J Hum Genet (2005) 50: 543–546
DOI 10.1007/s10038-005-0290-4

SHORT COMMUNICATION

**Daisuke Sawamura · Maki Goto · Kana Yasukawa
Kazuko Sato-Matsumura · Hideki Nakamura · Kei Ito
Hiroyuki Nakamura · Yuki Tomita · Hiroshi Shimizu**

Genetic studies of 20 Japanese families of dystrophic epidermolysis bullosa

Received: 30 June 2005 / Accepted: 27 July 2005 / Published online: 28 September 2005

Abstract Dystrophic EB (DEB) is clinically characterized by mucocutaneous blistering in response to minor trauma, followed by scarring and nail dystrophy, and is caused by mutations in the *COL7A1* gene encoding type VII collagen. DEB is inherited in either an autosomal dominant (DDEB) or recessive (RDEB) fashion. DDEB basically results from a glycine substitution mutation within the collagenous domain on one *COL7A1* allele, while a combination of mutations such as premature stop codon, missense, and splice-site mutations on both alleles causes RDEB. In this study, mutation analysis was performed in 20 distinct Japanese DEB families (16 RDEB and four DDEB). The result demonstrated 30 pathogenic *COL7A1* mutations with 16 novel mutations, which included four missense, five nonsense, one deletion, two insertion, one indel, and three splice-site mutations. We confirmed that Japanese *COL7A1* mutations were basically family specific, although three mutations, 5818delC, 6573 + 1G > C, and E2857X, were recurrent based on previous reports. Furthermore, the Q2827X mutation found in two unrelated families would be regarded as a candidate recurrent Japanese *COL7A1* mutation. The study furthers our understanding of both the clinical and allelic heterogeneity displayed in Japanese DEB patients.

Keywords Type VII collagen · Mutation · COL7A1 · Blister · Glycine substitution

D. Sawamura (✉) · M. Goto · K. Yasukawa
K. Sato-Matsumura · H. Nakamura · K. Ito · H. Nakamura
Y. Tomita · H. Shimizu
Department of Dermatology, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan
E-mail: smartdai@med.hokudai.ac.jp
Tel.: +81-11-7067387
Fax: +81-11-7067820

Introduction

Epidermolysis bullosa (EB) comprises a group of cutaneous hereditary mechanobullous disorders that can be classified into three major categories, the simplex, the junctional, and the dystrophic forms, on the basis of the level of tissue separation within the basement membrane zone (BMZ; Fine et al. 2000). Dystrophic EB (DEB) is clinically characterized by mucocutaneous blistering in response to minor trauma, followed by scarring and nail dystrophy, in which patients exhibit tissue separation beneath the lamina densa at the level of the anchoring fibrils. It occurs as either an autosomal dominant (DDEB) or recessive (RDEB) trait, each form having a different specific clinical presentation and severity (Fine et al. 2000).

Both DDEB and RDEB are caused by mutations in the *COL7A1* gene encoding type VII collagen, the major component of anchoring fibrils (Uitto et al. 1995; Fine et al. 2000). The most severe RDEB subtype, the Hallopeau–Siemens (HS) type, shows a complete lack of expression of type VII collagen, whereas some collagen expression is found in the non-Hallopeau–Siemens (nHS) type. Clinical features of DDEB are comparatively milder than those of RDEB. To date, several hundred pathogenic mutations within the collagenous and noncollagenous domains of type VII collagen gene have been identified in different forms of DEB (Christiano et al. 1995; Uitto et al. 1995; Shimizu et al. 1996; Pulkkinen and Uitto 1999; Whittock et al. 1999). Although particular molecular and phenotypic characteristics of DEB have been elucidated, we cannot always expect DEB clinical manifestations precisely from genetic information of *COL7A1*. Furthermore, no systematic study has thus far revealed detailed delineation of *COL7A1* mutations in Japanese DEB patients apart from several recurrent *COL7A1* mutations (Tamai et al. 1999; Murata et al. 2004).

In this study, we performed mutational analysis of 20 Japanese DEB families and have demonstrated the

characteristic features of *COL7A1* mutations in Japanese DEB patients.

