Reproductive alternatives for patients with dystrophic epidermolysis bullosa

Opções reprodutivas para pacientes com epidermólise bolhosa distrófica

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ABSTRACT

Epidermolysis bullosa describes a group of skin conditions caused by mutations in genes encoding proteins related to dermal-epidermal adhesion. In the United States, 50 cases of epidermolysis bullosa per 1 million live births are estimated, 92% of which classified as simplex, 5% dystrophic, 1% junctional and 2% non-classified. Dystrophic epidermolysis bullosa is associated with autosomal, dominant and recessive inheritance. Epidermolysis bullosa causes severe psychological, economic and social impacts, and there is currently no curative therapy, only symptom control. Embryonic selection is available for epidermolysis bullosa patients in order to prevent perpetuation of the condition in their offspring.

Keywords: Epidermolysis bullosa dystrophica; Collagen type VII; Basement membrane; Heredity; Genetic counseling

RESUMO

O termo “epidermólise bolhosa” descreve um grupo de afecções cutâneas causadas por mutações em genes que codificam proteínas relacionadas à aderência dermoepidérmica. Nos Estados Unidos, estima-se a ocorrência de 50 casos de epidermolise bolhosa por 1 milhão de nascidos vivos, sendo 92% deles da forma simples, 5% da forma distrófica, 1% da forma junctural e 2% não classificados. A epidermolise bolhosa do tipo distrófica foi associada a padrões autosômicos, dominante e recessivo. A epidermolise bolhosa causa sérios impactos psicológicos, econômicos e sociais, e não há tratamento curativo atualmente — apenas controle dos sintomas. A seleção embrionária é disponível para portadores de epidermolise bolhosa, a fim de evitar a perpetuação da condição em seus descendentes.

Descritores: Epidermolise bolhosa distrófica; Colágeno tipo VII; Membrana basal; Hereditariedade; Aconselhamento genético

INTRODUCTION

The expression “hereditary epidermolysis bullosa” (EB) describes a group of skin conditions characterized by the appearance of mechanobullous dermatoses in response to minimal trauma, limited to skin and/or mucous membranes. There are four main EB types (simplex, dystrophic, junctional and Kindler syndrome) classified according to the level of tissue separation.
in the basement membrane zone (BMZ), observed by electron microscopy and indirect immunofluorescence mapping.\(^2\,^3\) In the simplex type, the separation occurs in the basal keratinocytes; in the dystrophic type, it occurs in the dermis below the dense lamina of the BMZ; and in the junctional type, it is located within the BMZ lamina lucida.\(^2\,^3\) In addition, EB subtypes can be classified according to mode of transmission, clinical severity and molecular findings.\(^4\)

Pathogenic variants in 20 different genes cause change in the adhesion among keratinocytes or in the dermal-epidermal junction and lead to the genetic and allelic heterogeneity observed in EB.\(^4\) The broad phenotypic spectrum of patients ranges from severe cutaneous and extracutaneous involvement, caused by the absence of adhesion proteins (due to loss of function mutations), to moderate skin fragility caused by minimal molecular defects, such as amino acid substitution.\(^3\) New genes and mechanisms associated with the condition have been published on a regular basis.\(^4\)

Epidermolysis bullosa simplex (EBS) has an autosomal dominant inheritance pattern in most cases and occurs as a consequence of variations in 14 different genes, with KRT14 and KRT5 accounting for 60 to 70% of EBS cases. Patients may have localized, generalized, intermediate or severe blisters.\(^4\) Recessive forms of EBS are caused by mutations in the plectin gene (PLEC).\(^2\) Most recessive cases are characterized by generalized blisters and muscular dystrophy, with muscular weakness observed in the first decade of life.\(^2\)

The main clinical characteristic of dystrophic EB (DEB) is the presence of serous or hemorrhagic tense blisters that develop into scarring. It may present the autosomal recessive inheritance pattern or the autosomal dominant form. All forms are associated with variations in the COL7A1 gene responsible for encoding type VII collagen.\(^5\)

All forms of junctional EB (JEB) are inherited in an autosomal recessive manner. They are caused by variations in the LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4 and ITGA3 genes. The phenotype varies according to the gene and the variations observed.\(^3\)

In Kindler syndrome, localized or generalized blisters, poikiloderma, photosensitivity and involvement of mucous membranes are observed as phenotypic characteristics. It is caused by variations in the FERMT1 gene, which encodes the Kindlin-1 protein, with an autosomal recessive inheritance pattern.\(^3\)

Thus, the diagnosis of EB is determined by clinical and pathology examinations. However, for reproductive genetic counseling and embryonic genetic research, it is critical to know the mutation responsible for the condition and its pattern of inheritance.

