DOI: 10.1159/000330331

A Recurrent Nonsense Mutation Occurring as a de novo Event in a Patient with Recessive Dystrophic Epidermolysis Bullosa

N. Cuadrado-Corrales [a, e], *C. Sánchez-Jimeno* [a, e], *M. García* [a, e], *C. Ayuso* [b, e], *R. De Lucas* [c], *J.L. Vicario* [d], *C.J. Conti* [g], *G. Zambruno* [h], *M.J. Escamez* [a, e, f], *M. Del Rio* [a, e, f]

[a] Basic Research Department, Epithelial Biomedicine Division, Regenerative Medicine Unit, [b] Department of Genetics, Fundación Jiménez Díaz, [c] Department of Dermatology, La Paz University Hospital, [d] HLA Laboratory, Regional Transfusion Center, [e] Centro de Investigaciones Biomédicas en Red en Enfermedades Raras (CIBERER), U704 and U714, Madrid, and [f] Department of Bioengineering, Universidad Carlos III (UC3M), Leganés, Spain; [g] The University of Texas M.D. Anderson Cancer Center, Science Park Research Division, Smithville, Tex., USA; [h] Laboratory of Molecular and Cell Biology, Istituto Dermopatico dell' Immacolata, IDI-IRCCS, Rome, Italy

Key Words
De novo mutation · Dystrophic epidermolysis bullosa · Genetic counseling · Skin disease

Dystrophic epidermolysis bullosa (DEB) is a rare genodermatosis characterized by persistent blister development on the skin and mucous membranes in response to minor trauma. Both autosomal-dominant (MIM 131750, 131800) and autosomal-recessive (RDEB; MIM 226600) forms of DEB are caused by mutations in the *COL7A1* gene (MIM 120120), encoding type VII collagen, a protein that assembles into anchoring fibrils [1]. To date, more than 550 mutations, most of which are family-specific, have been described in the *COL7A1* gene (Human Gene Mutation Database; www.hgmd.cf.ac.uk). Herein, we report a *COL7A1* deletion mutation in compound heterozygosity with the recurrent nonsense p.R1763X mutation [2] in a Spanish patient affected with severe generalized RDEB. Of note, mutation R1763X occurred as a de novo event in our proband.

The patient is a 38-year-old woman born to non-consanguineous parents and with no family history of skin diseases. The generalized cutaneous blistering with scarring from birth and the presence of pseudosyndactyly on the hands and feet since early infancy supported a clinical diagnosis of severe generalized RDEB (fig. 1a–d). Oral screening revealed ulcerations, ankyloglossia, excessive caries, premature loss of teeth and microstomia (fig. 1e). The patient also complained of ocular pain, which was in agreement with the findings of blisters, erosions and scarring of the cornea on ophthalmic examination (fig. 1f). She presented other physical deformities, such as joint contractures, and exhibited esophageal strictures, chronic anemia and growth retardation.

Since the age of 33 years, she had undergone repeated surgical interventions for the excision of primary and recurrent squamous cell carcinomas (SCC) of both hands/arms (fig. 1g) and of the left foot and leg. In all cases, histopathological examination showed well to moderately differentiated SCC (fig. 1h, i), as usually described in RDEB patients. At the time of the last surgery, complete staging investigations – including ultrasound, CT and PET scans – were negative. Following written informed consent, skin and blood samples were obtained from the patient. Routine histological examination of a skin biopsy showed subepidermal blisters and indirect immunofluorescence, using the monoclonal antibody LH7.2, complete absence of VII collagen protein (fig. 1j, k) in accordance with the clinical presentation. Genomic DNA was extracted from peripheral blood lymphocytes of the patient and her parents by standard methods, and mutations were identified and characterized by PCR amplification and direct sequencing as described previously [3]. The patient was a heterozygous carrier for the R1763X mutation located in exon 60 and the c.6266delCCCC mutation (also designated as c.6266_6269del) located in exon 75 (fig. 2a) and predicted to result in a frameshift and a stop codon in exon 82. Thus, the patient bore two premature-termination-codon-causing mutations in her *COL7A1*. Further mutation analysis in the patient's parents revealed that the mother was a carrier of the c.6266delCCCC mutation, this deletion has already been reported by our group in this patient [patient No. 25 in reference 3]; however, the second disease-causing mutation (the de novo mutation) had not been disclosed at that time. In contrast, the father did not carry either mutation, suggesting that the p.R1763X mutation occurred de novo on the paternal allele. Paternity was therefore confirmed by human leukocyte antigen (HLA) haplotype analysis (fig. 2b) and intragenic SNP genotyping (fig. 2c) [4]. The nonsense mutation p.R1763X, previously identified in a DEB patient from Central Europe [2] is due to a C→T transition that affects a CpG dinucleotide. CpG dinucleotides are frequent sites of mutations due to hypermutability of 5-methyl-cytosine to thymine [5]. The occurrence of the same mutation in DEB patients of a different geographical origin suggests that the residue 1763 of the *COL7A1* represents a mutation 'hotspot', probably due to the vulnerability of the nucleotide sequence.

