JCI The Journal of Clinical Investigation

A homozygous mutation in the integrin alpha6 gene in junctional epidermolysis bullosa with pyloric atresia.

L Ruzzi, L Gagnoux-Palacios, M Pinola, S Belli, G Meneguzzi, M D'Alessio, G Zambruno

J Clin Invest. 1997;99(12):2826-2831. https://doi.org/10.1172/JCI119474.

Research Article

The alpha6 integrin subunit participates in the formation of both alpha6beta1 and alpha6beta4 laminin receptors, which have been reported to play an important role in cell adhesion and migration and in morphogenesis. In squamous epithelia, the alpha6beta4 heterodimer is the crucial component for the assembly and stability of hemidesmosomes. These anchoring structures are ultrastructurally abnormal in patients affected with junctional epidermolysis bullosa with pyloric atresia (PA-JEB), a recessively inherited blistering disease of skin and mucosae characterized by an altered immunoreactivity with antibodies specific to integrin alpha6beta4. In this report, we describe the first mutation in the alpha6 integrin gene in a PA-JEB patient presenting with generalized skin blistering, aplasia cutis, and defective expression of integrin alpha6beta4. The mutation (791delC) is a homozygous deletion of a single base (C) leading to a frameshift and a premature termination codon that results in a complete absence of alpha6 polypeptide. We also describe the DNA-based prenatal exclusion of the disease in this family at risk for recurrence of PA-JEB. Our results demonstrate that, despite the widespread distribution of the alpha6 integrin subunit, lack of expression of the alpha6 integrin chain is compatible with fetal development, and results in a phenotype indistinguishable from that caused by mutations in the beta4 chain, which is expressed in a more limited number of tissues.

A Homozygous Mutation in the Integrin α6 Gene in Junctional Epidermolysis Bullosa with Pyloric Atresia

Laura Ruzzi,* Laurent Gagnoux-Palacios,‡ Mari Pinola,* Serena Belli,§ Guerrino Meneguzzi,‡ Marina D'Alessio,* and Giovanna Zambruno*

**Laboratory of Molecular and Cell Biology, Istituto Dermopatico dell'Immacolata, IRCCS, 00167 Rome, Italy; ‡U385 INSERM, Faculté de Médicine, 06107 Nice Cedex 2, France; and §U.O. Neonatologia, Ospedale S. Chiara, 38100 Trento, Italy*

Abstract

The α6 integrin subunit participates in the formation of both α6β1 and α6β4 laminin receptors, which have been reported to play an important role in cell adhesion and migration and in morphogenesis. In squamous epithelia, the α6β4 heterodimer is the crucial component for the assembly and stability of hemidesmosomes. These anchoring structures are ultrastructurally abnormal in patients affected with junctional epidermolysis bullosa with pyloric atresia (PA-JEB), a recessively inherited blistering disease of skin and mucosae characterized by an altered immunoreactivity with antibodies specific to integrin α6β4. In this report, we describe the first mutation in the α6 integrin gene in a PA-JEB patient presenting with generalized skin blistering, aplasia cutis, and defective expression of integrin α6β4. The mutation (791delC) is a homozygous deletion of a single base (C) leading to a frameshift and a premature termination codon that results in a complete absence of α6 polypeptide. We also describe the DNA-based prenatal exclusion of the disease in this family at risk for recurrence of PA-JEB. Our results demonstrate that, despite the widespread distribution of the α6 integrin subunit, lack of expression of the α6 integrin chain is compatible with fetal development, and results in a phenotype indistinguishable from that caused by mutations in the β4 chain, which is expressed in a more limited number of tissues. (*J. Clin. Invest.* 1997. 99:2826–2831.) Key words: inherited epidermolysis bullosa • keratinocyte • hemidesmosome • integrin α6 gene • mutation

Introduction

Integrins are α/β heterodimeric transmembrane receptors that mediate cell–cell and cell–matrix interactions, and regulate cell growth, differentiation, and death through signal transduction processes (1, 2). The α6 integrin subunit associates with either β1 or β4 chains to form the laminin receptors α6β1 and α6β4 (1–4). The α6β1 heterodimer is expressed on platelets, lymphocytes, epithelial cells, and a variety of other cell types, and is involved in morphogenetic processes such as kidney tubule formation (5–8). The α6β4 complex is found predominantly in epithelia, and is the only integrin component of hemidesmosomes (9–11), the dense cytoplasmic devices that anchor basal cells of stratified and pseudostratified epithelia to the underlying basement membrane. Hemidesmosomes connect the cytoskeleton keratin intermediate filaments to the lamina densa of the basement membrane through anchoring filaments, thread-like structures which span the lamina lucida (12). The α6β4 integrin binds with high affinity to laminin 5, a putative component of anchoring filaments, and the major adhesion ligand of epithelial cells (13).

Defective expression of the α6β4 heterodimer has been described in a variant of junctional epidermolysis bullosa (JEB)[1] associated with congenital gastric outlet obstruction, pyloric atresia JEB, (PA-JEB) (MIM 226730) (14–16). These immunohistochemical findings have been supported by the identification of two distinct mutations in the β4 integrin gene (*ITGB4*, on chromosome 17q11-qter) in a patient affected with PA-JEB (17). JEB is a clinically and genetically heterogeneous group of autosomal recessively inherited blistering disorders of the skin and mucous membranes, characterized by mesenchymal–epithelial separation within the lamina lucida of the basement membrane zone (18). Typical ultrastructural findings in JEB are abnormalities in the hemidesmosomes. Mutations in genes encoding for laminin 5 and the transmembrane hemidesmosomal component collagen XVII, also known as the 180-kD bullous pemphigoid antigen (BPAG2), have been identified in different forms of JEB (19–21).

