

BRIEF ARTICLES

Mislocalization of Adherens Junction-Associated Proteins in a Patient with Darier Disease

George D. Glinos BS^a, Irena Pastar PhD^a, Marjana Tomic-Canic PhD^a,
Rivka C. Stone, MD PhD^a

^aDepartment of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL

ABSTRACT

Darier disease (DD) is an autosomal dominant keratinizing genodermatosis that manifests clinically with red-brown pruritic papules in a seborrheic distribution often in association with palmoplantar pits and dystrophic nail changes. It is caused by mutation in *ATP2A2* which encodes a sarco/endoplasmic reticulum calcium ATPase isoform 2 (SERCA2) pump that regulates calcium flux. Consequent alteration of intracellular calcium homeostasis is thought to impair trafficking of cellular adhesion proteins and to lead to aberrant keratinocyte differentiation, contributing to the characteristic histopathologic features of acantholysis and dyskeratosis in DD, though the precise mechanisms are incompletely understood. Previous studies have identified defective localization of desmosomal attachment proteins in skin biopsies and cultured keratinocytes from DD patients, but reports of effects on adherens junction proteins (including calcium-dependent E-cadherin) are conflicting. Here we describe a case of DD presenting with characteristic clinical and histologic features in which we performed immunofluorescence staining of four adherens junction-associated proteins (E-cadherin, α -catenin, β -catenin, and vinculin). In lesional (acantholytic) DD skin, we identified loss of distinctive bright membranous staining that was present at the periphery of keratinocytes throughout the epidermis in the healthy skin of a matched donor. Perilesional (non-acantholytic) portions of DD skin partially recapitulated the normal phenotype. Our findings support a role for SERCA2 dysfunction in impaired assembly of adherens junctions, which together with defective desmosomes contribute to acantholysis in DD.

INTRODUCTION

Darier disease (DD) (Darier-White disease; keratosis follicularis) is a rare keratinizing genodermatosis with autosomal dominant inheritance and complete penetrance but variable expressivity¹. DD manifests clinically as red-brown pruritic papules in a seborrheic distribution which often become secondarily infected and malodorous¹, in association with palmoplantar pits and characteristic dystrophic nail changes^{1,2}. DD is caused by mutation in *ATP2A2*, which encodes sarco/endoplasmic reticulum calcium ATPase isoform 2 (SERCA2), a pump located on the endoplasmic reticulum (ER) membrane that regulates calcium flux^{2,3}. Consequent defective calcium homeostasis is thought to lead to impaired ER trafficking of cellular adhesion proteins², aberrant keratinocyte differentiation^{2,4}, and disordered apoptosis^{2,3}. These defects may account for the histologic findings in DD: acantholysis, leading to the formation of suprabasal clefts^{2,3}, and dyskeratosis, with presence of corps ronds and grains^{2,3}. Prior studies have reported loss of membranous localization of desmosomal proteins in acantholytic DD lesions as well as in DD keratinocytes *ex vivo*, but conflicting findings have been reported for adherens junction-associated proteins⁵⁻⁷. Here we describe a case of DD with characteristic clinical presentation and histologic features on skin biopsy, in which we performed immunofluorescence staining of several adherens junction proteins in order to further characterize the *in vivo* effects of SERCA2 dysfunction on their cellular localization.

CASE REPORT

A 61 year old Caucasian man presented to our clinic for evaluation of a chronic eruption on the chest and legs that had appeared 15

years prior and had worsened over the preceding 3 years. The eruption was pruritic, exacerbated by heat and friction, and had not been previously treated. He also complained of fragile nails that broke easily with minimal trauma. Family history was notable for a similar eruption in the patient's father and paternal grandfather. Physical examination revealed erythematous keratotic crusted papules on the chest and back, excoriated lichenified plaques on the legs, and verrucous papules over the shins. White and red longitudinal bands in association with splitting, ridging, and distal V-nicking were observed on the nail plates of multiple fingernails (Figure 1). Bacterial cultures of the lesions grew *S. aureus*. Biopsies of involved skin on the chest, back, and legs revealed suprabasal acantholysis with dyskeratosis (Figure 2), and a diagnosis of DD was made.