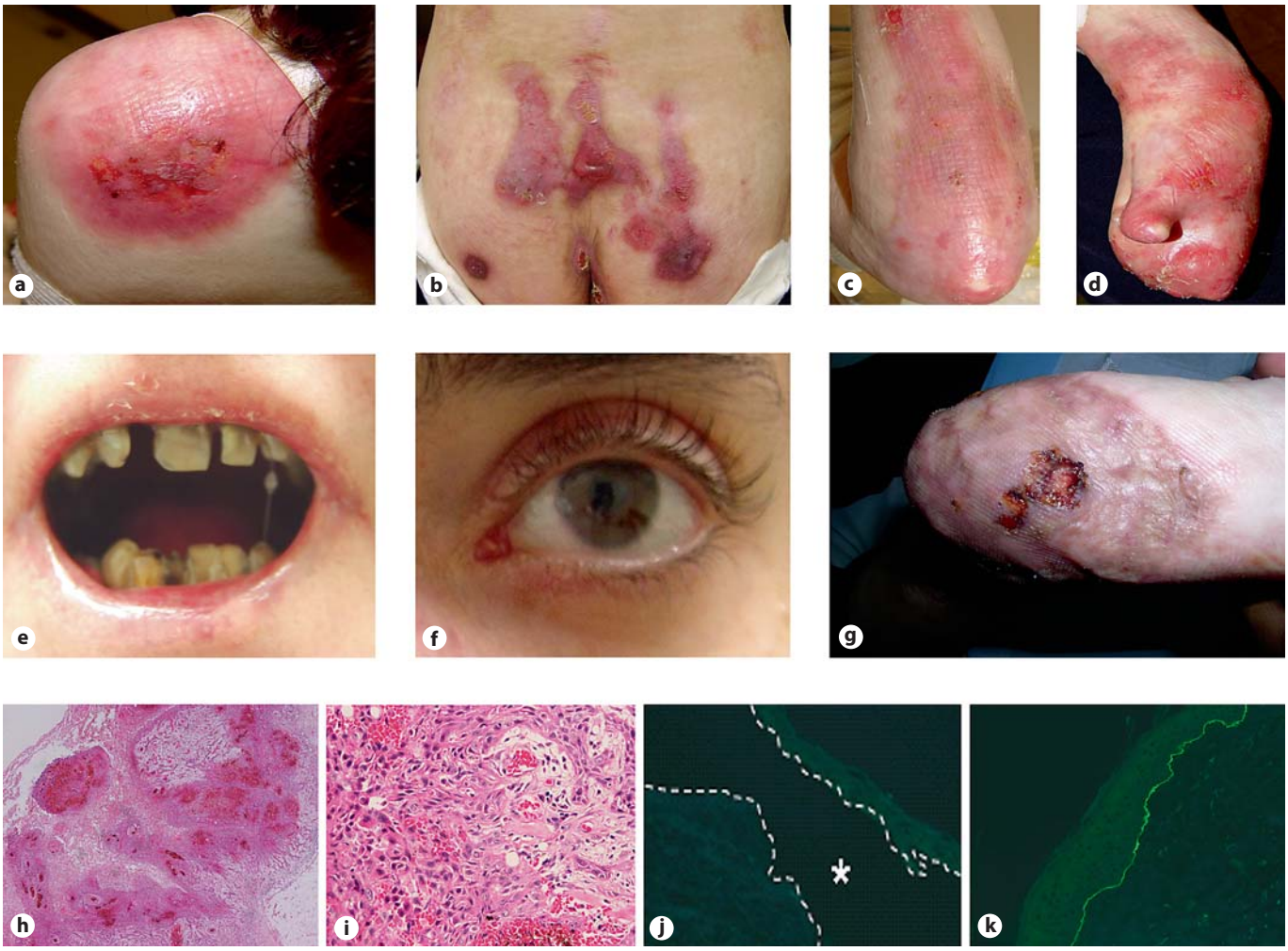
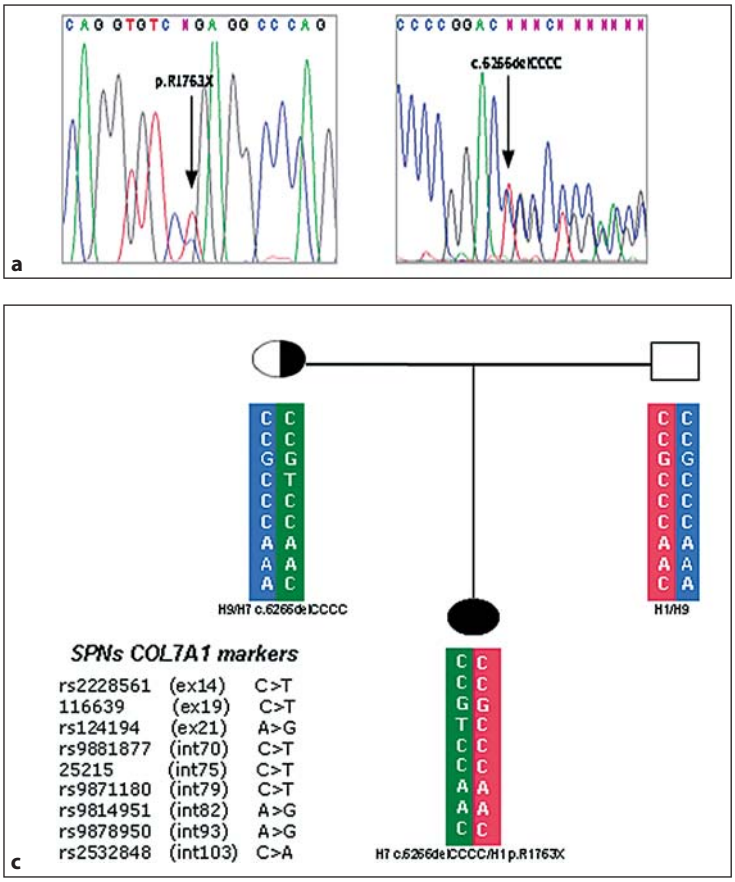


