Dystrophic epidermolysis bullosa: two case reports

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Abstract
We identified the mutations in two patients with different phenotypes of dystrophic epidermolysis bullosa (DEB). We performed molecular diagnosis to a patient aged 45 years who showed the typical severe generalized autosomal recessive DEB signs when admitted to the hospital. The other patient is a 4-month-old boy who showed a moderate clinical aspect of DEB, dominated by nail dystrophy. The molecular diagnosis disclosed in the first patient the presence of a heterozygous mutation consisting of a nucleotide substitution that lead to a splice site mutation, namely 425-2 A>G, associated to a premature termination codon, in exon 5, namely c.553 C>T, p.R185X and in the second patient a heterozygous substitution at nucleotide position 6100 that converts a glycine amino acid to arginine (6100G>A). The mutation is designated G2034R. We conclude that molecular diagnosis is the conclusive EBD investigation, maps the phenotype of a patient with his genotype and thus allows a better understanding of the disease mechanism and the development of gene therapy. Molecular diagnosis also enables genetic counseling and prenatal diagnosis.

Keywords: dystrophic epidermolysis bullosa, molecular diagnosis, type VII collagen gene mutation.

Introduction
Epidermolysis bullosa (EB) is a heterogeneous group of heritable skin disorders, characterized by blistering of the skin and of the mucous membranes, following minor trauma [1].

Dystrophic EB is caused by mutations in COL7A1, the gene coding for collagen VII, the major component of the anchoring fibrils.

The third International Consensus Meeting on Diagnosis and Classification of EB classified dystrophic EB into two types: DDEB (dominant dystrophic epidermolysis bullosa) and RDEB (recessive dystrophic epidermolysis bullosa) with their subtypes. The subtypes of DDEB are generalized DDEB, acral DDEB, preterminal DDEB, DDEB pruriginosa, DDEB nails only and DDEB bullous dermolyis of the newborn.

RDEB is subdivided into severe, generalized RDEB (the new name for Hallopeau–Siemens); generalized RDEB, other (the new name for non-Hallopeau–Siemens); inversa RDEB, preterminal RDEB, pruriginosa RDEB; centripetalis RDEB; bullous dermolysis RDEB of the newborn [2].

Materials and Methods
After informed consent, genomic DNA was extracted from a peripheral blood sample from the affected patients. Genomic DNA was extracted from EDTA blood samples using a DNA isolation kit according to the manufacturer’s protocol. Polymerase chain reaction (PCR) amplification was carried out. For sequencing the same primers as for PCR were used and the products were sequenced directly on an 8-capillary genetic analyzer.

Results
Case No. 1
The first case was a female patient aged 45 years who showed the typical signs of RDEB – severe generalized when admitted to the hospital.

The clinical features included ulceration, severe scars that caused mutilations of hands and feet, fibrosis of the oral cavity, weight loss, edentia, esophageal dysmotility and strictures, anemia, constipation and squamous cell carcinoma (Figures 1–3). A biopsy from the lesion suspected as squamous cell carcinoma was prelevated and the diagnostic was confirmed by the histopathological examination, which shows a proliferation of lobules of glassy, eosinophilic keratinocytes and keratin pearls (Figure 4). No other family member showed any cutaneous or extracutaneous lesions and her parents were not consanguine.

The treatment was based on skin care. We used special dressings, which comprises a silicone mesh, providing a good rate of epithelization. Bacterial infection was treated with systemic antibacterial agents, according to the antibiogram. The food was liquidized and the anemia was treated with iron supplements. The patient refused surgical treatment for squamous cell carcinoma.

The molecular diagnosis disclosed the presence of a heterozygous mutation consisting of a nucleotide
substitution that lead to a splice site mutation, namely 425-2 A>G (Figure 5), associated to a premature termination codon (PTC), in exon 5, namely c.553 C>T, p.R185X (Figure 6).

The first mutation, namely 425-2 A>G, on exon 3, causes damage to the donor site. This also causes a premature termination codon, the result being a lower quantity of synthesized protein. This mutation was previously published by Gardella R et al. (1996) in “American Journal of Human Genetics” [3]. In this case the splicing mutation was associated with another splicing mutation, namely 7344 G>A, situated on the exon 95, the patient being affected by the localized type of recessive EBD [3, 4].