Immunofluorescence staining of four cellular adherens junction-associated proteins (α -catenin, β -catenin, intracellular (cytoplasmic) tail of E-cadherin, and vinculin) was performed on formalin-fixed paraffin-embedded sections from the patient's biopsy specimen featuring "lesional" acantholytic clefting in association with histologically normal-appearing "perilesional" skin (Figure 3). Localization of each protein in lesional and perilesional DD was compared with localization in normal skin obtained from an age- and gender- matched healthy control. In the healthy control, E-cadherin, α -catenin, and β -catenin were localized to the cell membrane as expected, displaying a "chicken wire" pattern of uniformly bright staining around the keratinocyte borders. Vinculin localized to the membrane and perinuclear areas with a thicker peripheral staining. In contrast, in DD, the intracellular

portion of E-cadherin displayed intense membranous and associated diffuse cytoplasmic staining at the acantholytic cleft, with loss of membranous staining and faint cytoplasmic staining in the underlying epidermis. Perilesional skin showed restoration of membranous staining throughout the epidermis, with enhanced staining intensity of keratinocytes below the stratum corneum. Similar patterns were observed for α - and β -catenin, with more subtle signal enhancement in the upper portion of the epidermis. Vinculin remained perinuclear in lesional skin but there was loss of distinct strong peripheral staining, which was recapitulated in keratinocytes of lower portions of the epidermis in perilesional skin.

Management options for DD were discussed with the patient. He declined treatment with systemic retinoids due to concern over medication toxicities. He deferred genetic testing for himself and for other family members because of financial considerations. He was given a 1-week course of oral antibiotics for impetiginization. Topical triamcinolone 0.1% ointment was initiated to involved areas on the chest in combination with tretinoin 0.025% ointment to lichenified lesions on the legs. The patient was satisfied with his response to this regimen and was subsequently lost to follow-up.

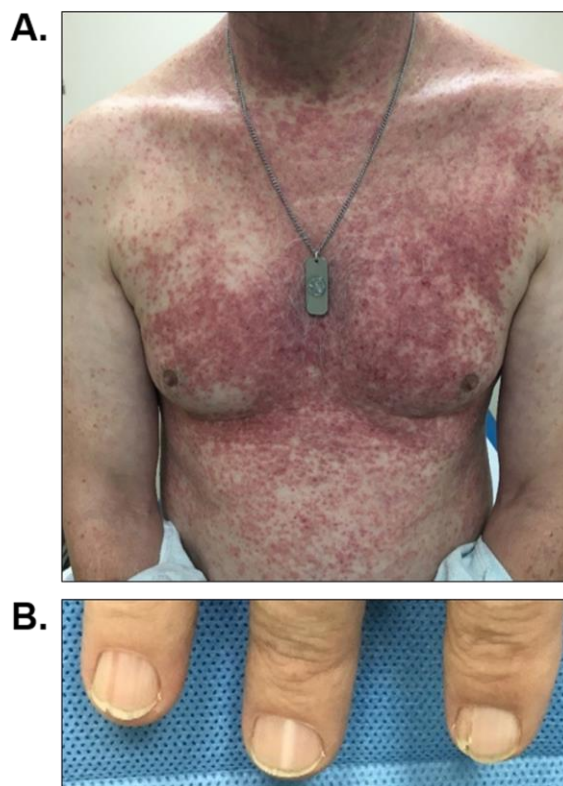


Figure 1. Darier Disease skin and nail findings. (A) Red-brown keratotic coalescing papules in a seborrheic distribution. (B) Red and white longitudinal bands, splitting, and distal V shaped nicks were most apparent in the nail plates of the patient's right hand.

