online for further details). Written informed consent, including for photographs and publication of their images, was obtained from study participants.

Stata statistical software (version 14.0, StataCorp, College Station, TX) was used for analyses. Intra-class correlation coefficients were calculated to determine intra-rater reliability between the two raters, and Cohen's \( \kappa \) coefficients were calculated to determine the inter-rater reliability. Cohen's \( \kappa \) coefficient scores of 0–0.2 correspond to slight agreement, 0.21–0.4 correspond to fair agreement, 0.41–0.6 correspond to moderate agreement, 0.61–0.8 correspond to substantial agreement, and 0.81–1 correspond to almost perfect agreement.

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CONFLICTS OF INTEREST

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A HOMOZYGAUS NONSENSE MUTATION IN THE DSG3 GENE CAUSES ACANTHOLYTIC BLISTERS IN THE ORAL AND LARYNGEAL MUCOSA


TO THE EDITOR

Keratinocytes bind to adjacent keratinocytes via desmosomes. Desmosomal cadherins, the transmembrane proteins of desmosomes, consist of four types of desmogleins (DSG1–4) and three types of desmocollins (DSC1–3), which form heterodimers or homodimers in the intercellular space (Delva et al., 2009). Among them, DSG1 is expressed throughout the skin epidermis, whereas DSG3 is expressed predominantly in the basal layer. In the mucosal epithelium, however, little DSG1 is expressed, whereas DSG3 is expressed throughout the epidermis (Mahoney et al., 1999). These findings explain the differential effects of DSG1 and DSG3.
Mucosal Suprabasal Blisters in DSG3 Deficiency

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Figure 1. Identification of a hereditary nonsense mutation in DSG3 in the patient. (a) Extensive erosions and deformity of the tongue were observed. (b) Laryngeal erosions were observed on laryngoscopy. Black arrowheads indicate the sites of erosion. (c) Biopsy sample of the oral mucosa showed suprabasal acantholytic blisters (hematoxylin and eosin). Scale bar = 25 μm. (d) Pedigree of the family. The square denotes a male member, and circles denote female members. The filled symbol denotes an affected individual. (e) PCR-restriction fragment length polymorphism assays of family members and healthy individuals were performed with the restriction enzyme TaqI. N indicates amplified pooled DNA from 20 healthy individuals. The wild-type sequence showed two fragments of 247-bp and 249-bp, whereas the PCR product containing c.859C>T mutation resulted in a 596-bp fragment. (f) Direct sequencing of DSG3 identified heterozygous c.859C>T mutations in the parents (I-1 and I-2) and a homozygous c.859C>T mutation in the patient (II-1). bp, base pair.

DSG3 inactivation on epidermal blister formation. DSG1 inactivation causes blisters in the superficial layers of the skin but not in the mucosa, where DSG3 compensates for DSG1 inactivation. On the contrary, DSG3 inactivation causes blisters in the mucosa but not in the skin, where DSG1 compensates for DSG3 inactivation (Mahoney et al., 1999). Inactivation of DSG3 can be caused by autoimmune dysfunction and genetic loss of DSG3. Autoimmune dysfunction of DSG3 is observed in the mucosal type of pemphigus vulgaris, caused by autoantibodies against DSG3, which manifests as suprabasal blisters in the oropharyngeal cavity. However, to our knowledge, there are no reports of patients with genetic loss of DSG3.

In this study, we investigated a Korean family with a 1-year-old girl having recurrent blisters and erosions in the oral mucosa since birth. The proband was the first child born to healthy parents who denied consanguinity. A deformed tongue due to recurrent erosions was noted (Figure 1a), and erosions were also detected in the laryngeal mucosa (Figure 1b). However, skin, conjunctival and genital mucosa, nail folds, and nails were unaffected. The girl’s hair was growing without hypotrichosis (see Supplementary Figure S1a and b online). Suprabasal acantholytic blisters were present in the epidermis of oral mucosa (Figure 1c), and normal structure was observed in the scalp epidermis and hair follicle (see Supplementary Figure S1c and d). Results of direct immunofluorescence performed on the oral mucosa were negative.

We then carried out whole-genome sequencing using the patient’s blood to identify genetic mutations contributing to the phenotype. The output data were filtered by selecting stop-gain, stop-loss, and frameshift single nucleotide polymorphisms, and these filtering steps showed two stop-gain and 10 frameshift single nucleotide polymorphisms (see Supplementary Table S1 online). Among them, we identified a single homozygous mutation in the DSG3 gene (chromosome 18: 29,041,235 for c.859C>T). To determine whether this mutation was inherited or de novo, PCR-restriction fragment length polymorphism analysis and direct sequencing were performed in the patient and her parents. The heterozygous c.859C>T mutations in DSG3 were identified in both parents, confirming that this mutation was hereditary (Figure 1d–f). This mutation was not present in the 1000 Genomes Project or the National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project.