Fig. 1. Clinical and histopathological features. **a–c** Characteristic cutaneous blistering with scarring of left shoulder (**a**), sacral area (**b**) and forearm (**c**). **d** Pseudosyndactyly and mitten deformity of the left hand. **e** Severe microstomia with tooth deformities and caries. **f** Ocular findings: corneal scarring and blistering of the lower lid. **g** SCC on the right hand stump. **h** Panoramic view of a section from the tumor shown in **g**. Several tumoral masses with an aggressive pattern of infiltration into the adipose tissue are depicted. The masses have minimal cornification, but present extensive hemorrhagic foci. HE. **i** Higher magnification of the same tumor showing atypical keratinocytes with marked nuclear polymorphism, cellular dyskeratosis and minimal areas of keratinization. Dilated blood vessels and hemorrhage are seen throughout the tumor. Small foci of clear cells can also be observed. **j**, **k** Indirect immunofluorescence shows the absence of collagen VII in the blister patient's biopsy (**j**) versus the normal control's biopsy (**k**).

Finally, the presence of p.R1763X mutation in the patient's lymphocyte DNA, together with the generalized clinical presentation, argues against somatic mosaicism and in favour of a de novo mutation event in a single gamete or a germline mosaicism of the father. De novo mutations are infrequent in RDEB and, to our knowledge, only two have been reported to date [6, 7]. This case emphasizes the importance of mutation verification in the family as a part of the molecular diagnostic procedure so that de novo mutations can be identified and accurate genetic counseling provided to couples at risk of recurrence. The risk of de novo mutations in DEB, although small, should be considered when planning prenatal diagnosis.

Disclosure Statement

The authors declare no conflict of interest.



