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Article

Novel ATP2A2 Gene Mutation c.118G>A Causing Keratinocyte and Cardiomyocyte Disconnection in Darier Disease.

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Abstract: Darier disease (DD) is an autosomal dominant disorder due to mutation of ATP2A2 gene causing an isolated skin manifestation based on keratinocyte disconnection and apoptosis. Systemic manifestations of DD have not been demonstrated so far although a high incidence of neuropsychiatric syndromes suggest an involvement of central nervous system. A Novel ATP2A2 Gene Mutation c.118G>A, causing keratinocyte and cardiomyocyte disconnection in Darier disease, is reported. Their common pathologic pathway, still unreported, was documented by both skin and left ventricular endomyocardial biopsy because of cardiac dilatation and dysfunction appeared several decades after skin manifestations. Keratinocyte disconnection was paralleled by cardiomyocyte separation at lateral junction. Cardiomyocyte separation was associated to cell disarray, sarcoplasmic reticulum dilatation and increased myocyte apoptosis. Clinically, hyperkeratotic skin papules were associated to chest pain, severe muscle exhaustion and ventricular arrhythmias that improved following administration of aminophylline, a phosphodiesterase inhibitor enhancing SERCA2 protein phosphorylation. Specific mutations of ATP2A2 gene may cause cardiac involvement in patients with DD. Cardiac pathologic changes are similar to those documented in the skin including cardiomyocyte disconnection that promotes precordial pain and cardiac arrhythmias. Phosphodiesterase inhibitors, enhancing SERCA2 protein phosphorylation may substantially attenuate the symptoms.

Keywords: Darier disease; Gene Mutation; SERCA2; cardiomyocyte disconnection

Introduction

Darier disease (DD) is an autosomal dominant disorder caused by mutation of *ATP2A2* gene, located on chromosome 12q23-24.1 and encoding for sarco/endoplasmic reticulum Ca⁺⁺ ATPase type 2 isoform. Mutations in the *ATP2A2* gene have been shown to cause severe disruption of calcium homeostasis by altering protein expression and/or the calcium transport function.

Clinically, DD manifests with hyperkeratotic papules in seborrheic areas, palmo-plantar pits and distinctive nail dystrophy. At histology it is characterized by loss of adhesion and apoptosis of keratinocytes (acantolysis) [1-2].

Consistent extra-cutaneous manifestations of Darier disease have not been documented so far [2] and specifically the heart has been reported as non-affected [3].

In the present study a novel mutation of *ATP2A2* is reported, severely affecting the skin and the myocardium. Clinical manifestations are supported by histological evidence of keratinocyte and cardiomyocyte disconnection. Clinical symptoms of muscle exhaustion, chest pain and cardiac arrhythmias have been attenuated by phosphodiesterase inhibitor administration.

Case Study

A 62-year-old lady was admitted because of palpitations and chest pain at rest, increasing with effort and limiting remarkably the quality of life and resulting in a depressive syndrome with a failed attempt of suicide. She was affected since the age of nine by Darier disease manifesting with extensive greasy, crusted, yellow-brown papules on the seborrheic areas. Beyond skin lesions, clinical examination was unremarkable, she was afebrile and her blood pressure was normal. Routine blood and chemical tests were within normal limits. Indices of inflammation like erythrocyte sedimentation rate and C reactive protein were normal. ECG showed sinus rhythm at 54 bts/min with abnormal ST segment deflection (-1,-1.5 mm) in the infero-lateral leads and negative T wave (Figure 2 Panel G).

Methods

Cardiac Studies

They included non-invasive (stress ECG, Holter monitoring, 2D-echo and Magnetic Resonance) and invasive investigations (Coronary angiography and left ventricular endomyocardial biopsy) after written informed consent. Specifically, five endomyocardial samples were drawn from left ventricular septum and processed for histology and electron microscopy as previously described [4]. Apoptosis of cardiomyocytes was assessed by hairpin probe as described [4].

Skin Biopsy

IT was obtained from an affected skin area after local anesthesia. It was used to confirm histological diagnosis of DD and compare skin with endomyocardial biopsy features.

Molecular Analyses

Genomic DNA was isolated from peripheral blood using the NucleoSpin Blood (Macherey-Nagel, Duren, Germany) according to the manufacturer's protocols. An opportunely designed custom TruSight One sequencing panel kit (Illumina, San Diego, CA, USA) was used to analyze a panel of 112 selected OMIM genes, including *ATP2A2* (MIM #108740) as well as other 111 genes involved in cardiac disorders (see Supp. Figure S1) for the list of cardiac genes included in the next-generation-sequencing [NGS] panel used for this analysis). The enriched libraries were sequenced by a NextSeq 500 instrument (Illumina, San Diego, CA, USA). NGS data analysis was performed using an in-house implemented pipeline as previously described [5,6].