In this report, we describe the first mutation in the α6 integrin gene (*ITGA6*, on chromosome 2q) in a patient with PA-JEB. We also describe the DNA-based prenatal exclusion of PA-JEB using allele-specific probes. Our results indicate that, despite the widespread distribution of the α6 integrin subunit, lack of expression of the α6 integrin chain is compatible with in utero development, and results in a phenotype indistinguishable from that caused by mutations in the β4 chain.

Address correspondence to G. Zambruno, Laboratory of Molecular and Cell Biology, Istituto Dermopatico dell'Immacolata, Via Monti di Creta, 104, 00167 Rome, Italy. Phone: 39-6-66464738; FAX: 39-6-66464705; E-mail: zambruno@idi.it

Received for publication 17 January 1997 and accepted in revised form 20 March 1997.

J. Clin. Invest.

0021-9738/97/06/2826/06 $2.00
Volume 99, Number 12, June 1997, 2826–2831

1. *Abbreviations used in this paper:* ASO, allele-specific oligonucleotide; BPAG1, 230-kD bullous pemphigoid antigen; BPAG2, 180-kD bullous pemphigoid antigen; DEJ, dermal-epidermal junction; JEB, junctional epidermolysis bullosa; PA-JEB, junctional epidermolysis bullosa with pyloric atresia.

Methods

Immunofluorescence analysis. Frozen 5-μm-thick skin sections obtained from skin biopsies of the proband, of the fetus, of six patients affected with other JEB variants, and of healthy volunteers, were processed for immunofluorescence using a three-step biotin-streptavidin-fluorescein procedure, as described (22). The following mAb and polyclonal antisera were used: G0H3 (rat mAb to the extracellular domain of the α6 integrin subunit, gift from A. Sonnenberg, the Netherland Cancer Institute, Amsterdam, The Netherlands) (23), 450-30A1 (mouse mAb to α6 subunit; obtained from the Fifth Workshop and Conference on Human Leukocyte Differentiation Antigens) (24), 3E1 (mouse mAb to β4; Telios Pharmaceuticals, San Diego, CA), 450-11A1 (mouse mAb against the cytoplasmic domain of β4 subunit; obtained from the Fifth Workshop and Conference on Human Leukocyte Differentiation Antigens) (24), GB3 (mouse mAb to laminin 5; from J.P. Octonne, U385 INSERM, Nice, France) (25), FP1 (rabbit antiserum to 230-kD bullous pemphigoid antigen [BPAG1], gift from J. Stanley, University of Pennsylvania, Philadelphia PA) (26), 1A8C (mouse mAb to cytoplasmic domain of BPAG2; gift from K. Owaribe, Nagoya University, Nagoya, Japan) (27), J143 (mouse mAb to α3 integrin subunit; gift from L.J. Old, Ludwig Institute for Cancer Research, New York) (28), K20 (mouse mAb to β1 integrin subunit; Immunotech International, Marseille, France), LH7:2 (mouse mAb to type VII collagen; Cymbus Bioscience, Southampton, United Kingdom). The rabbit antiserum to type IV collagen was purchased from Institut Pasteur (Lyon, France). The following secondary sera and detection systems were employed: biotinylated horse anti–mouse IgG serum (Vector Laboratories, Burlingame, CA), biotinylated rabbit anti–rat IgG serum (Vector), biotinylated goat anti–rabbit IgG serum (Vector), and fluorescein-streptavidin (Amersham International, Little Chalfont, United Kingdom).

Electron microscopy. Skin specimens were fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, embedded in Epon resin, and then sectioned on an ultramicrotome (Reichert Ultracut E; Leica, Wien, Austria). Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a transmission electron microscope (CM100; Philips, Eindhoven, The Netherlands).

Keratinocyte cultures. Human epidermal keratinocytes were obtained from skin biopsies of the proband parents and healthy volunteers, and were cultivated on a feeder layer of lethally irradiated 3T3-J2 murine fibroblasts (a gift from H. Green, Harvard Medical School, Boston, MA), as described (29).

Northern analysis. Total RNA from primary cultures of epidermal keratinocytes was prepared in guanidine isothiocyanate, as described (30). For each sample, 20 μg of total RNA was separated by electrophoresis through a 1% agarose/formaldehyde gel, and transferred to Hybond-N^+ nylon membrane in 20× SSPE, as described by the supplier (Amersham International). Membranes were hybridized with ^{32}P-labeled probes phβ4 (31) and phα6 (4) to detect the mRNAs for the β4 and α6 integrin subunits, respectively. For loading control, membranes were hybridized with a probe corresponding to the ubiquitously expressed gene glyceraldehyde-3-phosphate dehydrogenase.

Mutation detection. Total RNA was prepared from 3-mm skin biopsies of the PA-JEB patient and healthy controls following the manufacturer's recommendations (Micro-Scale total RNA Separator Kit; Clontech Laboratories, Palo Alto, CA) and was reverse-transcribed in a volume of 20 μl (SuperScript RNAse H free reverse transcriptase; Life Technologies Italia, San Giuliano Milanese, Milan, Italy). The cDNA obtained was used in PCR reactions with specific primers producing nine overlapping fragments (∼ 500 bp) covering the entire open reading frame of the α6 cDNA. The product of each PCR reaction, analyzed by agarose gel, was subcloned into the PCR-TM II plasmid vector (Invitrogen, NV Leek, The Netherlands), following the manufacturer's instructions, and sequenced with the dideoxy termination method. The oligonucleotide primers used to identify the mutation 791delC were: (L) 5′ TGTTATGTCCTGAGTCAGAA 3′ and (R) 5′ TCTCTCTTCAGCAAAACCAC 3′, which amplify nucleotides (nts) 442-866 of the α6 integrin cDNA sequence. PCR cycling conditions were: 94°C for 5 min, followed by 94°C for 45 s; 58°C for 45 s; 72°C for 45 s (35 cycles), and extension at 72°C for 10 min.

