einstein
Official Publication of the Instituto Israelita de Ensino e Pesquisa Albert Einstein

ISSN: 1679-4508 | e-ISSN: 2317-6385

CASE REPORT

Reproductive alternatives for patients with dystrophic epidermolysis bullosa

Opções reprodutivas para pacientes com epidermólise bolhosa distrófica

Denise Maria Christofolini[1,2], José Ricardo Magliocco Ceroni[2], Giovanna Guimarães Soares[3], Gustavo Bertollini Lamy[3], Ana Carolina Nemeth Calvo[3], Tamara Alba dos Santos[3], Bianca Del Bel Sonoda[3], Bianca Bianco[1,2], Caio Parente Barbosa[1,2]

[1] Departamento de Saúde da Coletividade, Faculdade de Medicina do ABC, Santo André, SP, Brazil.
[2] Instituto Ideia Fértil de Saúde Reprodutiva, Santo André, SP, Brazil.
[3] Faculdade de Medicina do ABC, Santo André, SP, Brazil.

DOI: 10.31744/einstein_journal/2019RC4577

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 epidermólise bolhosa por 1 milhão de nascidos vivos, sendo 92% deles da forma simples, 5% da forma distrófica, 1% da forma juncional e 2% não classificados. A epidermólise bolhosa do tipo distrófica foi associada a padrões autossômicos, dominante e recessivo. A epidermólise 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 epidermólise bolhosa, a fim de evitar a perpetuação da condição em seus descendentes.

Descritores: Epidermólise 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.[1] There are four main EB types (simplex, dystrophic, junctional and Kindler syndrome) classified according to the level of tissue separation

How to cite this article:
Christofolini DM, Ceroni JR, Soares GG, Lamy GB, Calvo AC, Santos TA, et al. Reproductive alternatives for patients with dystrophic epidermolysis bullosa. einstein (São Paulo). 2019;17(3):eRC4577. http://dx.doi.org/10.31744/einstein_journal/2019RC4577

Corresponding author:
Denise Maria Christofolini
Avenida Lauro Gomes, 2,000
Edifício CEPES, room 101 – Vila Sacadura Cabral
Zip code: 09060-870 – Santo André, SP, Brazil
Phone: (55 11) 4993-5464
E-mail: denise.christofolini@fmabc.br

Received on:
June 18, 2018

Accepted on:
Dec 20, 2018

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] 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 intracytoplasmic 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

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.[6]

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.[7] 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.[7]

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. [8]

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.

AUTHORS' INFORMATION

Christofolini DM: http://orcid.org/0000-0001-9589-6417
Ceroni JR: http://orcid.org/0000-0001-6192-5339
Soares GG: http://orcid.org/0000-0003-2984-9078
Lamy GB: http://orcid.org/0000-0001-9976-1739
Calvo AC: http://orcid.org/0000-0001-7470-4703
Santos TA: http://orcid.org/0000-0002-2551-4708
Sonoda BD: http://orcid.org/0000-0001-6398-9176
Bianco B: http://orcid.org/0000-0001-8669-3562
Barbosa CP: http://orcid.org/0000-0002-2922-0264

REFERENCES

1. Sampaio SA, Rivitti EA. Dermatologia. 3a ed. São Paulo: Artes Médicas; 2007. p. 1063-72.
2. Sawamura D, Nakano H, Matsuzaki Y. Overview of epidermolysis bullosa. J Dermatol. 2010;37(3):214-9. Review.
4. Has C, Fischer J. Inherited epidermolysis bullosa: new diagnostics and new clinical phenotypes. Exp Dermatol. 2018 Apr 20. doi: 10.1111/exd.13668. [Epub ahead of print]. Review.
3. Fine JD, Bruckner-Tuderman L, Eady RA, Bauer EA, Bauer JW, Has C, et al. Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. J Am Acad Dermatol. 2014;70(6):1103-26.
5. Cserhalmi-Friedman PB, Tang Y, Adler A, Krey L, Grifo JA, Christiano AM. Preimplantation genetic diagnosis in two families at risk for recurrence of Herlitz junctional epidermolysis bullosa. Exp Dermatol. 2000;9(4):290-7.
6. Oliveira ZN, Périgo AM, Fukumori LM, Aoki V. Immunological mapping in hereditary epidermolysis bullosa. An Bras Dermatol. 2010;85(6):856-61. Review.
7. Chao SC, Lee JY. Mutation analyses of COL7A1 gene in three Taiwanese patients with severe recessive dystrophic epidermolysis bullosa. J Formos Med Assoc. 2007;106(1):86-91.
8. Vendrell X, Bautista-Llácer R, Alberola TM, García-Mengual E, Pardo M, Urries A, et al. Pregnancy after PGD for recessive dystrophic epidermolysis bullosa inversa: genetics and preimplantation genetics. J Assist Reprod Genet. 2011;28(9):825-32.