Bidirectional Sanger sequencing was used to validate the identified variants and perform segregation studies. Sanger sequencing was performed using the ABI BigDye Terminator Sequencing Kit v.3.1 (ThermoFisher Scientific) and an ABI 3130 (ThermoFisher Scientific). Primer sequences and PCR conditions are available upon request.

RNA Studies

Total RNAs was extracted from PAXgene Blood RNA Tubes (Qiagen, Hilden, Germany) using the PAXgene Blood miRNA Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. cDNA was synthesized using the Superscript III first-strand synthesis kit (ThermoFisher, Waltham, MA, USA). A SYBR green-based real-time quantitative PCR (RT-qPCR) assay was used to measure *ATP2A2* mRNA (NM_001681.3) levels in peripheral blood using two primer sets spanning the junctions between exons 1-2 and 17-18, respectively (the primer sequences are available upon request). Each primer set was run in triplicate in two independent experiments using a 7900 HT Fast

Real-Time PCR Systems (Thermo Fisher, Waltham, MA, USA). The expression ratio of the target genes was calculated by using the $2^{-\Delta\Delta C_t}$ method [7] considering actin as reference gene.

Protein Isolation and Western Blot

Heart tissue samples were treated as described [8]. The expression of sarcoplasmic or endoplasmic reticulum calcium (SERCA-2) antibody was visualized by using a mouse monoclonal antibody (1:100, Santa Cruz Biotechnology) and Anti- α -sarcomeric actin (1:500, Sigma-Aldrich), antibody was used for normalization. Signal was visualized using a secondary horseradish peroxidase-labeled goat anti-mouse antibody (goat anti-mouse IgG-HRP 1:5000, Santa Cruz Biotechnology) and enhanced chemiluminescence (ECL Clarity Biorad). The purity as well as equal loading of the fraction was determined by measuring β -actin protein levels. Digital images of the resulting bands were quantified by the Image Lab software package (Bio-Rad Laboratories, Munchen, Germany) and expressed as arbitrary densitometric units.

Results

Cardiac Studies

Stress ECG was stopped at 2.5 min of 25 Watts (heart rate 60/bts/min, double product 6000) because of muscle exhaustion and worsening of precordial pain associated to increased (- 2 mm) deflection of ST segment in V4-V6 leads. (Figure 2, panel G).

Holter monitoring (24 hs) registered frequent (3200) supraventricular and polymorphic ventricular (2830) ectopic beats with some couples and triplets.

Cardiac Magnetic Resonance observed normal thickness of left ventricular wall (9 mm for ventricular septum and posterior wall) and normal myocardial mass (64.5 g/m² BSA; nv 34-70), apical hypokinesis with depressed LV function (EF 40%) (Figure 2 panel A,B),while failed to show signal abnormalities in T1 , T2 mapping and LGE after gadolinium infusion.

Skin biopsy showed loss of adhesion with dystrophic changes of dermal cells (Figure 1, panel A,B) confirming the diagnosis of DD.