Mutation verification. The mutation detected in the proband's integrin α6 cDNA was verified at the genomic level. Genomic DNA was extracted from peripheral blood by standard procedures. The forward primer, 5′ GTTTTTCTTTGGACTCAGGG 3′, and the reverse primer, 5′ CTCCACTGTGATTGGCTCT 3′, were synthesized and used in a 25-μl PCR reaction to amplify from 100 ng of total genomic DNA a 87-bp fragment extending from cDNA nts 758 to nts 844. PCR conditions were: 95°C for 7 min, followed by 94°C for 1 min, 56°C for 1 min, 72°C for 2 min (35 cycles), and extension at 72°C for 10 min. The amplified product was subcloned into the PCR-TM II plasmid vector. 10 independent clones were sequenced on both DNA strands using universal primers. Allele-specific oligonucleotide (ASO) analysis was carried out to confirm the homozygous state of the mutation in the proband, and to assess the inheritance in the kindred. Genomic DNA from each family member was amplified to produce the 87-bp PCR fragment as described above. 10 μl of the PCR reaction was transferred onto a Hybond-N^+ membrane using a dot blot apparatus. The membranes were prehybridized in a solution of 5× Denhardt's, 6× SSC, 0.1% SDS for 10 min at 65°C, and then hybridized for 2 h at room temperature with either the ^{32}P-labeled wild-type ASO with the sequence 5′ GTATTGTTTCTAAAGATGAG 3′, or the mutated ASO 5′ GGTATTGTTTTAAAGATGAG 3′. Filters were washed twice in 6× SSC, 0.1% SDS for 5 min at room temperature, and for 15 min at the T_m of the oligonucleotides (52°C), and were exposed for autoradiography.

Results

The proband was born from clinically normal Moroccan parents who denied consanguinity and referred to a previous, stillborn infant with extensive skin defects. At birth, the patient presented cutaneous aplasia of both legs, feet, hands, and both

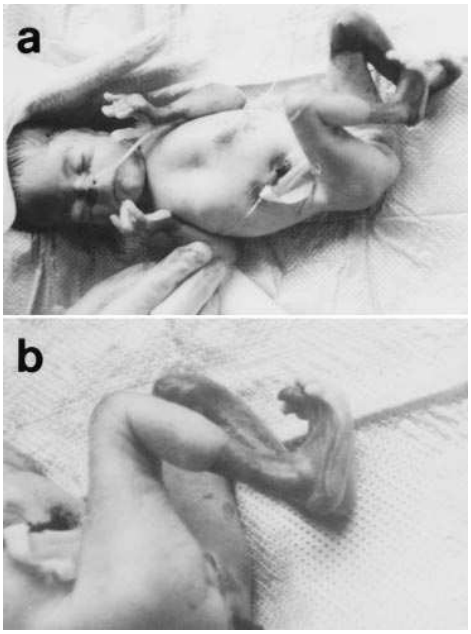

Figure 1. Clinical appearance of the newborn with junctional epidermolysis bullosa with pyloric atresia. (*a*) Blisters and erosions are present over the abdomen and the right knee. Extensive cutaneous aplasia affects the legs, feet and hands. (*b*) Closer view of the legs showing well-demarcated, depressed areas of aplasia cutis.

