Int. J. Oral Maxillofac. Surg. 2014; 43: 491–497
http://dx.doi.org/10.1016/j.ijom.2013.10.004, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Clinical Paper
Oral Medicine

Genotype–oropharyngeal phenotype correlation in Mexican patients with dystrophic epidermolysis bullosa

G. Fortuna[1,2,*], A. Pollio[3], M. Aria[4], M. G. Moreno-Trevino[5], F. Marasca[6], J. C. Salas-Alanís[2,5]

[1]Department of Oral Medicine, Carolinas Medical Center, Charlotte, NC, USA; [2]DebRA Mexico Foundation, Guadalupe NL, Monterrey, Nuevo León, Mexico; [3]Department of Neurosciences, University of Padua, Padua, Italy; [4]Department of Economics and Statistics, Federico II University of Naples, Monte Sant'Angelo, Naples, Italy; [5]Department of Basic Science, Universidad de Monterrey, San Pedro Garza García, Nuevo León, Mexico; [6]Department of Stomatology, Cardarelli Hospital, Naples, Italy

G. Fortuna, A. Pollio, M. Aria, M. G. Moreno-Trevino, F. Marasca, J. C. Salas-Alanís: Genotype–oropharyngeal phenotype correlation in Mexican patients with dystrophic epidermolysis bullosa. Int. J. Oral Maxillofac. Surg. 2014; 43: 491–497.

Abstract. Previous investigations have attempted to correlate the genotype with the cutaneous phenotype in patients with epidermolysis bullosa (EB), but never with the oropharyngeal phenotype. Seventeen dystrophic EB (DEB) patients were genotyped for *COL7A1* gene mutations and divided into five distinct groups. Oropharyngeal disease severity was assessed with the Epidermolysis Bullosa Oropharyngeal Severity (EBOS) score by an oral medicine specialist. The genotype–phenotype correlation was calculated by Kruskal–Wallis analysis of variance using the Mann–Whitney test, applying the Bonferroni correction. The most severe oropharyngeal phenotype was found in the group with the 2470insG/3948insT mutation, with a mean disease severity score of 18.50 ± 2.12; the mildest was found in the 6862del16 mutation group, with a mean disease severity score of 0.57 ± 1.13. The most significant difference in median score was found in the total score ($P = 0.009$), followed by tongue ($P = 0.02$) and upper lip ($P = 0.021$), but no correlation was found between disease severity and the groups ($P > 0.005$, after Bonferroni correction). Multiple comparisons among the five different genotypic groups revealed no statistically significant genotype–oropharyngeal phenotype correlation; it was not possible to establish which group was more severe, or to associate a specific mutation to a specific oropharyngeal phenotype.

Keywords: epidermolysis bullosa (EB); oropharyngeal; mutation; genotype–phenotype.

Accepted for publication 1 October 2013
Available online 5 November 2013

Epidermolysis bullosa (EB) is an inherited mucocutaneous disorder characterized by the occurrence of blisters on the skin and mucous membranes following mild mechanical trauma.[1,2] Dystrophic epidermolysis bullosa (DEB) represents one of the four major types of EB. DEB can be inherited in a dominant (DDEB) or recessive (RDEB) manner, and a wide variety of mutations in the gene encoding type VII collagen, *COL7A1*, has been detected.[3]

0901-5027/040491 + 07 $36.00/0

Even though DEB is primarily a cutaneous disease, mucosal surfaces may be equally affected, resulting in a dramatic impairment of quality of life for EB patients. The oropharyngeal mucosa is one of the most affected mucosal areas, involving both hard and soft tissues, with different features and degrees of severity.[4] In all EB types the oral soft tissues are fragile, resulting in frequent blister formation and/or erosion, accompanied in some EB subtypes by a scarring phenotype and also, although rarely, oral milia.[5,6] Similarly, the oral hard tissues may show either marked developmentally compromised enamel or minor structural defects, with areas of surface pitting and furrowing.[7]

Considering the ever-increasing number of mutations discovered each year, a paradigm for genotype–phenotype correlation has, to some extent, emerged,[8–10] generally associating the most severe form of RDEB to premature termination codons (PTC) on both *COL7A1* alleles, and the dominant form to heterozygous glycine substitution (GS) within the collagenous triple helix.[10]

All these previous studies have attempted to correlate the genotype with the cutaneous phenotypic manifestations. The aim of this study was to verify whether or not a correlation between the type of mutation and the oropharyngeal phenotype exists, analyzing a Mexican population of patients with DEB.