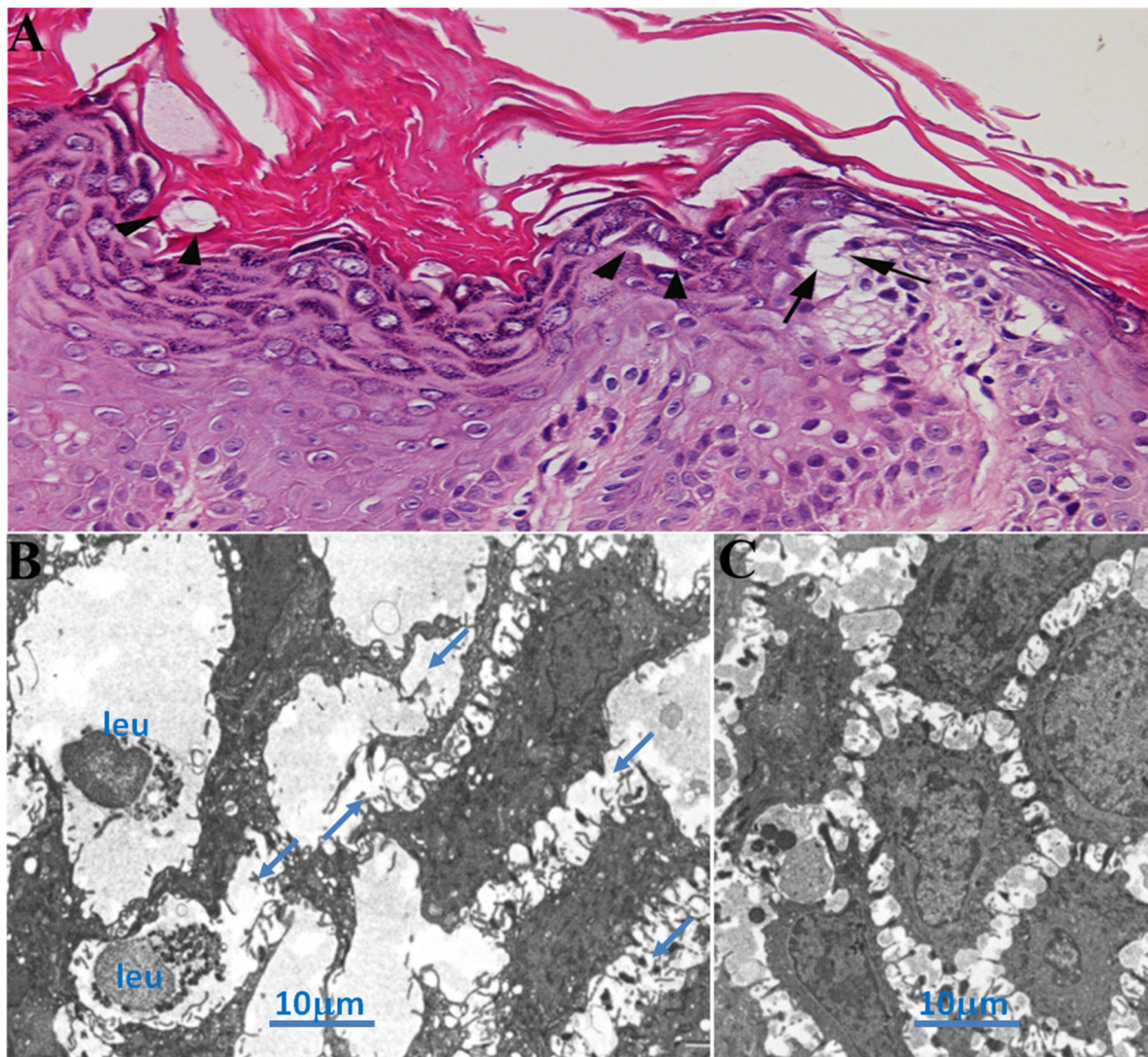


Figure 1. Panel A: Keratinocytes disconnection in Darier's disease. (A) Characteristic round corps (arrows) are present in the upper malpighian layer and stratum corneum. **Panel B:** Stratified squamous epithelium at level of dermatologic lesion. Cell processes from keratinocytes interacting through desmosomes appear mostly disorganized. Moreover, extracellular spaces appear broadly enlarged as compared to the control skin (**panel C**), containing some leukocytes (left side of the figure). Arrows: disorganized junction between keratinocytes, leu= leukocyte.

Likewise, endomyocardial biopsy documented at histology focal disconnection of cardiomyocytes (Figure 2, panel C), losing their spatial organization because of unendothelialized spaces and appearing often in total disarray (Figure 2, panel D). Affected myocytes showed also some swelling and cytoplasm vacuolization.