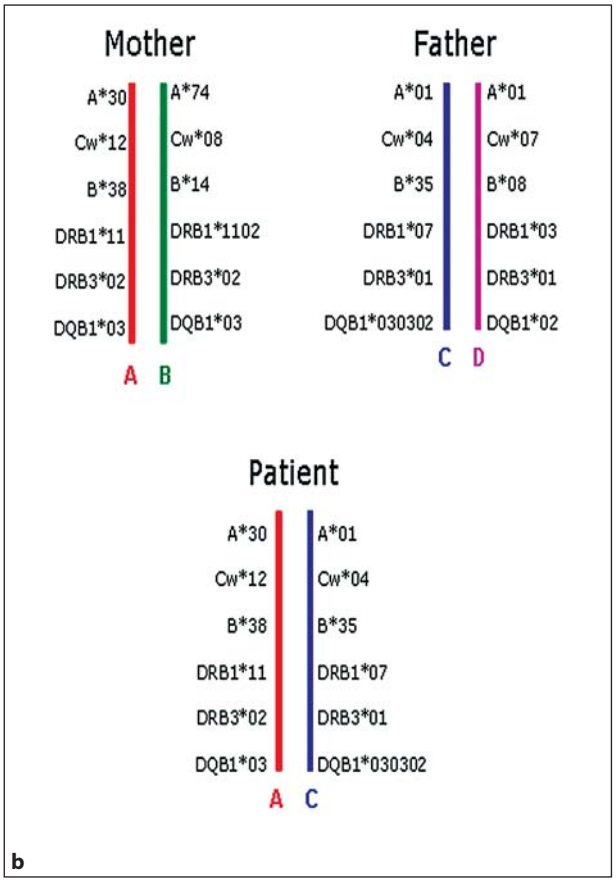


Fig. 2. Molecular characterization of the proband. **a** C→G transversion at nucleotide position 5287 leading to the missense mutation (p.R1763X) falls in exon 60 of the COL7A1 gene. The c.6266CCCC deletion (c.6266_6269del) in exon 75 leads to a premature termination codon in exon 82. **b** Pedigree with haplotype assignment of HLA typing results of the family. The proband inherited one HLA haplotype from each parent. **c** Haplotyping assay based on intragenic SNP selection shows that the haplotype 7 (H7) cosegregated with the deletion mutation inherited from her mother. The haplotype 1 (H1) was inherited from the father who does not carry the R1763X mutation.

References

1 Christiano AM, Hoffman GG, Chung-Honet LC, et al: Structural organization of the human type VII collagen gene (*COL7A1*) composed of more exons than any previously characterized gene. Genomics 1994; 21:169–179.

2 Kern JS, Grüninger G, Imsak R, et al: Forty-two novel *COL7A1* mutations and the role of a frequent single nucleotide polymorphism in the MMP1 promoter in modulation of disease severity in a large European dystrophicmepidermolysis bullosa cohort. Br J Dermatol 2009;161: 1089–1097.

3 Escámez MJ, García M, Cuadrado-Corrales N, et al: The first *COL7A1* mutation survey in a large Spanish dystrophic epidermolysis bullosa cohort: c.6527insC disclosed as an unusually recurrent mutation. Br J Dermatol 2010;163:155–161.

4 Cuadrado-Corrales N, Sánchez-Jimeno C, García M, et al: A prevalent mutation with founder effect in Spanish recessive dystrophic epidermolysis bullosa families. BMC Med Genet 2010;11:139.

5 Labuda D, Striker G: Sequence conservation in Alu evolution. Nucleic Acids Res G 1989;11:2477–2479.

6 Dunnill MG, McGrath JA, Richards AJ, et al: Clinicopathological correlations of compound heterozygous *COL7A1* mutations in recessive dystrophic epidermolysis bullosa. J Invest Dermato 1996;107:171–177.

7 Kern JS, Kohlhase J, Bruckner-Tuderman L, Has C: Expanding the *COL7A1* mutation database: novel and recurrent mutations and unusual genotype-phenotype constellations in 41 patients with dystrophic epidermolysis Bullosa. J Invest Dermatol 2006;126:1006–1012.

Marcela Del Rio
Universidad Carlos III de Madrid
Avda. de la Universidad, 30
ES–28911 Campus de Leganés
Madrid (Spain)
Tel. +34 91 624 9123, E-Mail mrnechae@ing.uc3m.es