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De Novo COL7A1 mutation in a patient with trisomy 21: coexistence of dystrophic epidermolysis bullosa and Down syndrome

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Abstract

Background Down syndrome (DS) is the most common autosomal chromosomal disorder. Epidermolysis bullosa (EB) is a rare genodermatosis characterized by skin and mucous membrane fragility, with formation of blisters and erosions after minor trauma. Dystrophic EB (DEB) is inherited as an autosomal dominant (DDEB) or recessive (RDEB) trait. Both forms are caused by mutations in *COL7A1*, the gene coding for the type VII collagen. We report a patient affected by both conditions: DS and DDEB.

Methods A patient with DS developed generalized blisters at the age of three months. Cytogenetic study was performed to confirm DS. Skin biopsies were examined with immunohistochemical and electron microscopy techniques to determine EB subtype. Genomic DNA was extracted from peripheral blood samples. *COL7A1* mutations were screened by heteroduplex analysis using conformation-sensitive gel electrophoresis and sequencing.

Results Karyotype analysis revealed trisomy 21. Histological study agreed with a DEB diagnosis. Mutational analysis showed a heterozygous c.6127G>T mutation in *COL7A1*, which is compatible with DDEB. Parental study suggests that c.6127G>T arises as a *de novo* mutation.

Conclusions This report demonstrates that EB can be associated with other common conditions and reports the case of a patient who suffered two *de novo* independent genetic conditions. It also contributes to expanding the knowledge and database of clinical and molecular aspects of DDEB.

Introduction

Down syndrome (DS) due to trisomy 21 (OMIN 190685) is the most common autosomal chromosomal disorder causing mental impairment, with an incidence of 1 in 404 live births in Chile.[1] The phenotype of DS is variable; some of the more frequent physical features include hypotonia, epicanthal folds, upslanting palpebral fissures, flat nasal bridge, small mouth and ears, large tongue, excess nuchal skin, a single transverse palmar crease, and clinodactyly of the fifth fingers.[2] There is an increased risk of congenital malformations of different organs and many other physical and psychosocial disabilities.[2–4]

DS is associated with rare dermatological disorders (e.g. elastosis perforans serpiginosa and leukemia cutis) and increased frequency of some common dermatoses (e.g. xerosis, palmoplantar hyperkeratosis, seborrheic dermatitis, onychomycosis, cheilitis, fissured and geographic tongue, psoriasis, folliculitis, and alopecia areata).[3,5]

The term epidermolysis bullosa (EB) represents a heterogeneous group of genetic disorders characterized by the formation of blisters and erosions in the skin and mucous membranes after minor trauma. Based on the ultrastructural level in which blisters develop in the skin, EB has been classified into four main types: simplex, junctional, dystrophic, and Kindler syndrome.[6] In addition to cutaneous manifestations, patients with dystrophic epidermolysis bullosa (DEB) might present secondary clinical complications such as growth retardation, nail dystrophy, corneal erosions, esophagus strictures, and anemia.[6,7] DEB is inherited as an autosomal dominant (DDEB) or recessive (RDEB) trait (OMIM 131750 and 226600, respectively). Both forms are caused by mutations in *COL7A1* (NCBI NG_007065.1), the gene coding

for the type VII collagen (NCBI NP_000085.1).[8] This collagen chain is missing or reduced in DEB skin. As it is the major component of the anchoring fibrils, they are absent or morphologically altered and functionally defective in patients with DEB.[9]

We report a patient affected by both conditions: DS and DDEB. To our knowledge, this is the first report of the coexistence of both conditions in the scientific literature.

Materials and methods

The patient is a Chilean boy born in 1995 and is the second child of healthy nonconsanguineous parents. There was no history of other cases of EB or DS in the family. At the time of conception, the mother was 30 and the father 25 years old. Pregnancy was uneventful and at term delivery, the newborn had the phenotypic characteristic of DS (Fig. 1a), including an atrioventricular septal defect, which was surgically corrected at the age of four months. At the age of three months, he began to develop blisters on hands, arms, legs, thorax, abdomen, and perineal region. The diagnosis of DEB was confirmed at the age of five months by skin biopsy.

The infant was admitted to the DEBRA Chile Foundation, where he has received multidisciplinary care and has been on follow-up since then. Over the years, he has continuously developed blisters on hands and feet and occasionally generalized blisters. Over time he presented nail dystrophy and subsequently complete anonychia (Fig. 2a). On examination, he had milia cysts, blisters on hands, knees (Fig. 2b), and feet, and a keloid in the region of his sternotomy (Fig. 1a). He reported having mild constipation and the following surgeries: adenotonsillectomy, left inguinal hernia, right cryptorchidia, and cholecystectomy.

A cytogenetic study was performed in metaphase chromosomes prepared from cultured lymphocytes according to routine protocols. Chromosomes were GTG banded by standard methods.