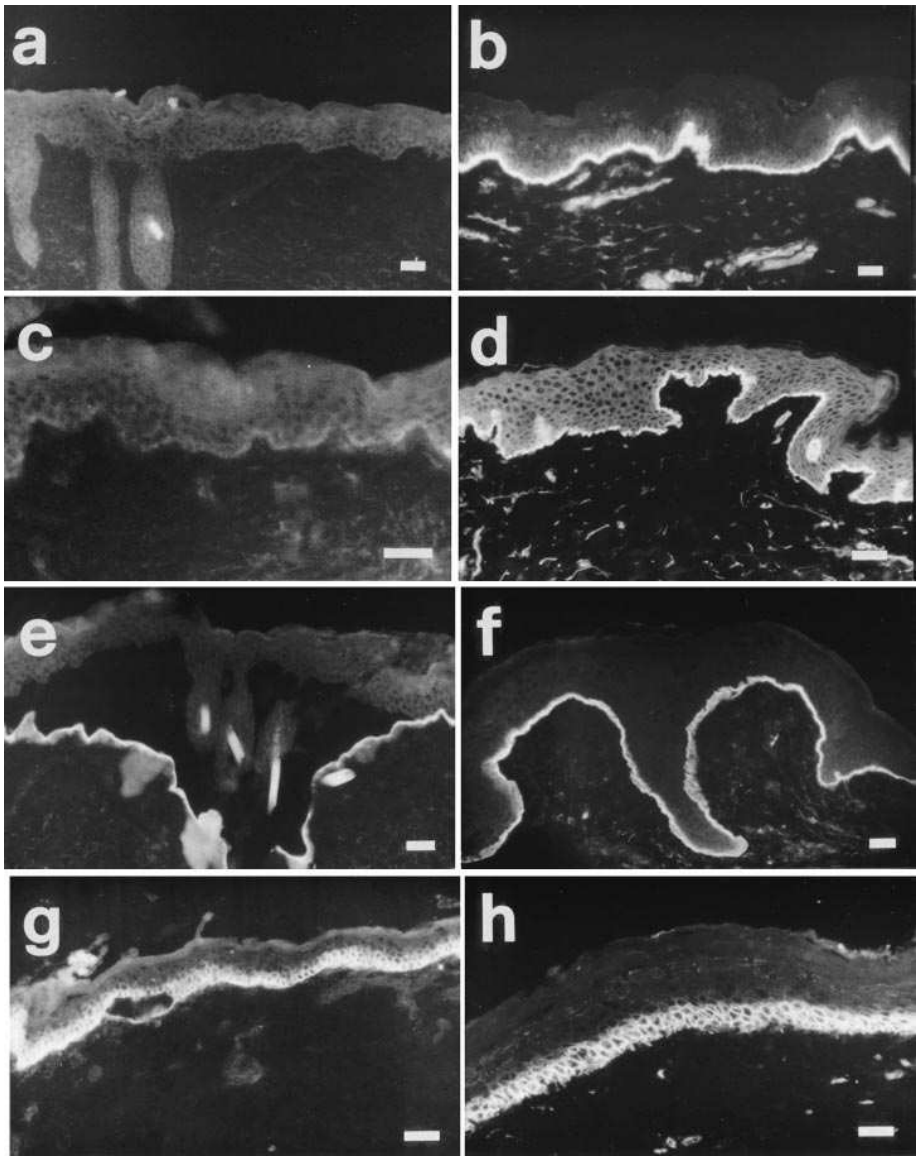

Figure 2. Immunofluorescence microscopy of the DEJ. Labeling of the PA-JEB skin with mAb G0H3 to α6 integrin subunit (*a*) is completely negative, while normal skin (*b*) shows bright linear staining at the DEJ. Labeling of PA-JEB skin with mAb 3E1 against the β4 integrin chain (*c*) also appears strongly reduced compared to normal skin (*d*). In contrast, the labeling of the DEJ with mAb GB3 against laminin 5 is similar in normal (*f*) and PA-JEB skin (*e*), where it is localized at the blister floor. J143 mAb against the α3 integrin subunit gives the expected staining of lateral and apical sides of basal keratinocytes in the skin of a patient affected from the generalized atrophic benign form of JEB (21) (*h*), but it also labels the basal pole of basal keratinocytes in PA-JEB skin (*g*). Bar, 20 μm.

sides of the scalp, as well as several blisters and erosions distributed over the trunk and extremities (Fig. 1). The pinnae of both ears were small and malformed. The infant was also affected with pyloric atresia and severe esophageal stenosis. Eruption of new cutaneous and mucosal blisters and multisystem failure led to death 23 d after birth.

Immunofluorescence analysis of perilesional skin with mAb G0H3 and 450-30A1 against the α6 integrin subunit revealed a complete absence of reactivity, whereas normal control skin showed the expected linear labeling of the dermal-epidermal junction (DEJ) (Fig. 2, *a* and *b*). With mAb 3E1 and 450-11A1 against β4 integrin, a strongly reduced staining of the DEJ was observed (Fig. 2, *c* and *d*). In contrast, antibodies recognizing type VII collagen, laminin 5 (Fig. 2, *e* and *f*), and the hemidesmosomal components BPAG1 (230-kD bullous pemphigoid antigen) and BPAG2 presented a labeling pattern comparable to controls. Interestingly, the α3 and β1 integrin subunits, detected on the lateral sides of basal keratinocytes in normal skin and other JEB variants, were also expressed along the basal pole of basal keratinocytes in PA-JEB skin (Fig. 2, *g* and *h*).

Ultrastructural examination of PA-JEB skin showed the cleavage plane localized within the lamina lucida of the basement membrane (Fig. 3 *a*) and revealed the presence of hemidesmosomes. The hemidesmosomes were reduced in number and did not contain a sub-basal dense plate. In most of them, the inner cytoplasmic plaque was also markedly reduced or even absent, although a limited number of keratin filaments inserting on cytoplasmic electron-dense plaques were detected regularly (Fig. 3 *b*).

Given the paucity of the biopsy material available from the proband, we could not examine the level of expression of α6 and β4 mRNA in PA-JEB skin. We therefore performed Northern blot analysis of total RNA from primary cultures of epidermal keratinocytes obtained from the proband's parents who were expected to be healthy carriers of the genetic defect. A reduced expression of α6 mRNA was found in both parents, whereas the signal intensity obtained with a specific β4 probe was comparable to controls (not shown). Thus, we concluded that *ITGA6* was the candidate gene in this PA-JEB patient, and initiated the search for mutations.