Materials and methods

Subjects

Twenty unrelated Japanese DEB families, who had been referred to Hokkaido University Hospital's special clinic for inherited skin disorders from January 2000 to December 2004, were studied (Table 1). DEB was at first clinically diagnosed and later confirmed by immunofluorescence antigen mapping that demonstrated tissue separation beneath the lamina densa. Clinical features and inheritance modes also helped to differentiate most, though not all, cases into recessive or dominant DEB subtypes. Immunofluorescence expression of type VII collagen was of significant diagnostic value in determining HS-RDEB and nHS-RDEB.

Immunohistochemical analysis

Skin biopsies were taken from DEB patients and subjected to a routine immunofluorescence antigen mapping study (Shimizu et al. 1996). The specimens were embedded in OCT compound, and 10-μm thick sections were cut. The following monoclonal antibodies (mAbs) against BMZ components were used: mAbs HD1-121 for plectin; GoH3 and 3E1 (Chemicon International, CA, USA) for the α6 and β4 integrins, respectively; GB3 (Sera-lab, Cambridge, UK) for laminin 5; LH7.2 (Sigma, St. Louis, MO, USA) for type VII collagen; and S1193 and HDD20 for BPAG1 and BPA2, respectively. The antibodies GoH3, S1193, and HDD 20 were kind gifts from Dr. A. Sonnenberg of the Netherlands Cancer Institute. The antibody HD1-121 was also a kind gift from Dr. K. Owaribe of Nogoya University. The bound antibodies were detected with FITC-conjugated goat anti-mouse IgG antibody. In some cases, nuclei were counterstained with propidium iodide.

All DEB patients in this study were evaluated by several experienced dermatologists. This study was approved by the Ethical Committee at Hokkaido University Graduate School of Medicine. Informed consent was obtained from individual patients or their parents.

Mutation analysis

Genomic DNA was isolated from peripheral lymphocytes of patients and their families using standard procedures. *COL7A1* segments including all 118 exons, all exon-intron borders, and the promoter region were amplified by PCR using pairs of oligonucleotide primers synthesized on the basis of intronic sequences according to the report by Christiano et al. (1997; GenBank numbers L02870 and L23982). The PCR products were examined on 2% agarose gel and subjected to direct automated nucleotide sequencing using the BigDye

Table 1 Clinical phenotype, type VII collagen expression, and *COL7A1* mutations in patients in this study

Family	Age/sex (proband)	Phenotype	VII expression	Mutation	Effect
1	1 year/F	nHS-RDEB	+	R1340X/C2875F	PTC/Mis
2	44 years/F[a]	nHS-RDEB	+	G1815R/5818delC	GS/PTC
3	40 years/M	nHS-RDEB	+	E2857X/5604 + 2G > C	PTC/SS
4	7 years/M[a]	nHS-RDEB	+	G1595R/Q2827X	GS/PTC
5	1 year/F	nHS-RDEB	+	8109 + 2T > A/6573 + 1G > C	SS/SS
6	9 years/M	nHS-RDEB	+	8358 + 1G > T/G2366C	SS/GS
7	5 years/M	nHS-RDEB	+	R1957Q/6573 + 1G > C	Mis/SS
8	37 years/M	nHS-RDEB	+	Q2827X/ND	PTC/ND
9	7 days/F	nHS-RDEB	+	R236X/ND	PTC/ND
10	32 years/F	nHS-RDEB	+	R1978X/ND	PTC/ND
11	2 months/M[b]	HS-RDEB	–	434insGCAT/R2261X	PTC/PTC
12	1 week/M	HS-RDEB	–	R137X/Q641X	PTC/PTC
13	1 year/M	HS-RDEB	–	1474del8/5818delC	PTC/PTC
14	3 days/M	HS-RDEB	–	R1683X/6081insC	PTC/PTC
15	3 years/F	HS-RDEB	–	5818delC/ND	PTC/ND
16	7 years/M	HS-RDEB	–	ND/ND	ND/ND
17	1 month/F	DDEB	+[c]	G2037E	GS
18	4 years/F	DDEB	+	G2064E	GS
19	23 years/M	DDEB	+	G2034R	GS
20	26 years/M	DDEB	+	8069del17insGA	GS

Novel mutations are underlined

RDEB recessive dystrophic epidermolysis bullosa; *DDEB* dominant dystrophic epidermolysis bullosa; *HS* Hallopeau-Siemens; *nHS* non-Hallopeau-Siemens; *GS* glycine substitution mutation; *PTC* premature stop codon mutation; *Mis* missense mutation; *SS* splice-site mutation; *ND* not detected

[a] Families 2 and 4 with unusual clinical phenotype were published in a case report (Sato-Matsumura et al. 2002; Tomita et al. 2003)

[b] Family 11 was also published (Sato-Matsumura et al. 2003)

[c] Retention of type VII collagen in keratinocytes

Terminator System (Applied Biosystems, Foster City, CA, USA).

