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Letter to the Editor

## Analysis of the *COL7A1* gene in Czech patients with dystrophic epidermolysis bullosa reveals novel and recurrent mutations

Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous group of heritable skin disorders. Fine et al. separated EB into 4 major types – epidermolysis bullosa simplex, junctional epidermolysis bullosa, dystrophic epidermolysis bullosa (DEB), and Kindler syndrome – on the basis of distinguishing ultrastructural sites of blister formation [1]. Inheritance patterns of DEB may be autosomal dominant (DDEB) or autosomal recessive (RDEB). Both DDEB and RDEB result from mutations in the type VII collagen gene (*COL7A1*) [2]. DDEB is usually associated with glycine substitutions within the collagenous domain of type VII collagen. In RDEB, the allelic variants include "silent" glycine substitutions (mutations manifested in a recessive state when inherited with another mutation), non-glycine missense variants, nonsense mutations, splice site mutations, deletions, and insertions [3].

We performed DNA analysis of *COL7A1* in 6 DDEB and 27 RDEB probands at the EB Centre, University Hospital Brno, Czech Republic. From 27 RDEB patients; 17 patients suffered from RDEB-severe generalised (RDEB-sev gen), 3 patients had RDEB-generalised other (RDEB-O), 5 patients had RDEB-inversa (RDEB-I), 1 patient suffered from RDEB-acral (RDEB-ac), and 1 patient had RDEB-pretibial (RDEB-Pt). In case of RDEB patient 12, DNA was unavailable (patient died 10 years ago) and so DNA from his parents was examined. The promoter region (from -449 nucleotide) and 118 exons of the *COL7A1* gene, as well as adjacent intron regions, were amplified and sequenced.

Twenty-nine different sequence variants were found, nine of which have not been reported previously (Table 1). The most common mutation was the transition c.425A>G. This mutation was detected in 10 probands in a heterozygous state, and in 3 probands in a homozygous state (29.6% of mutant alleles). In the study of Csikos et al. [4], *COL7A1* mutations were analysed in 43 unrelated patients with DEB phenotypes from the registries of DEBRA Hungary and DEBRA Germany and the mutation c.425A>G was identified in 10 of them (11 of 86 alleles, 12.8%). The transition c.425A>G at the −2 donor splice site of exon 3 cause aberrant splicing and at least two abnormal transcripts with premature termination codons are generated downstream of this mutation [5]. Other common mutations detected in our set of DEB patients were p.Gly2049Glu (5 RDEB probands), p.Arg2069Cys (5 RDEB probands), and p.Arg1343X (4 RDEB probands).

The novel mutations comprise "silent" glycine substitutions (p.Gly1845Arg, p.Gly2296Glu, and p.Gly2557Arg), splice site mutations (c.3894+1G>A, c.5856+1G>A, and c.6751-2delAG), the deletion c.4556delG, the insertion c.5644insA, and the missense mutation p.Lys1981Arg. The presence of the last-named mutation was analysed in the control group using PCR-RFLP (Restriction Fragment Length Polymorphism). The mutation was not found in any of 200 control alleles (data not shown).

In patients 26 and 27, we detected only one *COL7A1* mutation (on the basis of literature data associated with RDEB-sev gen [5] and RDEB-inversa [6], respectively), the second mutation was not found. It is possible that these patients could have a *COL7A1* rearrangement or a sequence change within the primer binding sites to prevent PCR amplification or a pathogenic mutation within an intron not detected by our sequencing approach. Results of *COL7A1* gene analysis were correlated with clinical, electron-microscopical, and immunohistochemical findings and some of these correlations are shown in Table 1.

A missense mutation of Lys has not been described in DEB association so far. The patient's phenotype associated with p.Lys1981Arg is milder in comparison with patients' phenotypes associated with substitutions of Gly and Arg detected in our DEB patients and corresponds with the subtype RDEB-acral with distinct affliction of fingers (Fig. 1). Type VII collagen is the main constituent of anchoring fibrils where it is present as a homotrimer composed of three identical alpha chains [7]. The central collagenous domain of type VII collagen consists of characteristic Gly-X-Y repeat sequences. The Gly-X-Y repeat is a prerequisite for the formation of the collagen triple helix, which is stabilised by the presence of hydroxyproline and hydroxylysine [8]. The hydroxyl groups of hydroxylysine residues have two important functions: they serve as attachment sites for carbohydrate units and they are crucial for the stability of the intramolecular and intermolecular collagen crosslinks. It is possible that Lys1981, localised in the first Y-position of 38-triplet Gly-X-Y stretch flanked by non-collagenous sequences of 39 and 6 amino acids, has a part in assembly of collagen fibrils, and so mutations in this position will be associated with DEB phenotype.