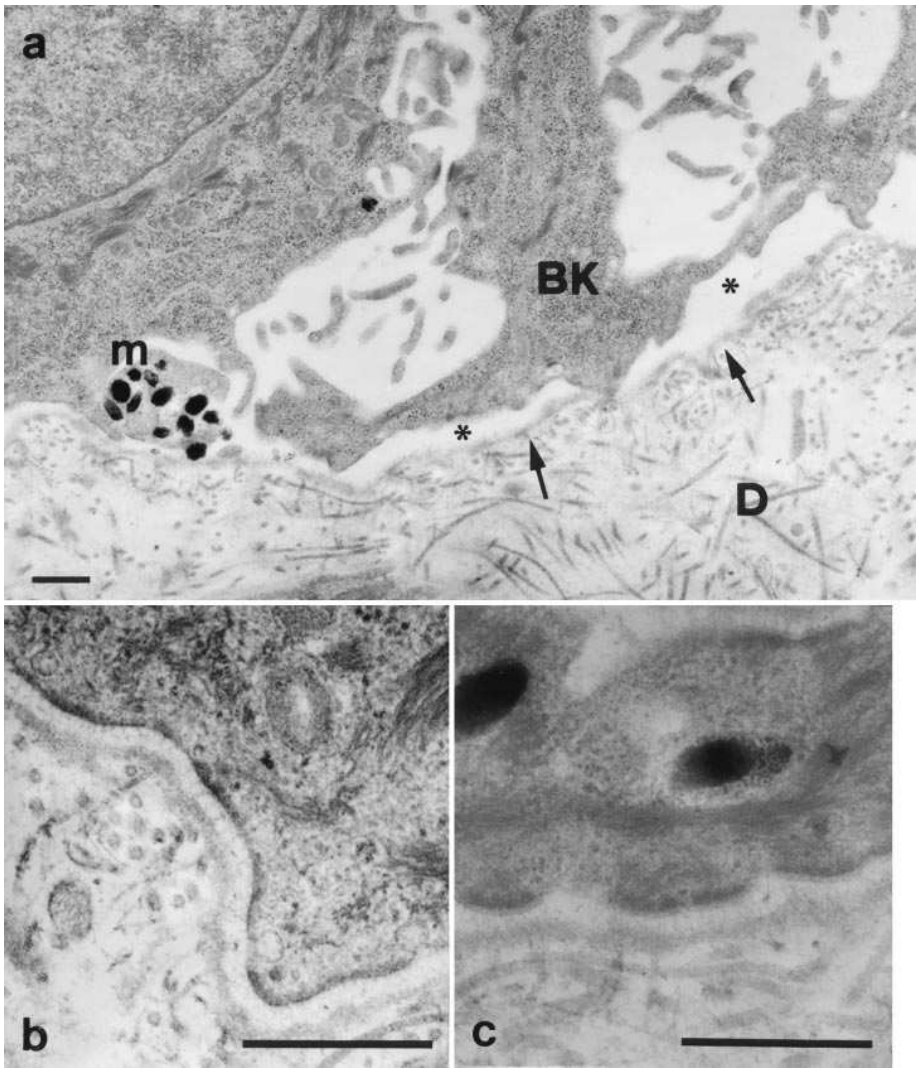

Figure 3. Electron microscopy of PA-JEB skin. (*a*) In an initial cleavage area, the blister formation at the DEJ occurs within the lamina lucida (*star*), and the electron-dense lamina densa forms the blister floor (*arrow*) (*BK*, basal keratinocytes; *D*, dermis; *m*, melanosomes). Higher magnification of the DEJ of uninvolved PA-JEB skin (*b*) reveals the presence of rudimentary hemidesmosomes lacking the sub-basal dense plaque. The cytoplasmic plaque of hemidesmosomes with insertion of keratin filaments is clearly detectable, although markedly reduced compared to normal skin (*c*). Bar, 0.5 μm.

Overlapping cDNA fragments spanning the entire open reading frame for the α6 polypeptide were obtained by RT-PCR amplification of total RNAs from the proband and healthy control skin biopsies using nine pairs of oligonucleotides. Sequence analysis of a 5′ PCR product (nt 442–866) showed a deletion of a single base (C) at position 791, leading to a frameshift and a premature termination codon (TGA) 66 bp downstream from the deletion site. This mutation, designated 791delC, predicts a truncated α6 polypeptide terminating at residue 268. Sequencing of the corresponding region of the proband genomic DNA demonstrated the presence of the C deletion in all the clones analyzed (Fig. 4 *a*), suggesting a homozygous mutation. ASO analysis of genomic DNA confirmed the homozygous state of the mutation in the proband and showed both parents to be heterozygous healthy carriers of this mutation (Fig. 4 *b*). Screening by the same technique of genomic DNA from 50 unrelated healthy subjects and from eight patients with different forms of JEB showed hybridization signals only with wild-type oligonucleotide, indicating that this mutation is neither a polymorphic variation in the population, nor the basis of JEB in the other patients studied.

The family requested a prenatal diagnosis for a new pregnancy. ASO analysis performed on genomic DNA extracted from a skin biopsy obtained at 16 wk gestation revealed that the fetus was a heterozygous healthy carrier (Fig. 4 *b*), as confirmed by the clearly detectable expression of α6 and β4 integrins in the fetus skin sections (not shown).

Discussion

In this report, we describe the identification of the first mutation in the *ITGA6* gene which encodes for α6 integrin in a patient presenting with PA-JEB. In the proband, the presence of a homozygous premature termination codon (PTC) predicts the synthesis of an α6 polypeptide truncated at amino acid 268, and therefore lacking most of the extracellular domain and the whole transmembrane and intracellular domains. It has been shown that PTCs generated by genetic defects occurring in a variety of genes cause decay of the aberrant mRNA transcripts (32, 33). In the family described in this report, a degradation of the mutated α6 mRNA would be consistent with the reduced steady state level of integrin α6 transcripts detected in keratinocytes obtained from the proband's parents. Therefore, little if any truncated α6 polypeptide would be synthesized by the patient's keratinocytes. Indeed, no staining for the α6 integrin subunit was detected in the patient's skin. Absence of α6 integrin subunit also explains the dramatic decline in the immunohistochemical expression of the β4 integrin subunit which asso-