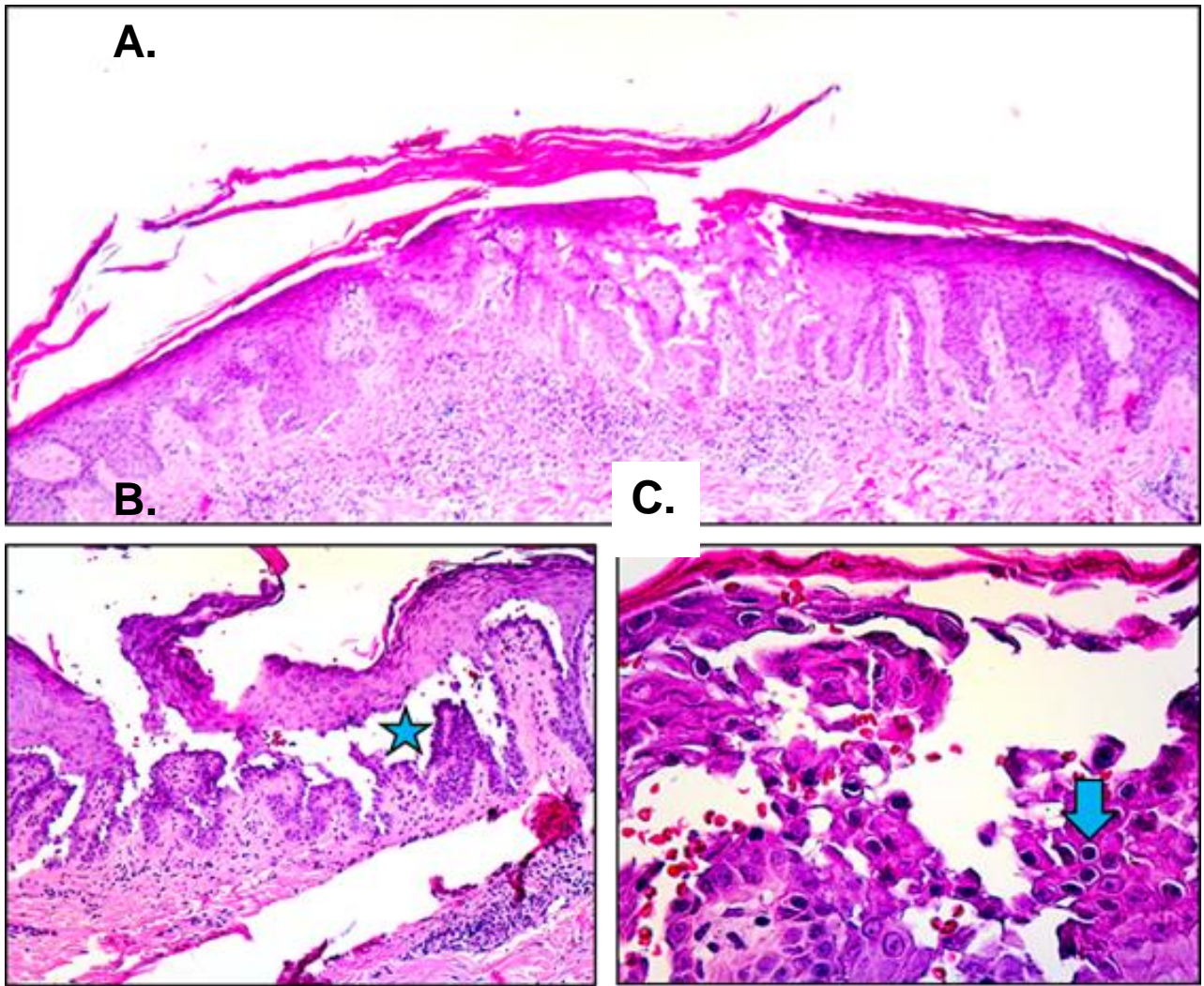


Figure 2. (A) Histologic findings in DD skin lesions. Predominant features include (B) acantholysis with suprabasal clefting (star) and (C) dyskeratosis with presence of epidermal corps ronds (arrow) and grains.