In patients 16 and 17, we detected heterozygous and homozygous occurrence of the novel mutation c.6751-2delAG, respectively. Patient 17 has Czech citizenship but is a descendant of unrelated Russian parents, patient 16 is descendant of Czech parents. It seems that the mutation c.6751-2delAG could be specific for the Slavonic population. A similar mutation c.6751-2delA was found by Posteraro et al. in an Italian patient. This mutation affected the acceptor splice site of intron 85 and led to aberrant splicing of exon 86 [9].

In summary, this study represents a high mutation detection rate in the *COL7A1* gene in Czech DEB families. Besides mutations detected also in other countries, we described mutations specific for our patients. In the set of our new mutations, the mutation p.Lys1981Arg seems to be most interesting. The reason is that (i) a missense mutation of Lys was not described in DEB patients so far and (ii) the phenotype associated with p.Lys1981Arg is milder in comparison with patient phenotypes associated with Gly and Arg substitutions detected in our DEB patients.

**Table 1**
Mutation data and phenotypes of Czech RDEB and DDEB patients.

| No | Subtype of EB | Age | Mutation on cDNA level | Mutation on protein level | Mutation on cDNA level | Mutation on protein level | Affliction of skin, hair, nails; pseudosyndactyly | Other afflictions |
|---|---|---|---|---|---|---|---|---|
| 1 | RDEB-sev gen | 18 | c.425A>G | Splice site | c.425A>G | Splice site | Aplasia cutis, generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, defluvium, loss of nails | Microstomia, ankyloglossia, oral cavity erosions, corneal erosions |
| 2 | RDEB-sev gen | 41 | c.425A>G | Splice site | c.425A>G | Splice site | Generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, defluvium, loss of nails, spinocellular carcinoma | Microstomia, ankyloglossia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 3 | RDEB-sev gen | 7 | c.425A>G | Splice site | c.425A>G | Splice site | Generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, pruritus, defluvium | Microstomia, ankyloglossia, oral cavity erosions, oesophageal stenosis |
| 4 | RDEB-sev gen | 4 | c.425A>G | Splice site | c.682+1G>A | Splice site | Aplasia cutis, generalised blistering, atrophic scarring, skin contractures, partial pseudosyndactyly, pruritus, loss of nails | Ankyloglossia, oral cavity erosions, dysphagia |
| 5 | RDEB-sev gen | 25 | c.425A>G | Splice site | c.3551-3T>G | Splice site | Generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, pruritus, defluvium, loss of nails, spinocellular carcinoma | Microstomia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 6 | RDEB-sev gen | 5 | c.425A>G | Splice site | c.4027C>T | p.Arg1343X | Generalised blistering, atrophic scarring, skin contractures, partial pseudosyndactyly, defluvium, loss of nails | Ankyloglossia, oral cavity erosions |
| 7 | RDEB-sev gen | 14 | c.425A>G | Splice site | c.6146G>A | p.Gly2049Glu | Aplasia cutis, generalised blistering, atrophic scarring, skin contractures, partial pseudosyndactyly, defluvium, loss of nails | Ankyloglossia, oral cavity erosions, oesophageal stenosis |
| 8 | RDEB-sev gen | 16 | c.425A>G | Splice site | c.6146G>A | p.Gly2049Glu | Generalised blistering, atrophic scarring, skin contractures, partial pseudosyndactyly, pruritus, onychodystrophy | Microstomia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 9 | RDEB-sev gen | 12 | c.425A>G | Splice site | c.6187C>T | p.Arg2063Trp | Aplasia cutis, generalised blistering, atrophic scarring, skin contractures, partial pseudosyndactyly, pruritus, defluvium, loss of nails | Microstomia, ankyloglossia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 10 | RDEB-sev gen | 14 | c.682+1G>A | Splice site | c.1826C>G | p.Ser609X | Generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, defluvium, loss of nails | Microstomia, ankyloglossia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 11 | RDEB-sev gen | 21 | c.2638del25 | PTC | c.4027C>T | p.Arg1343X | Generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, defluvium, loss of nails | Microstomia, ankyloglossia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 12 | RDEB-sev gen | 22 | c.4027C>T | p.Arg1343X | **c.7669G>A** | **p.Gly2557Arg** | Aplasia cutis, generalised blistering, pseudosyndactyly, loss of nails | Extracutaneous involvement, corneal dystrophy |
| 13 | RDEB-sev gen | 18 | c.6081insC | PTC | **c.4556delG** | **PTC** | Generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, defluvium, loss of nails | Ankyloglossia, oral cavity erosions, corneal erosion, dysphagia |
| 14 | RDEB-sev gen | 21 | c.6146G>A | p.Gly2049Glu | **c.5644insA** | **PTC** | Aplasia cutis, generalised blistering, atrophic scarring, skin contractures, partial pseudosyndactyly, pruritus, defluvium, loss of nails | Microstomia, ankyloglossia, oral cavity erosions, oesophageal stenosis |
| 15 | RDEB-sev gen | 34 | c.6146G>A | p.Gly2049Glu | **c.5856+1G>A** | **Splice site** | Generalised blistering, atrophic scarring, skin contractures, pruritus, defluvium, loss of nails, spinocellular carcinoma | Ankyloglossia, oral cavity erosions, oesophageal stenosis, corneal erosion |
| 16 | RDEB-sev gen | 34 | c.6146G>A | p.Gly2049Glu | **c.6751-2delAG** | **Splice site** | Generalised blistering, atrophic scarring, skin contractures, pseudosyndactyly, loss of nails | Ankyloglossia, oral cavity erosions, oesophageal stenosis |
| 17 | RDEB-sev gen | 6 | **c.6751-2delAG** | **Splice site** | **c.6751-2delAG** | **Splice site** | Aplasia cutis, generalised blistering, atrophic scarring, skin contractures, partial pseudosyndactyly, pruritus, loss of nails | Ankyloglossia, oral cavity erosions |
| 18 | RDEB-I | 9 | c.6205C>T | p.Arg2069Cys | c.425A>G | Splice site | Predominant blistering in intertriginous, lumbosacral, and axial distribution; atrophic scarring | Microstomia, oral cavity erosions, dysphagia |
| 19 | RDEB-I | 2 | c.6205C>T | p.Arg2069Cys | c.425A>G | Splice site | Aplasia cutis, mild blistering in intertriginous, lumbosacral, and axial distribution; atrophic scarring | Oral cavity erosions |