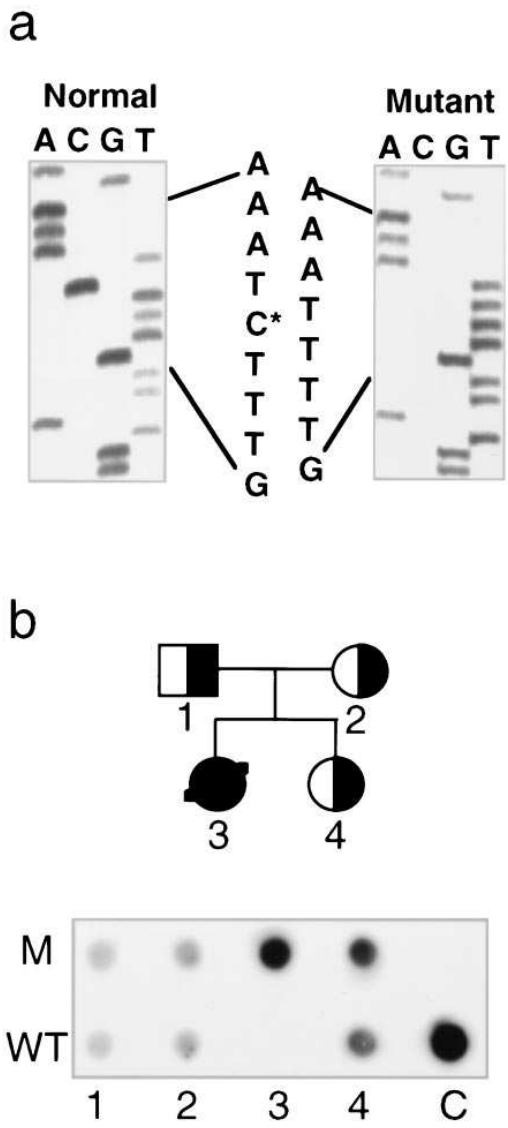

Figure 4. Identification of the homozygous 791delC mutation in the proband's *ITGA6* gene and inheritance in the kindred. (*a*) Total genomic DNA was subjected to PCR reaction using primers which amplify an 87-bp fragment extending from cDNA nts 758 to nts 844. In comparison with the DNA of a normal control, nucleotide sequencing of the PA-JEB patient's DNA reveals a single base deletion (*C** at position 791) resulting in a frameshift and a premature termination codon (TGA) 66 bp downstream. The mutation is designated 791delC. (*b*) Pedigree of the PA-JEB family (*top*): proband died at 23 d (*3*), fetus of 16 wk gestation (*4*), clinically unaffected parents (*1*, *2*). ASO analysis (*bottom*): hybridization of the 87-bp PCR product with a wild-type (*WT*) and mutated (*M*) ASO shows the homozygous state of the mutation in the proband, whereas both parents and the fetus are heterozygous carriers of the mutated allele. *C*, unaffected control individual.

ciates exclusively with the α6 chain. Since α6β4 integrin plays a crucial role in hemidesmosome assembly and stability (11, 34, 35), the absence of this heterodimer correlates well with the reduction in the number and the rudimentary shape of hemidesmosomes in PA-JEB skin.

The recurrent blistering and erosions of the gastrointestinal, urinary, and respiratory tracts, which are additional clinical hallmarks of PA-JEB (36), are also consistent with an altered expression of α6β4 in simple epithelia that do not possess hemidesmosomes (5, 6), as well as with in vitro data indicating that α6β4 mediates cell adhesion, even in the absence of formed hemidesmosomes (35). At present, it is still unclear whether esophageal and urethral stenosis and pyloric atresia, which are recurrent findings in PA-JEB patients, result from the fusion of the detached epithelial linings.

Mainiero et al. have shown recently that EGF, a growth factor crucial for the regulation of keratinocyte growth and migration, modifies the functions of α6β4 with a consequent disruption of hemidesmosomes and an upregulation of α6β4-mediated cell migration towards laminins (37). The recurrent finding of extensive areas of aplasia cutis in PA-JEB patients (36) correlates well with a role for the α6β4 heterodimer in mediating epidermal cell migration in wound healing. The absence of α6β4 in PA-JEB skin might in fact impair the reepithelialization of skin blisters formed during embryonic and fetal life when the EGF receptor is expressed strongly in the epidermis (38).

The α6 integrin is known to combine with both β4 and β1 chains. While the α6β4 heterodimer is restricted mainly to the basal pole of basal epithelial cells, the α6β1 laminin receptor is expressed on a variety of cell types, and, during morphogenesis, co-appears with laminin 1 at the four-cell stage, when the first basement membrane is assembled (3–11). Our findings demonstrate that, despite the widespread distribution of the α6 chain, a genetic defect in the α6 integrin leads to a PA-JEB phenotype similar to that associated with mutations in the *ITGB4* gene (17). Therefore, these observations suggest that during embryonic and fetal development, other laminin receptors, possibly of the β1 integrin family, can compensate for the absence of the α6 integrin subunit. In this respect, the α3β1 integrin displays a basal polarization in the basal keratinocytes of our PA-JEB patient. Since this basal localization of α3β1 integrin is not observed in other JEB variants, nor in the skin of healthy controls, this integrin, which is also a laminin 5 receptor (39), may partially compensate for the lost functions of α6β4 integrin.

Our data are consistent with the recent demonstration that the inactivation of the genes for α6 and β4 causes extensive detachment of epithelia in mice (40–42). Several distinct features, however, distinguish PA-JEB from the phenotype observed in α6 and β4 null mice. Rudimentary and sparse hemidesmosomes are clearly detectable in the skin of PA-JEB patients, whereas no hemidesmosomal adhesion structure is observed in the knockout animals. In addition, pyloric atresia and aplasia cutis are clinical hallmarks specific to humans. These differences may imply a similar but not identical role of α6β4 integrin in epithelial adhesion and signal transduction in the human and in the mouse, and raise the question of the suitability of animal systems as models for the faithful reproduction of human diseases.

Acknowledgments

The authors thank N. De Luca, A. Bucci, and A. Pisani Spadafora for technical assistance, D. Castiglia for helpful discussions, A. Nicolini (Clinica Mangiagalli, Milano), G. Tadini, and A. Brusasco (Department of Clinical and Pediatric Dermatology, Milano) for performing fetal skin biopsy, and C. Secci for artwork.

This work was supported by grants from Ministero della Sanità,

Ministero per l'Università e la Ricerca Scientifica e Tecnologica, Istituto Superiore di Sanità (Progetto Sostituzioni funzionali, Organi Artificiali e Trapianti di Organo), EEC BIOMED 2 (n. BMHG-CT97-2062), INSERM-CNAMTS (France), Programma Galileo, the Programme Hospitalier de Recherche Clinique (France), the DEBRA Foundation (United Kingdom), the Association Francaise contre les Myopathies (France), the Ministère de l'Education National (ACCSV, France), and the Association pour la Recherche sur le Cancer (France).

