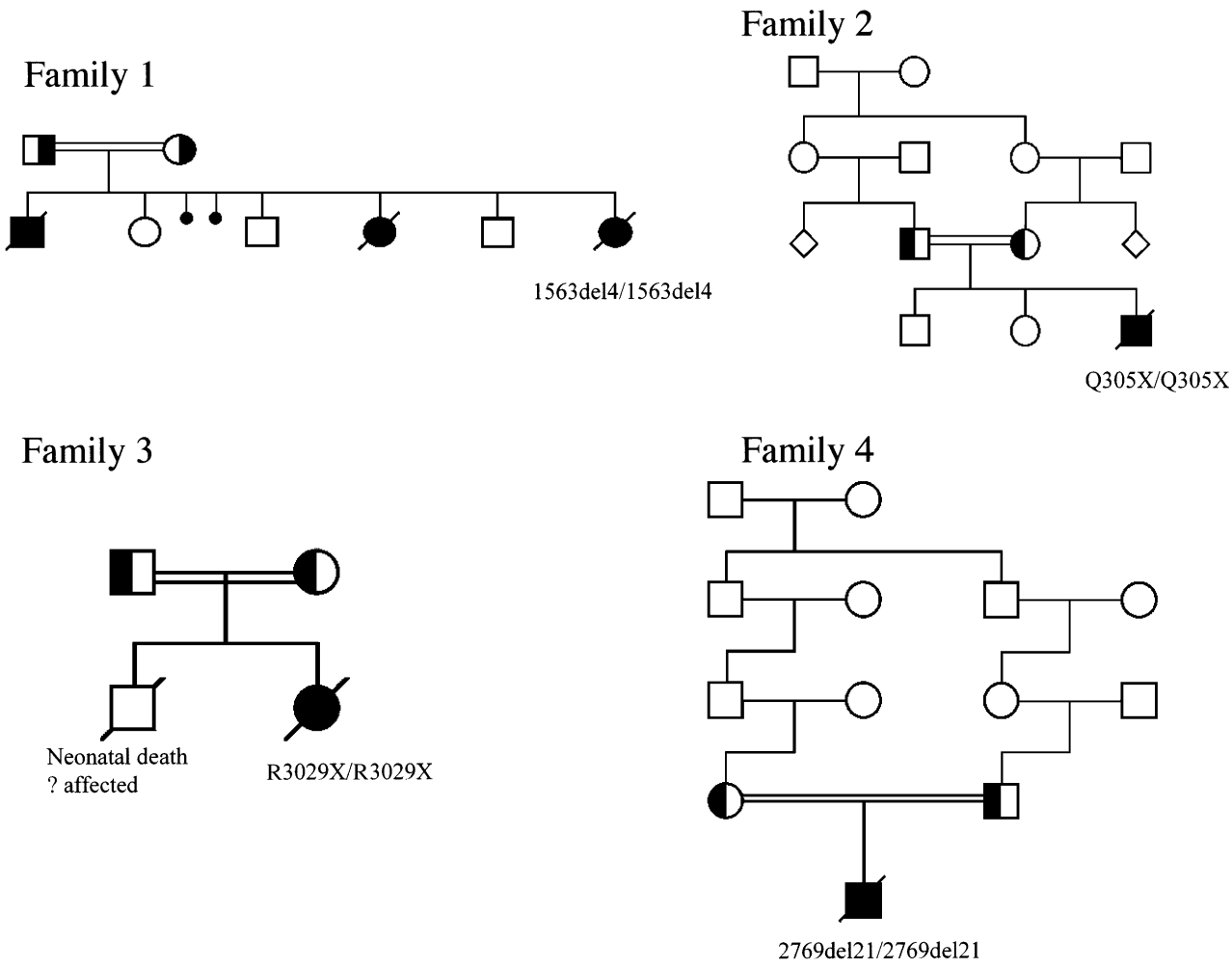

Figure 1
Nuclear pedigrees of the families with epidermolysis bullosa with pyloric atresia (EB-PA). Solid symbols denote children who died from complications of the disease shortly after birth. Note consanguinity in each family.

Table I. Plectin gene mutations in families with epidermolysis bullosa with pyloric atresia (EB-PA)

Family no.	Ethnic origin	Consanguinity (parents)	Clinical features of the proband	Diagnostic skin[a] pathology	Mutations[b] maternal/paternal
1	Pakistani	Distant cousins	Blistering and PA at birth; two similarly affected older siblings	EM: low basal cell cytolysis; attenuation of anchoring filaments; rudimentary hemidesmosomes	1563del4/1563del4 (1614del4/1614del4)
2	Lebanese	First cousins	Extensive blistering; aplasia cutis of abdomen and legs; ear abnormalities	IF: laminin 5, uncein, and type VII collagen expressed at the base of an intra-epidermal cleft	Q305X/Q305X
3	Saudi Arabian	First cousins	Blistering and PA at birth; another sibling with neonatal demise	IF: $\alpha 6\beta 4$ staining normal	R3029X/R3029X
4	Caucasian	Second cousins	Extensive blistering and aplasia cutis at birth; poly-hydramnion	EM: lamina lucida cleavage; hypoplastic hemidesmosomes; IF: collagen XVII/BPAG2 staining negative; collagen VII staining normal	2769del21/2769del21 (2820del21/2829del21)

[a]EM, electron microscopy; IF, immunofluorescence; Please note that immunofluorescence for plectin was not done in any of the cases.
[b]The mutations in the plectin gene (*PLEC1*; GeneBank U53204) refer to nucleotide positions of the gene counting the translation initiation codon ATG as 1–3, as published by McLean *et al* (1996); the numbers in parentheses refer to positions of the corresponding nucleotides counting the beginning of the published gene sequence (−51) as 1.