**Table 1** (*Continued*)

| No | Subtype of EB | Age | Mutation on cDNA level | Mutation on protein level | Mutation on cDNA level | Mutation on protein level | Affliction of skin, hair, nails; pseudosyndactyly | Other afflictions |
|---|---|---|---|---|---|---|---|---|
| 20 | RDEB-I | 57 | c.6205C>T | p.Arg2069Cys | **c.3894+1G>A** | **Splice site** | Predominant blistering in intertriginous, lumbosacral, and axial distribution; atrophic scarring; defluvium, onychodystrophy; basocellular carcinoma | Ankyloglossia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 21 | RDEB-I | 29 | c.6205C>T | p.Arg2069Cys | c.4018C>T | p.Arg1340X | Aplasia cutis, predominant blistering in axial distribution, atrophic scarring, partial pseudosyndactyly | Microstomia, ankyloglossia, oral cavity erosions, dysphagia |
| 22 | RDEB-I | 27 | c.6205C>T | p.Arg2069Cys | c.4027C>T | p.Arg1343X | Predominant blistering in intertriginous, lumbosacral, and axial distribution; atrophic scarring; defluvium, loss of nails | Oral cavity erosions, oesophageal stenosis |
| 23 | RDEB-O | 25 | c.425A>G | Splice site | **c.5533G>A** | **p.Gly1845Arg** | Mild generalised blistering, atrophic scarring, onychodystrophy | Ankyloglossia, oral cavity erosions, oesophageal stenosis, corneal erosions |
| 24 | RDEB-ac | 36 | c.497insA | PTC | **c.5942A>G** | **p.Lys1981Arg** | Mild generalised blistering predominant in acral and knee distribution; atrophic scarring; partial pseudosyndactyly; defluvium; loss of nails | Oral cavity erosions, dysphagia |
| 25 | RDEB-O | 15 | c.1573C>T | p.Arg525X | **c.6887G>A** | **p.Gly2296Glu** | Aplasia cutis, generalised blistering, atrophic scarring, pruritus, onychodystrophy | Microstomia, ankyloglossia, oral cavity erosions, dysphagia, corneal erosions |
| 26 | RDEB-O | 6 | c.425A>G | Splice site | Not found | | Generalised blistering, atrophic scarring | Not detected |
| 27 | RDEB-Pt | 17 | c.7864C>T | p.Arg2622Trp | Not found | | Mild localised blistering, atrophic scarring, onychdystrophy | Not detected |
| 28 | DDEB | 7 | c.6007G>A | p.Gly2003Arg | | | Aplasia cutis | Not detected |
| 29 | DDEB-gen | 2 | c.6119G>A | p.Gly2040Asp | | | Mild generalised blistering, atrophic scarring, onychodystrophy | Oral cavity erosions |
| 30 | DDEB-gen | 30 | c.6127G>C | p.Gly2043Arg | | | Mild generalised blistering, atrophic scarring | Not detected |
| 31 | DDEB | 1 | c.6190G>A | p.Gly2064Arg | | | Localised mild blistering | Oral cavity erosions |
| 32 | DDEB | 1 | c.6208G>A | p.Gly2070Arg | | | Aplasia cutis, localised mild blistering, onychodystrophy | Not detected |
| 33 | DDEB-gen | 21 | c.6227G>A | p.Gly2076Asp | | | Aplasia cutis, mild generalised blistering, onychodystrophy, loss of nails | Not detected |