References

1. Hynes, R.O. 1992. Integrins: versatility, modulation and signaling in cell adhesion. *Cell.* 69:11–25.

2. Dedhar, S., and G.E. Hannigan. 1996. Integrin cytoplasmic interactions and bidirectional transmembrane signalling. *Curr. Opin. Cell Biol.* 8:657–669.

3. Tamura, R.N., C. Rozzo, L. Starr, J. Chambers, L.F. Reichardt, H.M. Cooper, and V. Quaranta. 1990. Epithelial integrin α6β4: complete primary structure of α6 and variant forms of β4. *J. Cell Biol.* 111:1593–1604.

4. Hogervorst, F., I. Kuikman, A.G. Van Kessel, and A. Sonnenberg. 1991. Molecular cloning of the human α6 integrin subunit. Alternative splicing of the α6 mRNA and chromosomal localization of the α6 and β4 genes. *Eur. J. Biochem.* 199:425–433.

5. Sonnenberg, A., C.J.T. Linders, J.H. Daams, and S.J. Kennel. 1990. The α6β1 (VLA-6) and α6β4 protein complexes: tissue distribution and biochemical properties. *J. Cell Sci.* 96:207–217.

6. Hogervorst, F., L.G. Admiraal, C. Niessen, I. Kuikman, H. Janssen, H. Daams, and A. Sonnenberg. 1993. Biochemical characterization and tissue distribution of the A and B variants of the integrin α6 subunit. *J. Cell Biol.* 121: 179–191.

7. Collo, G., S.Z. Domanico, G. Klier, and V. Quaranta. 1995. Gradient of integrin α6A distribution in the myocardium during early heart development. *Cell. Adh. Comm.* 3:101–113.

8. Falk, M., K. Salmivirta, M. Durbeej, E. Larsson, M. Ekblom, D. Vestweber, and P. Ekblom. 1996. Integrin α6Bβ1 is involved in kidney tubulogenesis in vitro. *J. Cell Sci.* 109:2801–2810.

9. Stepp, M.A., S. Spurr-Michaud, A. Tisdale, J. Elwell, and I.K. Gipson. 1990. α6β4 integrin heterodimer is a component of hemidesmosomes. *Proc. Natl. Acad. Sci. USA.* 87:8970–8974.

10. Sonnenberg, A., J. Calafat, H. Janssen, H. Daams, L.M.H. van der Raaij-Helmer, R. Falcioni, S.J. Kennel, J.D. Aplin, J. Baker, M. Loizidou, and D. Garrod. 1991. Integrin α6/β4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. *J. Cell Biol.* 113:907–917.

11. Jones, J.C.R., M.A. Kurpakus, H.M. Cooper, and V. Quaranta. 1991. A function for the integrin α6β4 in the hemidesmosomes. *Cell Regul.* 2:427–438.

12. Borradori, L., and A. Sonnenberg. 1996. Hemidesmosomes: roles in adhesion, signaling and human diseases. *Curr. Opin. Cell Biol.* 8:647–656.

13. Dewel, G.O., and A. Sonnenberg. 1996. Laminin isoforms and their integrin receptors. *In* Adhesion Receptors as Therapeutic Targets. M. Horton, editor. CRC Press, Inc., Boca Raton, FL. 9–36.

14. Gil, S.G., T.A. Brown, M.C. Ryan, and W.G. Carter. 1994. Junctional epidermolysis bullosis: defects in expression of epiligrin/nicein/kalinin and integrin β4 that inhibit hemidesmosome formation. *J. Invest. Dermatol.* 103:31s–38s.

15. Shimizu, H., K. Suzumori, N. Hatta, and T. Nishikawa. 1996. Absence of detectable α6 integrin in pyloric atresia-junctional epidermolysis bullosa syndrome. *Arch. Dermatol.* 132:919–925.

16. Niessen, C.M., L.M.H. van der Raaij-Helmer, E.H.M. Hulsman, R. van der Neut, M.F. Jonkman, and A. Sonnenberg. 1996. Deficiency of the integrin β4 subunit in junctional epidermolysis bullosa with pyloric atresia: consequences for hemidesmosome formation and adhesion properties. *J. Cell Sci.* 109:1695–1706.

17. Vidal, F., D. Aberdam, C. Miquel, A.M. Christiano, L. Pulkkinen, J. Uitto, J.-P. Ortonne, and G. Meneguzzi. 1995. Integrin β4 mutations associated with junctional epidermolysis bullosa with pyloric atresia. *Nat. Genet.* 10:229–234.

18. Fine, J.-D., E.A. Bauer, R.A. Briggaman, D.M. Carter, R.A.J. Eady, N.B. Esterly, K.A. Holbrook, S. Hurwitz, L. Johnson, A. Lin, et al. 1991. Revised clinical and laboratory criteria for subtypes of epidermolysis bullosa. A consensus report by the Subcommittee on Diagnosis and Classification of the National Epidermolysis Bullosa Registry. *J. Am. Acad. Dermatol.* 24:119–135.

19. Aberdam, D., M.-F. Galliano, J. Vailly, L. Pulkkinen, J. Bonifas, A.M. Christiano, K. Tryggvason, J. Uitto, E.H. Epstein, Jr., J.-P. Ortonne, and G. Meneguzzi. 1994. Herlitz's junctional epidermolysis bullosa is linked to mutations in the gene (LAMC2) for the γ2 subunit of nicein/kalinin (LAMININ–5). *Nat. Genet.* 6:299–304.

20. Pulkkinen, L., A.M. Christiano, D. Gerecke, D.W. Wagman, R.E. Burgeson, M.R. Pittelkow, and J. Uitto. 1994. A homozygous nonsense mutation in the β3 chain gene of laminin 5 (LAMB3) in Herlitz junctional epidermolysis bullosa. *Genomics.* 24:357–360.