Results and discussion

An increasing number of DEB mutations have elucidated some general genotype-phenotype correlations (Jarvikallio et al. 1997; Pulkkinen et al. 1999). DDEB patients basically harbor glycine substitution mutations within the collagenous domain on one *COL7A1* allele, leading to disruptions in anchoring fibril assembly and relatively mild clinical features. On the other hand, patients with RDEB in its most severe form, the Hallopeau–Siemens variant (HS-RDEB), frequently have premature termination codon (PTC) mutations on both alleles. These mutations characteristically lead to nonsense-mediated mRNA decay that manifests as a complete absence of type VII collagen protein and total loss of anchoring fibrils. On the other hand, patients with the non-Hallopeau–Siemens variant (nHS-RDEB) show milder phenotype, and type VII collagen can be generally detected immunohistologically. This DEB subtype is caused by a combination of mutations such as PTC, missense, and splice-site mutations on both alleles.

The routine immunofluorescence antigen mapping study in a blister site showed that all BMZ antigens were located in the roof of the blister, indicating tissue separation beneath the lamina densa. Also, linear type VII collagen expression was found along the dermal epidermal junction in nHS-RDEB and DDEB patients, whereas HS-RDEB cases showed no expression (Table 1). We found retention of type VII collagen within epidermal keratinocytes in a DDEB (family 17, data not shown).

Examination of 40 alleles of 20 families (10 nHS-RDEB, six HS-RDEB, and four DDEB) identified 30 pathogenic *COL7A1* mutations, including 16 novel mutations (Table 1). *COL7A1* mutations of nHS-RDEB included five missense mutations [G1595R(4783G > A), G1815R(5443G > A), R1957Q(5870G > A), G2366C (7096G > T), C2875F(8627G > T)], five nonsense mutations [R236X(706C > T), R1340X(4018C > T), R1978X (5932C > T), Q2827X(8479C > T), E2857X(8569G > T)], one insertion-deletion mutation (5818delC), and four splice-site mutations (5604 + 2G > C, 6573 + 1G > C, 8109 + 2T > A, 8358 + 1G > T). HS-RDEB patients showed four nonsense mutations [R137X(409C > T), Q641X(1921C > T), R1683X(5047C > T), R2261X (6781C > T)] and four insertion-deletion mutations (434insGCAT, 1474del8, 5818delC, 6081insC). As predicted by previous DEB mutation reports, all combinations of PTC mutations caused HS-RDEB, while nHS-RDEB resulted from compound heterozygous *COL7A1* mutations except for homozygous nonsense PTC/PTC mutations. Although we could not find positional effect of PTC mutations as suggested by the previous report (Tamai et al. 1999), final conclusions need further accumulation of RDEB patients with PTC mutations.

In DDEB patients, we identified three dominant glycine substitution mutations [G2034R (6100G > A), G2037E (6110G > A), G2064E (6191G > A)]. These glycine substitution mutations were previously reported, and, interestingly, the nucleotide changes were identical to those in previous reports (Kon et al. 1997; Rouan et al. 1998; Jonkman et al. 1999; Whittock et al. 1999; Lee et al. 2000). Glycine residues within the collagenous domain are critical for proper triple helix formation. Some *COL7A1* glycine substitution mutations, which cause RDEB in association with a second mutation on the other allele, are silent in patients with a normal COL7A1 allele. In addition, heterozygous glycine substitution mutations can cause DDEB through dominant negative interference of the collagen triple helix. Although this study also identified both dominant and recessive glycine substitution mutations (Table 1), we could not clarify positional effect of glycine substitution on the inheritance mode. A single indel mutation, 8069del17insGA, was novel. The 17-nucleotide deletion from 8069 to 8084 with GA insertion resulted in a 15-nucleotide deletion within the collagenous domain, which failed to change an open reading frame of *COL7A1* but interfered with the collagen triple helix (Gly-X-Y). This mutation causes a DDEB phenotype, probably in a dominant negative fashion.