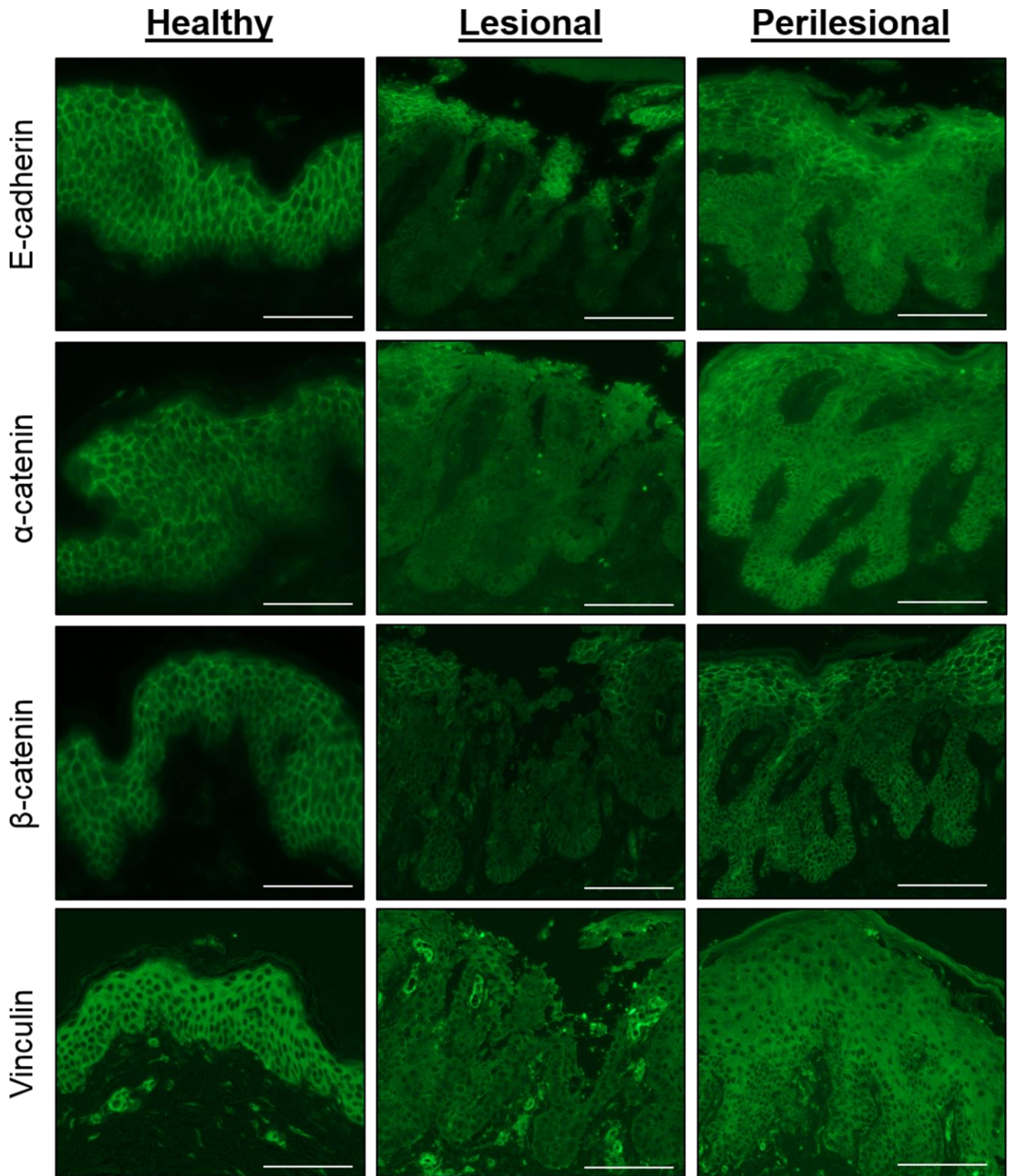


Figure 3. Adherens junction-associated proteins in DD. Immunofluorescence staining of E-cadherin, α -catenin, β -catenin, and vinculin in healthy donor skin, in DD lesional (acantholytic) skin, and in perilesional (non-acantholytic) DD skin. Original magnification 20x; scale bars = 100 μ m.