21. McGrath, J.A., B. Gatalica, A.M. Christiano, K. Li, K. Owaribe, J.R. McMillan, R.A.J. Eady, and J. Uitto. 1995. Mutations in the 180-kD bullous pemphigoid antigen (BPAG2), a hemidesmosomal transmembrane collagen (COL17A1), in generalized atrophic benign epidermolysis bullosa. *Nat. Genet.* 11:83–86.

22. Kanitakis, J., G. Zambruno, J. Viac, L. Tommaselli, and J. Thivolet. 1989. Expression of an estrogen receptor-associated protein (p29) in epithelial tumors of the skin. *J. Cutaneous. Pathol.* 16:272–276.

23. Sonnenberg, A., H. Janssen, F. Hogervorst, J. Calafat, and J. Hilgers. 1987. A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. *J. Biol. Chem.* 262:10376–10383.

24. Kennel, S.J., R.G. Epler, T.K. Lankford, V. Dickas, M. Canamucio, R. Cavalierie, M. Cosimelli, I. Venturo, and A. Sacchi. 1990. Second generation monoclonal antibodies to the human integrin α6β4. *Hybridoma.* 9:243–255.

25. Verrando, P., C. Blanchet-Bardon, A. Pisani, L. Thomas, F. Cambazard, R.A.J. Eady, O. Schofield, and J.-P. Ortonne. 1991. Monoclonal antibody GB3 defines a widespread defect of several basement membranes and a keratinocyte dysfunction in patients with lethal junctional epidermolysis bullosa. *Lab. Invest.* 64:85–92.

26. Tanaka, T., N.J. Korman, H. Shimizu, R.A.J. Eady, V. Klaus-Kovtun, K. Cehrs, and J.R. Stanley. 1990. Production of rabbit antibodies against carboxy-terminal epitopes encoded by bullous pemphigoid cDNA. *J. Investig. Dermatol.* 94:617–623.

27. Owaribe, K., Y. Nishizawa, and W.W. Franke. 1991. Isolation and characterization of hemidesmosomes from bovine corneal epithelial cells. *Exp. Cell Res.* 192:622-630.

28. Fradet, Y., C. Cordon-Cardo, T.M. Thomson, M.E. Daly, W.F. Whitmore, K.O. Lloyd, M.R. Melamed, and L.J. Old. 1984. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA.* 81:224–228.

29. Zambruno, G., P.C. Marchisio, A. Marconi, C. Vaschieri, A. Melchiori, A. Giannetti, and M. De Luca. 1995. Transforming growth factor-β1 modulates β1 and β5 integrin receptors and induces the de novo expression of the αvβ6 heterodimer in normal human keratinocytes: implications for wound healing. *J. Cell Biol.* 129:853–865.

30. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

31. Hogervorst, F., I. Kuikman, A.E.G. von dem Borne, and A. Sonnenberg. 1990. Cloning and sequence analysis of β4 cDNA: an integrin subunit that contains a unique 118 kD cytoplasmic domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:765–770.

32. McIntosh, I., A. Hamosh, and H.C. Dietz. 1993. Nonsense mutations and diminished mRNA levels. *Nat. Genet.* 4:219.

33. Cui, Y., K.W. Hagan, S. Zhang, and S.W. Peltz. 1995. Identification and characterization of genes that are required for the accelerated degradation of mRNAs containing a premature translation termination codon. *Genes Dev.* 9: 423–436.

34. Mainiero, F., A. Pepe, K.K. Wary, L. Spinardi, M. Mohammadi, J. Schlessinger, and F.G. Giancotti. 1995. Signal transduction by the α6β4 integrin: distinct β4 subunit sites mediate recruitment of Shc/Grb2 and association with the cytoskeleton of hemidesmosomes. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:4470–4481.

35. Spinardi, L., S. Einheber, T. Cullen, T.A. Milner, and F.G. Giancotti. 1995. A recombinant tail-less integrin β4 subunit disrupts hemidesmosomes, but does not suppress α6β4-mediated cell adhesion to laminins. *J. Cell Biol.* 129:473–487.

36. Lestringant, G.G., S.R. Akel, and K.I. Qayed. 1992. The pyloric atresia-junctional epidermolysis bullosa syndrome. *Arch. Dermatol.* 128:1083–1086.

37. Mainiero, F., A. Pepe, M. Yeon, Y. Ren, and F.G. Giancotti. 1996. The intracellular functions of α6β4 integrin are regulated by EGF. *J. Cell Biol.* 134: 241–253.

38. Zambruno, G., G. Girolomoni, V. Manca, A. Segre, and A. Giannetti. 1990. Epidermal growth factor and transferrin receptor expression in human embryonic and fetal epidermal cells. *Arch. Dermatol. Res.* 282:544–548.

39. Carter, W.G., M.C. Ryan, and P.J. Gahr. 1991. Epiligrin, a new cell adhesion ligand for integrin α3β1 in epithelial basement membranes. *Cell.* 65: 599–610.

40. Van der Neut, R., P. Krimpenfort, J. Calafat, C.M. Niessen, and A. Sonnenberg. 1996. Epithelial detachment due to absence of hemidesmosomes in integrin β4 null mice. *Nat. Genet.* 13:366–369.

41. Dowling, J., Q.-C. Yu, and E. Fuchs. 1996. β4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. *J. Cell Biol.* 134: 559–572.

42. Georges-Labouesse, E., N. Messaddeq, G. Yehia, L. Cadalbert, A. Dierich, and M. Le Meur. 1996. Absence of integrin α6 leads to epidermolysis bullosa and neonatal death in mice. *Nat. Genet.* 13:370–373.