*Note*: Mutations depicted by bold letters are novel, not described so far; No: number of patient; Subtype of EB: the subtype of EB determined in the patient on the basis of clinical findings; Age: age at phenotype assessment.

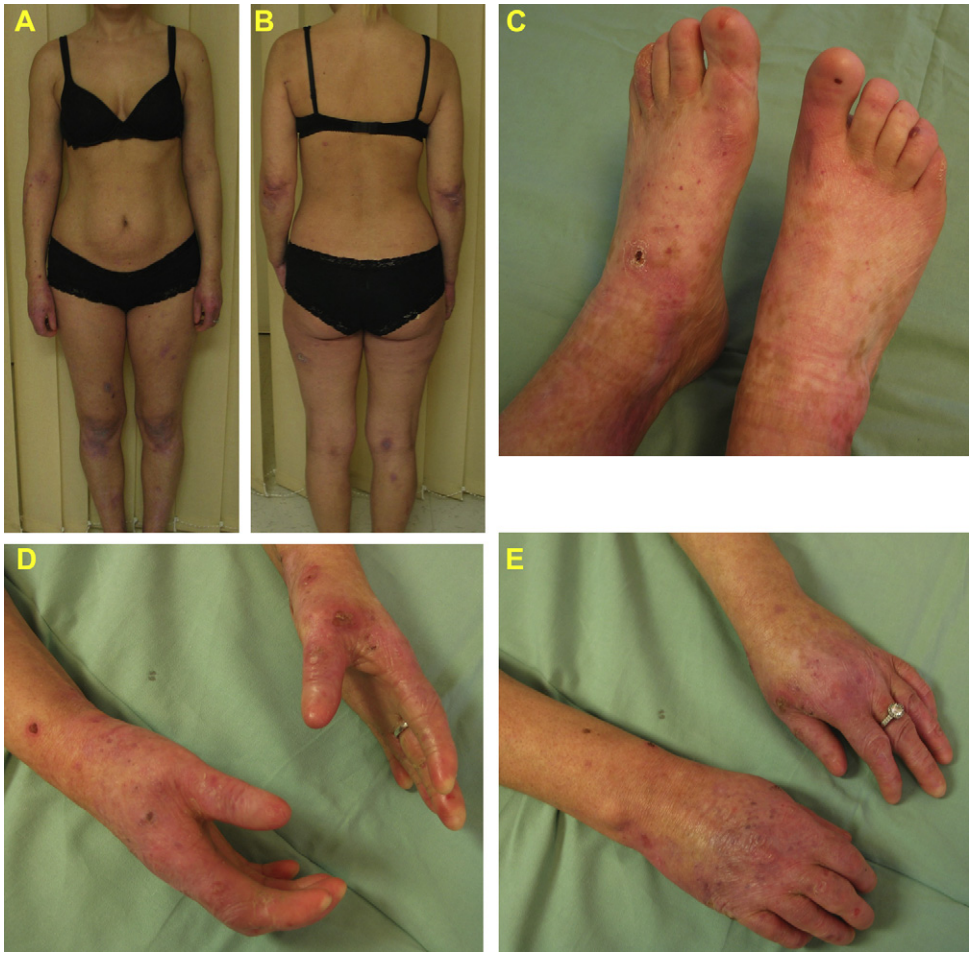


**Fig. 1.** The clinical photographs of patient 24 (detected mutations: p.Lys1981Arg/c.497insA). (A and B) Atrophic skin in acral areas and knees. (C) Loss of toe-nails, hypopigmentation, small hemorrhagic crusts. (D and E) Loss of finger-nails, semiflectional position of fingers, partial pseudosyndactyly (the right hand is more afflicted), sporadically small erosions and crusts. The photographs document that the novel p.Lys1981Arg mutation is associated with a milder phenotype (in comparison with patient phenotypes associated with Gly and Arg substitutions).