Immunohistochemical and electron microscopy study, to confirm EB diagnosis, was performed on a second skin biopsy obtained in 2005. Briefly, skin biopsies were sectioned (7 μm) in cryostate. Sections were incubated with monoclonal anticollagen IV antibody (Novocastra, Newcastle upon Tyne, England; dilution 1:40) and subsequently with a biotin conjugated antimouse IgG antibody (DAKO, Carpinteria, CA, USA; dilution 1 : 40). Then, skin sections were incubated with a streptavidin–biotin–peroxidase complex (DAKO; dilution 1 : 70) for development. Before observation under the light microscope, samples were hematoxylin stained.

The molecular study was approved by the Bioethics Committee of Facultad de Medicina, Clínica Alemana-Universidad del Desarrollo. Peripheral blood samples were obtained from the patient, his parents, and control subjects after written informed consent. Genomic DNA was isolated from blood lymphocytes using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) and amplified with pairs of primers spanning all *COL7A1* exons (118) and flanking introns.[10] Polymerase chain reaction products were screened by heteroduplex analysis using conformation-sensitive gel electrophoresis, and amplicons showing pattern shifts were sequenced as previously described.[10] Mutation c.6127G>T abolishes one of the two *Sma*I restriction sites (positions 433 and 478) present in an exon 72–73 amplicon (571 bp length). To confirm and screen for this mutation, genomic DNA was used as a template for polymerase chain reaction amplification of exons 72–73, and product was subjected to restriction endonuclease digestion with *Sma*I. Restriction fragments were observed in agarose gel stained with ethidium bromide.

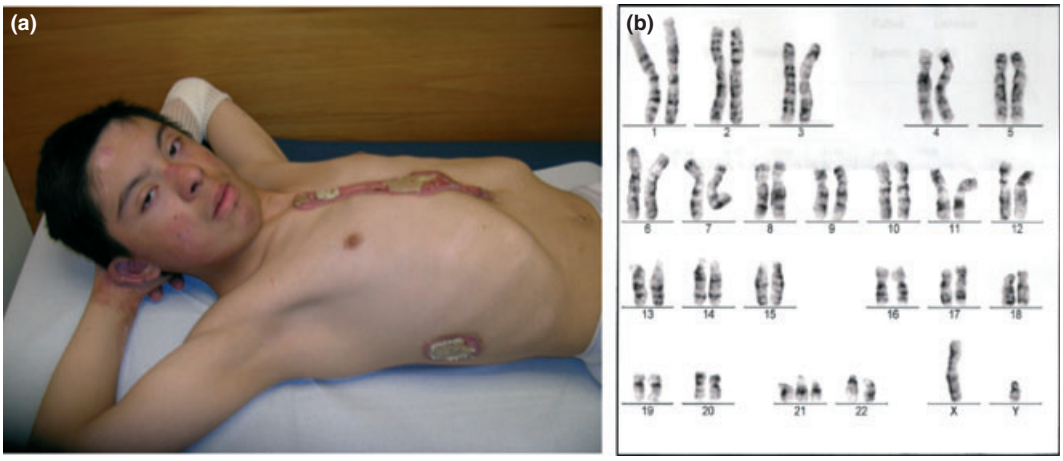

Figure 1 (a) Phenotypic appearance of the patient at the age of 14 years. The image shows epicanthal folds and upward slanting eyes, flat nasal bridge, and a keloid in the region of the sternotomy. (b) Karyotype analysis confirmed trisomy 21

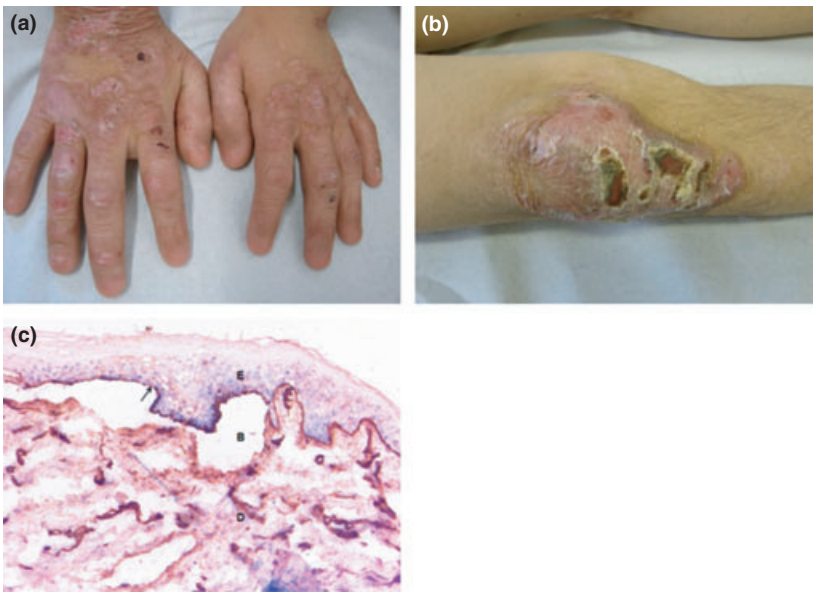

Figure 2 Characteristic features of dystrophic epidemolysis bullosa. (a) The hands show xerosis and complete anonychia. (b) Hypopigmented skin and erosions can be seen secondary to recent bullae at the right knee. (c) Immunohistochemical analysis of patient skin showed type IV collagen presence in blister roof (arrow); epidermis (E), blister (B), and dermis (D). (hematoxylin and eosin, magnification × 100)