DISCUSSION

Despite its rarity, DD has been well described in the medical literature for over 100 years. The causal *ATP2A2* mutation is known, but the pathophysiologic impact on clinical phenotype is incompletely understood³. One proposed mechanism is that reduced Ca^{2+} influx into the ER lumen induces a stress response, which causes mis-folding and impaired cellular trafficking of proteins². This has been supported by studies in DD keratinocytes demonstrating constitutive ER stress in association with cytoplasmic retention of junctional proteins and formation of immature adhesion complexes⁶. In fact, the loss of membranous localization of desmosomal attachment plaque proteins (e.g. desmoplakins, plakophilin, plakoglobin) as well as desmosomal cadherins (desmoglein 1/3, desmocollin 1) has been well-described in acantholytic portions of histologic sections from DD skin biopsies^{5,7,8}, with normal localization maintained in perilesional skin⁵ as well as relative maintenance of tight junctions⁸. However, there are conflicting reports regarding preservation versus loss of adherens junction protein localization in DD^{5,6,8}. In healthy skin, in response to calcium flux, the cytoplasmic (intracellular) tail of E-cadherin interacts with β -catenin which binds to α -catenin, associating E-cadherin with actin-binding proteins including vinculin to link the cytoskeleton and produce intercellular adhesion⁹. In contrast with previous findings of preserved membranous staining of E-cadherin, α - and β -catenin throughout the DD epidermis⁵, we observed alterations in the localization of these proteins in our patient, with intense membranous and cytoplasmic staining at the

acantholytic cleft and dim diffuse cytoplasmic staining below. Interestingly, while the membranous staining was maintained in healthy-appearing perilesional skin, there was enhanced intensity at the superior portion of the epidermis, perhaps indicating keratinocytes at the threshold of acantholysis and cleft formation. We similarly observed reduced peripheral staining of vinculin in lesional skin as well as in pre-acantholytic superior portion of perilesional skin. Though our study is limited to observations in a single patient, our findings support a role for SERCA2 dysfunction in the impaired cellular trafficking of at least four adherens junction-associated proteins which contributes to the loss of cell-to-cell adhesion in DD. Further studies in additional DD patients are warranted to confirm our findings.

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Conflict of Interest Disclosures: None

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Corresponding Author:

Rivka C. Stone, MD, PhD
 Department of Dermatology and Cutaneous Surgery
 University of Miami Miller School of Medicine
 1600 NW 10th Ave, RMSB 2029A
 Miami, FL 33136
 305-243-7295 (Office)
 rivka.stone@med.miami.edu

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SUPPLEMENTARY MATERIAL: METHODS

IMMUNOFLUORESCENCE STAINING

8 μm thick formalin-fixed, paraffin-embedded tissue sections from skin biopsies were de-paraffinized with xylene (EMD, Gibbstown, NJ) and rehydrated. For antigen retrieval, sections were heated at 95°C in 0.01 M sodium-citrate solution (Sigma-Aldrich, St. Louis, MO) for 30 minutes. Sections were blocked with 5% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO), and separate sections were subsequently incubated overnight with anti-vinculin (V9131; Sigma-Aldrich, St. Louis, MO), anti- α -catenin (ab3236; Cell Signaling Technology, Danvers, MA), anti- β -catenin (ab9587S; Cell Signaling Technology, Danvers, MA), and anti-E-cadherin (ab610181; BD Biosciences, San Jose, CA) primary antibodies. Signal was visualized with anti-rabbit and anti-goat Alexa-Fluor 488 secondary antibodies (Invitrogen, Carlsbad, CA) diluted 1:500 in BSA. Slides were mounted with media containing 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA) to visualize cell nuclei. Images were captured with the EVOS FL Auto Imaging System/EVOS FL Auto Software (Thermo Fisher Scientific, Waltham, MA).

SKIN SPECIMENS

Skin biopsies from a patient with Darier Disease were initially obtained for diagnostic purposes after informed consent was obtained, and the patient further consented to the use of these specimens for immunofluorescence staining as well as to the use of the clinical images in this study. Healthy human skin was obtained after informed consent from a donor undergoing an elective surgery, with approval of the University of Miami Institutional Review Board (Protocol# UM20070922).