Methods

Study design and patients

We retrospectively reviewed the data of 17 DEB patients (seven patients with DDEB, five with RDEB severe generalized (RDEB-sev gen), and five with RDEB other generalized (RDEB-O)) between June 2012 and September 2012. All patients provided their written informed consent to participate. The study was conducted in accordance with the principles of the Declaration of Helsinki.

All patients were enrolled based on the following inclusion criteria: (1) patients of either gender and of all ages and races, with the presence of typical mucocutaneous lesions of any EB type/subtype, as previously reported.[1] (2) Diagnosis of EB based on skin biopsy with routine histology, immunofluorescence antigen mapping, and DNA analysis. (3) Patients able to give consent if older than 18 years; for minor patients consent was given by their parents or guardian.

At the time of admission the exclusion criteria encompassed: Patients who had used topical corticosteroids and/or topical and/or systemic antifungal therapy during the 3 weeks prior to the study, as such medications might have a significant effect on the clinical appearance of the oropharyngeal mucosa.

Study protocol

Genomic DNA was isolated from peripheral blood leukocytes, and molecular screening of the *COL7A1* gene was performed as described in detail elsewhere,[11,12] as well as immunofluorescence antigen mapping.[13]

At admission, age, gender, type/subtype of EB, type of mutation, site of mutation, and the consequences of mutations were recorded, as well as the family, current, and past pathological medical history, and a general medical examination was performed. Data on recorded oropharyngeal lesions were reviewed and tabulated for all patients, who were grouped into five groups on the basis of their specific type of mutation. At the time of hospital attendance, an accurate intraoral and extraoral examination was performed by an oral medicine specialist (GF) with extensive experience diagnosing, treating, and managing patients with EB. In addition, at the time of the hospital appointment, no more than three to five EB patients were seen on a scheduled day.

The presence or absence of four physical signs were identified as key features of oropharyngeal manifestations in EB and deemed to reflect oropharyngeal disease activity: erythema, erosion/ulceration, atrophy, and blister. The analysis was done at 13 different anatomical sites of the oropharyngeal cavity.[4] Even though all EB patients exhibited variable degrees of activity, an analysis of the quantity of lesions was not performed, as this was considered a subjective (scorer-dependent) parameter. Along with the disease activity, the structural damage as a part of previous damage was recorded, including the presence or absence of four parameters: microstomia, ankyloglossia, presence of intraoral scars beyond microstomia and ankyloglossia, such as vestibule obliteration, and enamel hypoplasia. The method used for the evaluation of microstomia and ankyloglossia has been reported previously.[4] Disease activity and structural damage scores were recorded in a specific format, known as the Epidermolysis Bullosa Oropharyngeal Severity (EBOS) score, to give an overall evaluation of the oropharyngeal disease severity.[4]

Statistical analysis

Descriptive statistics of demographic characteristics were calculated as the mean ± standard deviation, along with the frequency of distribution of the EB types/subtypes and oropharyngeal lesions. The Kruskal–Wallis analysis of variance (ANOVA) test was applied to verify any statistical difference in the median score among the five groups, calculated for each oropharyngeal site. In addition, multiple comparisons among the five groups to verify any possible difference in terms of oropharyngeal severity were made by Kruskal–Wallis ANOVA using the Mann–Whitney test, adjusted with the Bonferroni correction. *P*-values of less than 0.05 were considered significant and were calculated using SPSS software (SPSS for Windows, version 17.0; SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Seventeen patients were analyzed during the study period; 11 (64.7%) were females and six (35.3%) were males, and the mean age was 31.7 years (range 6 months to 59 years; standard deviation 14.5 years; 95% confidence interval 24.3–39.2 years).

The frequency distribution of mutations, EB types, and oropharyngeal lesions are summarized in Table 1.

Genotypic data

The predominant *COL7A1* mutation was the heterozygous 6862del16 (41.2%), followed by the homozygous 2470insG (23.5%) (Table 2). The 6862del16 mutation was present in seven patients and was genotypically responsible for a 16-bp deletion within exon 87 of *COL7A1*, which surprisingly resulted in in-frame exon skipping with subsequent restoration of the reading frame, instead of the generation of a PTC.[11] This unexpected finding determined the dominant pattern of inheritance and was phenotypically indicative of a mild form of DEB.[11]

The homozygous 2470insG mutation was genotypically responsible for a 1-bp frameshift mutation in exon 19 of *COL7A1*, leading to a downstream PTC in exon 20,[12] and phenotypically was responsible for a recessive form. Intriguingly, this mutation led to an RDEB-O form in two patients and to an RDEB-sev gen form in two others; this disparity could be explained by the fact that the phenotype in the first two cases was milder, probably due to a partial restoration of the *COL7A1*