The second mutation, in exon 5 results in PTC. This mutation was published before by Hovnanian A et al. (1997) in “American Journal of Human Genetics” [4]. In this case the patient was a compound heterozygote for a missense mutation (G1982W) and a mutation (R185X) leading to a PTC and he was affected with severe RDEB. PTC leads to the absence of protein synthesis and these patients express only the mutated allele bearing the missense mutation.

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Case No. 2

The second case is a 4-month-old boy presented with nail dystrophy, milium on his hands and erosions on the thigh. Dystrophic changes were present in toes (Figures 7–9). There was a family history of nail dystrophy, as the father, grandfather and sister of his grandfather have been affected.

The treatment consisted of skin care, using special dressings and topical antimicrobial agents when needed.

The direct sequencing revealed a heterozygous substitution at nucleotide position 6100 that converts a glycine amino acid to arginine (6100G→A) (Figure 10). The mutation is designated G2034R and is localized on exon 73. The mutation was confirmed by repeating the entire procedure, and by reverse sequencing. The mutation was also found in the DNA of the father. The mutation has been previously published by Hammami-Haouasli N et al. [5].

The mutation affects the triple helicoidal domain and causes an interference with the assembly and secretion of collagen type VII. The patients who carried this mutation presented blistering of the skin, scars, milia and nail dystrophy, the lesions being localized on their hands, feet, and knee. The mutation causes the substitution of the glycine with arginine, which is a bigger amino acid, with the consequence of intracellular accumulation of procollagen VII and a low amount of the protein.
Discussion

These types of DEB show completely different clinical manifestation of the same gene mutation. If in the first case the diagnosis is easier because of the severe clinical aspects (mutilation of the hands and feet, squamous cell carcinoma), in the second case the clinical picture is mild, suggesting EBS (epidermolysis bullosa simplex), and which is usually inherited in a dominant pattern or DDEB. A significant number of families with DDEB, having nail dystrophy as the primary clinical feature and showing minimal blistering have been described in the literature. This is why we took into account the possibility of DDEB.

Cases like these ones have been described before. For example, Nakamura H et al. described ten members of a family, spanning four generations, who presented nail dystrophy of the toe, without cutaneous fragility. The mutation described, G2028R (exon 73) was also present at a patient with cutaneous fragility and blistering and also at a patient with pruriginous EB [6].

Other mutations associated with nail dystrophy were described, G1595R (exon 50), G1815R (exon 63), and they are inherited in an autosomal dominant pattern. Nail changes tend to be more prevalent to the toe [7].

Dharma B et al. described a family with a history of nail dystrophy inherited in an autosomal dominant pattern during a period of three generations. There was no cutaneous fragility or blistering induced by minor trauma. In the forth generation a child presented nail dystrophy, acral blistering and milia. The diagnostic of dominant DEB was confirmed by the COL7A1 gene analyzes. The mutation disclosed was G1776R, exon 61, which is more frequent in dominant DEB [8].

The mutations inherited in a dominant pattern and associated with nail dystrophy as a dominant feature of the clinical aspect are presented in Table 1.

Table 1 – Mutations inherited in a dominant pattern and associated with nail dystrophy

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Exon</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>G2623C</td>
<td>105</td>
<td>Christiano AM et al. (1995) [9]</td>
</tr>
<tr>
<td>G2251E</td>
<td>86</td>
<td>Hammami-Hauasli N et al. (1998) [10]</td>
</tr>
<tr>
<td>G2287R</td>
<td>87</td>
<td>Shimizu H et al. (1999) [12]</td>
</tr>
<tr>
<td>G2043R</td>
<td>73</td>
<td>Mallipeddi R et al. (2003) [13]</td>
</tr>
<tr>
<td>G1776R</td>
<td>61</td>
<td>Dharma B et al. (2001) [8]</td>
</tr>
<tr>
<td>G1595R</td>
<td>50</td>
<td>Sato-Matsumura KC et al. (2002) [7]</td>
</tr>
<tr>
<td>G1815R</td>
<td>63</td>
<td>Sato-Matsumura KC et al. (2002) [7]</td>
</tr>
</tbody>
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There may be multiple other cases of nail dystrophies misdiagnosed or overlooked, which are actually caused by a mutation. This is another proof of the value of the molecular diagnosis in epidermolysis bullosa.

Conclusions

The dystrophic epidermolysis bullosa family of diseases shows a wide range of clinical features, from mild to extremely severe, and the only positive diagnostic tool remains the DNA analysis. Because the therapeutic approaches are mainly symptomatic, and have limited success, prevention of the disease by risk assessment and genetic counseling is the only effective strategy for the moment.

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References


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