We failed to detect one allelic mutation in families 8, 9, 10, and 15, and both allelic mutations in RDEB family 16 (Table 1). Thus, this study could demonstrate *COL7A1* mutations in 30 out of 36 alleles that were expected to have *COL7A1* mutations, and the resulting ratio of successful mutation detection was 83%. Similar, large-scale *COL7A1* mutation reports using Italian patient data also failed to determine single allele mutations in 13 RDEB families out of a total of 49 families (Gardella et al. 2002). This suggests at least two possibilities: that pathogenic mutations lie in the other parts of the *COL7A1* gene that were not examined in these studies, and that genes other than *COL7A1* are responsible for the DEB phenotype.

Although *COL7A1* mutations are generally family specific, some recurrent mutations have been reported in several populations: R2814X, R578R, and 7786delG in British patients (Mellerio et al. 1997); 2470insG in Mexican patients (Salas-Alanis et al. 2000); and 8441-14del21, 4783-1G > A, 497insA, and G1664A in Italian patients (Gardella et al. 2002). In Japanese patients, the mutations 5818delC (nine out of 50 cases: 18%), 6573 + 1G > C (6/50:12%), and E2857X (9/50:18%) are present only in individuals of Japanese ethnic origin (Tamai et al. 1999). The present study also detected 5818delC in four families, 6573 + 1G > C in two families, and E2857X in one family out of total of 20 unrelated families. Furthermore, the Q2827X mutation was found in two unrelated families, and this mutation should be regarded as a candidate recurrent Japanese COL7A1 mutation. However, 16 mutations were novel out of a total of 30 pathogenic DEB mutations identified, indicating that Japanese *COL7A1* mutations are family

specific. This result furthers our understanding of both the clinical and allelic heterogeneity displayed in Japanese DEB patients.

Acknowledgements The authors wish to thank Akari Nagasaki and Megumi Sato for technical assistance and Dr. James R. McMillan for proofreading and comments concerning this manuscript. This work was supported in part by a Grant-In-Aid for Scientific Research from the Japanese Society for the Promotion of Science (15390337 to D.S., 13357008 to H.S., and 15390336 to H.S.) and by a Health and Labor Sciences Research Grant (Research on Measures for Intractable Diseases to H.S.). Informed consent both for the research and for publication of the photographs was obtained from the families in this study. We thank the patients and their families for their interest in our study. We thank the referring physicians at Kyushu University, Nara Medical University, Asahikawa Medical College, National Hospital Organization Okayama Medical Center, Shimada Municipal Hospital, the University of Yamanashi, Kakogawa Municipal Hospital, Kanazawa Medical University, and Osaka Red Cross Hospital for providing clinical information on the patients.

References

Christiano AM, Morricone A, Paradisi M, Angelo C, Mazzanti C, Cavalieri R, Uitto J (1995) A glycine-to-arginine substitution in the triple-helical domain of type VII collagen in a family with dominant dystrophic epidermolysis bullosa. J Invest Dermatol 104:438–440

Christiano AM, Hoffman GG, Zhang X, Xu Y, Tamai Y, Greenspan DS, Uitto J (1997) Strategy for identification of sequence variants in *COL7A1*, and a novel 2 bp deletion mutation in recessive dystrophic epidermolysis bullosa. Hum Mutat 10:408–414

Fine JD, Eady RA, Bauer EA, Briggaman RA, Bruckner-Tuderman L, Christiano A, Heagerty A, Hintner H, Jonkman MF, McGrath J, McGuire J, Moshell A, Shimizu H, Tadini G, Uitto J (2000) Revised classification system for inherited epidermolysis bullosa: report of the Second International Consensus Meeting on diagnosis and classification of epidermolysis bullosa. J Am Acad Dermatol 42:1051–1066

Gardella R, Castiglia D, Posteraro P, Bernardini S, Zoppi N, Paradisi M, Tadini G, Barlati S, McGrath JA, Zambruno G, Colombi M (2002) Genotype-phenotype correlation in Italian patients with dystrophic epidermolysis bullosa. J Invest Dermatol 119:1456–1462

Jarvikallio A, Pulkkinen L, Uitto J (1997) Molecular basis of dystrophic epidermolysis bullosa: mutations in the type VII collagen gene (*COL7A1*). Hum Mutat 10:338–347