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## Letter to the Editor

### Merkel cell polyomavirus in naevoid basal cell carcinoma syndrome-associated basal cell carcinomas and sporadic trichoblastomas

Merkel cell carcinoma (MCC) is a very aggressive neuroendocrine non-melanoma skin cancer (NMSC) of elderly or immunosuppressed individuals [1]. Approximately 80% of MCC harbor the recently detected Merkel cell polyomavirus (MCPyV) within the tumor DNA [2]. MCPyV-positive MCC reveal tumor specific truncating mutations within the large T antigen (LTAg) of MCPyV which are not found in the MCPyV wildtype [3]. Thus epidemiology, clonal integration and tumor specific mutations within the LTAg of MCPyV are strongly supporting an important role of MCPyV in the etiopathogenesis of human MCC. However, the presence of MCPyV has been reported in NMSC of immunosuppressed and immunocompetent patients, including squamous cell carcinomas (SCC), Bowen's disease (BD) and basal cell carcinoma (BCC) [4–7]. BCCs are among the most common malignant skin tumors and show differentiation towards germinative cells of the hair follicle. In addition to sporadic forms, BCCs may occur in the context of the naevoid basal cell carcinoma syndrome (NBCCS) which is a rare disease with an autosomal dominant trait with high penetrance and variable phenotype. The underlying molecular causes of NBCCS are germline mutations of the tumor suppressor gene PTCH1 located on the long arm of chromosome 9q22.3 [8]. It is of interest that sporadic trichoblastoma which is a relatively rare benign counterpart of BCC, also showing differentiation toward germinative cells of the hair follicle, has been linked to chromosome 9p22.3 [9].

Our recent findings of MCPyV in 37.5% of sporadic BCC of immunocompetent patients prompted us to study the possible association of MCPyV with BCC occurring in the context of the NBCCS syndrome [4]. In addition, we aimed to determine the presence of MCPyV in sporadic trichoblastomas. Using a number of different primer pairs targeting diverse parts of the MCPyV genome, applying mutational analysis and determining the viral

**Table 1a**
Detailed clinic pathological data of 26 BCC of 6 patients with NBCCS.

| Pat. ID | GE. | Age | DX | Local. | β-G | VP1 | LT3 |
|---|---|---|---|---|---|---|---|
| 1 | M | 72 | sol. BCC | Thorax | + | – | – |
| 1 | M | 72 | sol. BCC | Thorax | + | – | – |
| 1 | M | 72 | sol. BCC | Abdomen | + | – | + |
| 1 | M | 72 | scl. BCC | Forehead | + | – | – |
| 1 | M | 72 | sol. BCC | Face | + | – | – |
| 1 | M | 72 | sol. BCC | Thorax | + | – | – |
| 2 | F | 73 | sol. BCC | Nose | + | – | – |
| 2 | F | 73 | sol. BCC | Eyelid | + | – | + |
| 2 | F | 73 | sup. BCC | Upper arm | + | – | – |
| 2 | F | 73 | sol. BCC | Neck | + | – | – |
| 3 | M | 67 | sol. BCC | Head | + | – | – |
| 3 | M | 67 | sol. BCC | Head | + | – | – |
| 3 | M | 67 | sol. BCC | Eyebrow | + | – | – |
| 3 | M | 67 | sol. BCC | Chin | + | – | – |
| 3 | M | 67 | sup. BCC | Thorax | + | + | + |
| 3 | M | 67 | sup. BCC | Shoulder | + | – | – |
| 3 | M | 67 | sol. BCC | Nose | + | – | – |
| 3 | M | 67 | sol. BCC | Face | + | – | – |
| 3 | M | 67 | sol. BCC | Eye lid | + | – | – |
| 4 | F | 43 | sup. BCC | Chest | + | – | + |
| 4 | F | 43 | sol. BCC | Abdomen | + | – | – |
| 5 | M | 46 | scl. BCC | Ear | + | – | – |
| 5 | M | 46 | sol. BCC | Head | + | – | + |
| 6 | F | 38 | sup. BCC | Head | + | – | – |
| 6 | F | 38 | sup. BCC | Abdomen | + | – | – |
| 6 | F | 38 | sup. BCC | Abdomen | + | – | – |

GE. = gender, M = male, f = female, DX = diagnosis, BCC = basal cell carcinoma; sol. = solid, sup. = superficial, scl. = sclerodermiform, Local. = localisation, β-G = beta-globin PCR, LT3 = large t antigen 3 PCR, VP1 = viral protein 1 PCR.