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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