DSG3 is a 130-kDa protein composed of five large N-terminal extracellular domains (EC1–5), a small transmembrane, and C-terminal intracellular domains (Delva et al., 2009). The c.859C>T mutation is expected to result in premature termination of translation in the EC3 domain (p.R287*) (Figure 2a). To confirm whether a partial form of DSG3 is expressed in the epidermis, we used two monoclonal anti-DSG3 antibodies: (i) clone 5H10 reacting with EC1 and EC2 domains and (ii) clone 5G11 reacting with EC4 and EC5 domains (Lucchese et al., 2004). Immunofluorescence and immunoblotting studies with these two antibodies showed that DSG3 was not expressed in the epidermis of the skin and oral mucosa (Figure 2b–d, and see Supplementary Figure S2a online). Instead, in immunoblotting findings,
DSG1, DSC2, and DSC3 were expressed in the patient’s skin, and DSC2 and DSC3 in the patient’s mucosa, more than in healthy individuals (Figure 2c and d). Similar to the immunoblotting findings of mucosa, the immunofluorescence findings showed a higher expression of DSC2 and DSC3 in patient’s mucosa than in the normal mucosa (Figure 2b). In contrast to the protein expression levels, however, the mRNA levels of DSG1, DSC2, and DSC3 in skin and mucosal keratinocytes were similar between healthy individuals and the patient (see Supplementary Figure S2b). Components of the intracellular plaque of the desmosome (e.g., plakoglobin and desmoplakin) were normally expressed in the epidermis (see Supplementary Figure S2c). Electron microscopy showed normal desmosomal structures in the basal and granular layers (see Supplementary Figure S2d), indicating that the loss of DSG3 in skin does not affect the ultra-structure of the desmosome.

Loss-of-function mutations in desmosomal cadherins including DSG1, DSG2, DSG4, DSC2, and DSC3 have been reported in human disorders. DSG1 deficiency causes severe dermatitis, allergies, and metabolic problems, which is clinically distinct from pemphigus foliaceus, caused by autoantibodies against DSG1.
(Samuelov et al., 2013). DSG2 and DSC2 deficiencies are associated with cardiomyopathy (Pilichou et al., 2006; Syrris et al., 2006), and DSG4 and DSC3 deficiencies manifest as hypotrichosis (Ayub et al., 2009; Kljuic et al., 2003). Patients with DSC3 deficiency are reported to have intraepidermal blisters on the skin, but mucosal and skin blisters develop in patients with autoantibodies against DSC3 (Ayub et al., 2009; Mao et al., 2010; Payne, 2010). Suprabasal blisters in the mucosa are exclusively observed in DSG3 deficiency among the diseases with desmosomal cadherin mutations, suggesting that DSG3 plays a critical role in maintaining mucosal desmosomes.

DSG3−/− mice exhibit erosions of trauma-prone skin and hypotrichosis, as well as suprabasal blisters on mucous membranes including the mouth, genitilia, nostrils, and conjunctiva (Koch et al., 1997). Compared with DSG3−/− mice, the DSG3-deficient patient had a milder phenotype, suggesting that humans harbor superior compensation abilities with respect to desmosomal cadherins. Compensatory up-regulation of DSG3 and DSC3 was observed in Netherton syndrome caused by mutations in SPINK5, which encodes a protease inhibitor LEKTI, thereby showing protease-mediated degradation of DSG1 and DSC1 (Hachem et al., 2006). Both immunoblotting and immunofluorescence showed DSC2 and DSC3 up-regulation in the patient's mucosa, suggesting that loss of DSG3 may induce compensatory overexpression of other desmosomal cadherins.

Desmosplakin, plakoglobin, and plakophilin are the major components of cytoplasmic plaques in desmosomes. Suprabasal acantholytic blisters due to mutations in DSP (encoding desmosplakin), JUP (encoding plakoglobin), and PKP1 (encoding plakophilin 1) have been reported. Loss-of-function mutations in DSP and JUP cause lethal acantholytic blisters in the skin and mucosa (Jonkman et al., 2005; Pigors et al., 2011), whereas a loss-of-function mutation in PKP1 results in mild blisters in the skin (McGrath et al., 1997). Among the genetic suprabasal blistering diseases, mucosal involvement without skin lesions is a unique finding observed in DSG3 deficiency.

In conclusion, we report a patient with an autosomal recessive, homozygous nonsense mutation in DSG3, which is characterized by suprabasal acantholytic blisters limited to the oral and laryngeal mucosa. This case also shows the crucial role of DSG3 in maintaining epidermal integrity of human oral and laryngeal mucosa.

This study was approved by the institutional review board (no. 3-2017-0208) of the Yonsei University College of Medicine. The guardians provided written informed consent, complying with the principles of the Declaration of Helsinki.

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CONFLICT OF INTEREST
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SUPPLEMENTAL MATERIAL
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