Jonkman MF, Moreno G, Rouan F, Oranje AP, Pulkkinen L, Uitto J (1999) Dominant dystrophic epidermolysis bullosa (Pasini) caused by a novel glycine substitution mutation in the type VII collagen gene (*COL7A1*). J Invest Dermatol 112:815–817

Kon A, Nomura K, Pulkkinen L, Sawamura D, Hashimoto I, Uitto J (1997) Novel glycine substitution mutations in *COL7A1* reveal that the Pasini and Cockayne–Touraine variants of dominant dystrophic epidermolysis bullosa are allelic. J Invest Dermatol 109:684–687

Lee JY, Li C, Chao SC, Pulkkinen L, Uitto J (2000) A de novo glycine substitution mutation in the collagenous domain of *COL7A1* in dominant dystrophic epidermolysis bullosa. Arch Dermatol Res 292:159–163

Mellerio JE, Dunnill MGS, Allison W, Ashton GH, Christiano AM, Uitto J, Eady RA, McGrath JA (1997) Recurrent mutations in the type VII collagen gene (*COL7A1*) in patients with recessive dystrophic epidermolysis bullosa. J Invest Dermatol 109:246–249

Murata T, Masunaga T, Ishiko A, Shimizu H, Nishikawa T (2004) Differences in recurrent *COL7A1* mutations in dystrophic epidermolysis bullosa: ethnic-specific and worldwide recurrent mutations. Arch Dermatol Res 10:442–447

Pulkkinen L, Uitto J (1999) Mutation analysis and molecular genetics of epidermolysis bullosa. Matrix Biol 18:29–42

Rouan F, Pulkkinen L, Jonkman MF, Bauer JW, Cserhalmi-Friedman PB, Christiano AM, Uitto J (1998) Novel and de novo glycine substitution mutations in the type VII collagen gene (*COL7A1*) in dystrophic epidermolysis bullosa: implications for genetic counseling. J Invest Dermatol 116:1210–1213

Salas-Alanis JC, Amaya-Guerra M, McGrath JA (2000) The molecular basis of dystrophic epidermolysis bullosa in Mexico. Int J Dermatol 39:436–442

Sato-Matsumura KC, Yasukawa K, Tomita Y, Shimizu H (2002) Toenail dystrophy with COL7A1 glycine substitution mutations segregates as an autosomal dominant trait in 2 families with dystrophic epidermolysis bullosa. Arch Dermatol 138:269–271

Sato-Matsumura KC, Sawamura D, Goto M, Goto M, Nakamura H, Shimizu H (2003) A novel insertion mutation in COL7A1 identified in Hallopeau-Siemens recessive dystrophic epidermolysis bullosa. Acta Derm Venereol 83:137–138

Shimizu H, McGrath JA, Christiano AM, Nishikawa T, Uitto J (1996) Molecular basis of recessive dystrophic epidermolysis bullosa: genotype/phenotype correlation in a case of moderate clinical severity. J Invest Dermatol 106:119–124

Tamai K, Murai T, Mayama M, Kon A, Nomura K, Sawamura D, Hanada K, Hashimoto I, Shimizu H, Masunaga T, Nishikawa T, Mitsuhashi Y, Ishida-Yamamoto A, Ikeda S, Ogawa H, McGrath JA, Pulkkinen L, Uitto J (1999) Recurrent *COL7A1* mutations in Japanese patients with dystrophic epidermolysis bullosa: positional effects of premature termination codon mutations on clinical severity. J Invest Dermatol 112:991–993

Tomita Y, Sato-Matsumura KC, Sawamura D, Matsumura T, Shimizu H (2003) Simultaneous occurrence of three squamous cell carcinomas in a recessive dystrophic epidermolysis bullosa patient. Acta Derm Venereol 83:225–226

Uitto J, Hovnanian A, Christiano AM (1995) Premature termination codon mutations in the type VII collagen gene (*COL7A1*) underlie severe recessive dystrophic epidermolysis bullosa. Proc Assoc Am Phys 107:245–252

Whittock NV, Ashton GH, Mohammedi R, Mellerio JE, Mathew CG, Abbs SJ, Eady RA, McGrath JA (1999) Comparative mutation detection screening of the type VII collagen gene (*COL7A1*) using the protein truncation test, fluorescent chemical cleavage of mismatch, and conformation sensitive gel electrophoresis. J Invest Dermatol 113:673–686