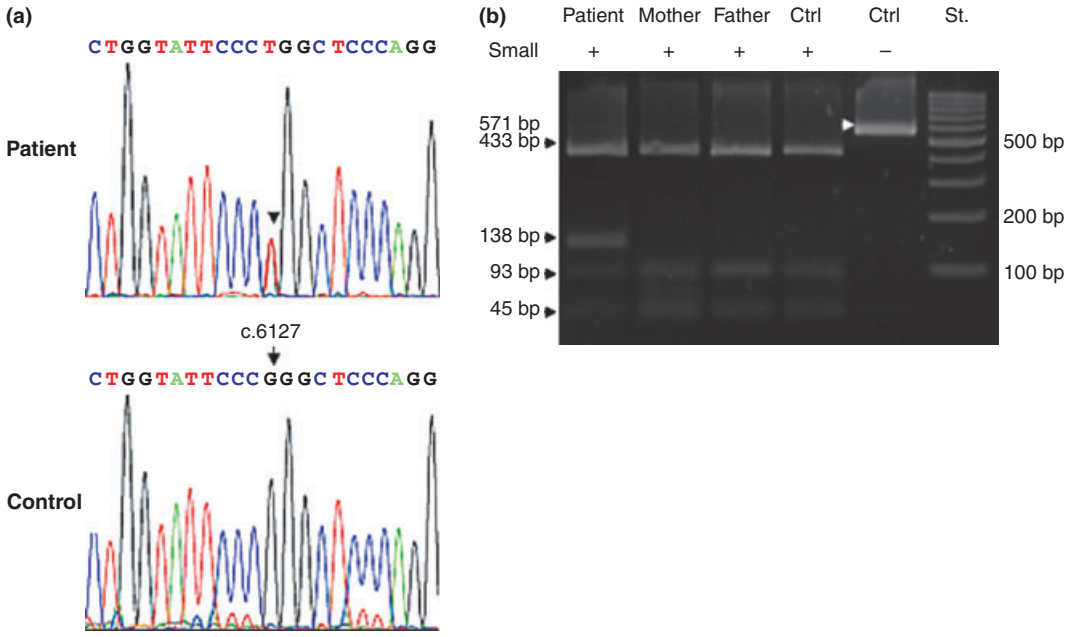

Figure 3 Sequence analysis of *COL7A1* exons 72–73 and flanking regions (a) showed a heterozygous substitution of guanine c.6127 (indicated by arrow in control) by thymine (indicated by arrowhead in patient). (b) *Sma*I endonuclease restriction analysis showed four fragments (433, 138, 93, and 45 bp) in patient, which results from wild-type amplicon digestion at positions 433 and 478 (fragments 433, 93, and 45 bp) and mutant amplicon digestion only at position 433 (fragments 433 and 138 bp). *Sma*I site at position 478 is abolished by c.6127G>T. Three fragments (433, 93, and 45 bp) were detected in the mother, father, and control. Undigested 571 bp control amplicon is shown next to 100 bp molecular weight standard (St.)

Results

Karyotype analysis revealed trisomy 21 (47,XY + 21) in all 25 cells scored (Fig. 1b). The skin biopsy showed a positive reaction against collagen type IV on the blister roof, indicating that the blister developed beneath the dense lamina, suggesting DEB (Fig. 2c).

Molecular analysis of genomic DNA from the patient showed a heterozygous substitution of guanine by thymine at position c.6127 of *COL7A1* (Fig. 3a). It predicts a change of glycine by tryptophan at position p.2043 (p.2043T>W) of the type VII collagen chain. Although c.6127G>T was confirmed in the patient by restriction analysis, it was not detected in genomic DNA from the patient's parents. In addition, substitution c.6127G>T was not detected in 100 alleles from 50 healthy unrelated controls, demonstrating that it is unlikely that this substitution represents a normal polymorphic variant in the Chilean population.

Discussion

The dominant form of DEB is predominantly associated with glycine substitutions in the collagenous domain of type VII collagen polypeptide and results in a relatively mild clinical phenotype.[11] The mutation detected in our patient was previously reported by Mecklenbeck *et al.*[12] in a patient with DDEB phenotype. The Gly>Trp substitution represents a shift in volume of the collagen molecule at triple helix domain (60.1Å^3 > 163Å^3), which should be deleterious considering the role of the small amino acid glycine as the core of the helix conformation.

Most patients have inherited the mutation from an affected progenitor, but it can also be caused by a *de novo* mutation.[13] As restriction analysis showed normal sequence in both progenitors (Fig. 3b), it suggests that c.6127G>T arose as a *de novo* mutation in our patient. Germline mosaicism could not be excluded.

This patient had two de *novo* genetic conditions, aneuploidy and a dominant mutation, which are normally related to parental age. In our case, however, parental age was not in the usual range consistent with an increased risk. Compared with other patients with the same *COL7A1* mutation, this child had similar phenotypic features and clinical course. This suggests that trisomy 21 does not have modifier effect in DEB.

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