### CASE REPORT

A 28-year-old female patient sought genetic counseling for a clinical diagnosis of EB and the wish to avoid her offspring inheriting the condition. She referred her father and sister were affected by the condition, and denied consanguinity between parents. All three presented a history of recurrent hand and feet blisters precipitated by trauma, dystrophic nails, atrophic scars, mucosal lesions and milia. They had no ophthalmological, gastrointestinal symptoms, or finger fusion.

Diagnostic workup consisted of KRT5 and KRT14 gene sequencing (associated with the simplex form) performed by the Sanger method, lesion biopsy and pathology investigation. Knowledge of the specific mutation is essential to perform the pré-implantation genetic testing for monogenic defects (PGT-M), formerly known as pré-implantation genetic diagnosis (PGD).

No pathogenic variants were observed in the KRT5 and KRT14 genes. Biopsy showed positive antibodies against bullous pemphigoid antigen, laminin-1 and type IV and VII collagens, suggesting DEB. Sequencing of exons 73 to 76 of the COL7A1 gene, performed by the Sanger method, showed the presence of the pathogenic variant c.6182G>A p.Gly2061Glu (NM_000094) in heterozygosity, confirming the biopsy result. Study of exons 73 to 76 of the COL7A1 gene was performed on remaining affected members of the family, and the same pathogenic variant was observed in heterozygosity.

Analysis of STR-like markers binding to a region adjacent to the mutation was performed for embryonic analysis. Developed by an external laboratory, the analysis combines direct genetic study (polymerase chain reaction - PCR amplification of the COL7A1 sequence) and indirect genetic study (PCR amplification of the polymorphic markers linked to the COL7A1 gene - 5’: D3S1568, D3S3629, D3S2384; 3’: D3S1581, D3S2420 and D3S1767), using fluorescent multiplex PCR. Fragment analysis of the PCR products was performed by capillary electrophoresis (CE) using AB 3130 (ThermoFisher).

The couple underwent assisted reproduction treatment with intrauterine sperm injection (ICSI). Ovarian stimulation was performed using 200IU of recombinant follicle-stimulating hormone (FSH) from the second day of the menstrual cycle. When the largest follicle reached 14mm, the gonadotrophin releasing hormone (GnRH) antagonist was associated to treatment. When the largest follicles reached 17mm, a dose of 5,000IU of human chorionic gonadotrophin was administered, and

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36 hours later oocyte retrieval was performed. In both cycles, 15 mature oocytes were recovered, resulting in 4 biopsied and vitrified embryos (3 reaching the blastocyst stage in D5, and 1 in D6). Biopsy products were cryopreserved and awaited embryonic genetic analysis.

**DISCUSSION**

The diagnosis of EB is based on clinical and laboratory findings, and immunohistochemistry is essential to check the subtype of the disease, since each variant shows different prognosis and risks. The family described in the present case report showed clinical features of EB simplex, as predominantly, this form has dominant transmission and affects hands, feet and mucosa. Immunohistochemical analysis, however, reclassified the family as dystrophic EB patients. The classification was compatible with the variant COL7A1 gene and corroborated with molecular findings (c.6182G>A). The dominant inheritance pattern and the lower complexity of the clinical presentation were suggestive of EB simplex and rendered clinical diagnosing more challenging.

The COL7A1 gene (gene ID: 1294), located at 3p21.3, encodes the alpha 1 chain of collagen VII, which is composed of three identical alpha chains, and the largest structural component of the anchoring fibrils of the dermal-epidermal junction. Variations in this gene are associated with all forms of DEB. Codon 2061 of the COL7A1 gene is located in a highly preserved region of the triple helix domain. Analysis by the *in silico* MutationTaster tool, which predicts the possible effect of the mutation on the protein, classified it as pathogenic. However, this mutation has not been reported in either the ClinVar or ExAC databases. There is only one report of this variant in the HGMD® database, which is the same present in the international registry of patients with DEB. Interestingly, in this report, the variant allele (6182A) was observed in heterozygosis along with another allele of the COL7A1 gene (a mutation in intron 116 with potential splicing abnormalities) and more severe clinical presentation.

It is common for couples with a family history of EB to seek preconception assessment. In this context, PGT-M is a safe and well-established procedure to prevent transmissibility of severe hereditary diseases, being an alternative to prenatal diagnosis and termination of pregnancy. By identifying the type of EB, genetic counseling helps to clarify the inheritance pattern in the family and guides the risk of offspring. Once identified, PGT-M can be performed, allowing selection and transfer of mutation-free embryos.

**CONCLUSION**

Taking into account clinical findings, immunohistochemical analysis, gene sequencing and familial allelic segregation, the case of epidermolysis bullosa could be reclassified as the dystrophic variant with autosomal dominant inheritance pattern, allowing pre-implantation diagnosis to select variant-free embryos.

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