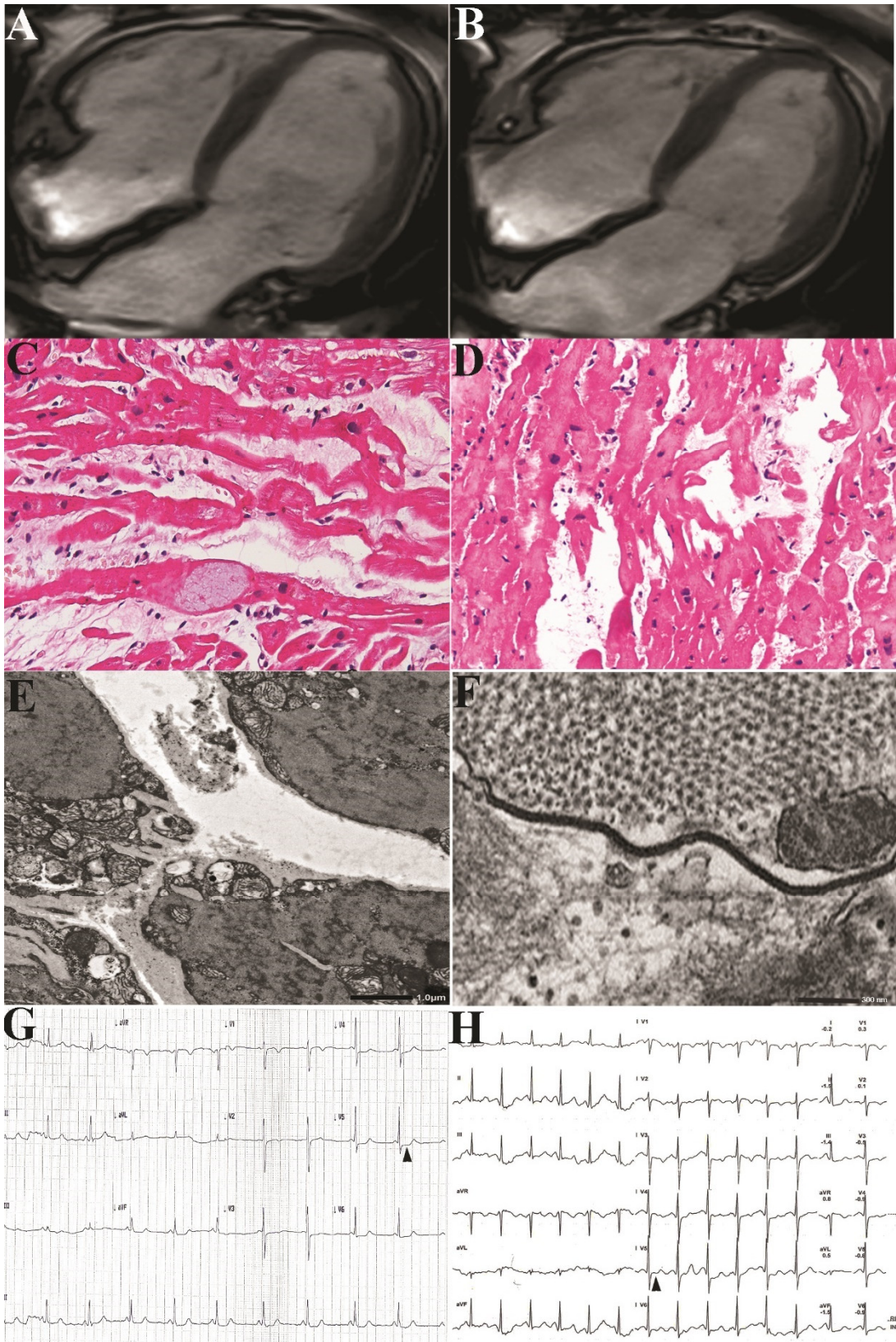


Figure 2. Panel A B:Cardiac magnetic resonance in diastole (A) and systole (B) showing reduction of left ventricular function (EF 40%). **Panel C-D :** show normal sized cardiomyocytes separated by unendothelialized spaces that cause cell disarray (D). **Panel E:** Electronmicroscopy of endomyocardial biopsy showing extracellular spaces between myocytes in Darier's disease compared with normal control (F). **Panel G:** Stress ECG showing ST segment deflection in V4-V6 precordial leads

associated to chest pain. **Panel H:** Painless increase in heart rate (117 bts/min) with isoelectric ST segment, following aminophylline administration.

At ultrastructural examination, myocyte detachment appeared to occur at cell lateral junctions while intercalated disks appeared structurally well preserved. Cytoplasm vacuoles were due to remarkable dilatation of sarcoplasmic reticulum that was the site of mutated *ATP2A2* protein and to perinuclear areas of myofibrilolysis. Myocyte apoptosis was significantly higher in our patient (1639±180 apoptotic nuclei) compared with normal controls 12±2 ($p < 0.05$).

Protein Studies (Western Blotting)

Protein expression of this sarcoplasmic protein failed to show significant quantitative differences compared with surgical control biopsies.

Gene Investigations

Exome sequencing on a sample from the proband identified a heterozygous G to A transition at the last nucleotide of exon 1 of *ATP2A2* gene (NM_170665.3:c.118G>A), which encodes the sarco/endoplasmic reticulum Ca(2+) ATPase type 2 isoform (SERCA2) (Supp. Figure S1). No pathogenic variant was found in the panel of cardiac genes (Supp. Table S2). Sanger sequencing confirmed segregation of the *ATP2A2* gene c.118G>A variant with DD phenotype in an affected sibling but not in two unaffected family members (Figure 3, panel A) This variant had not previously been reported in public databases (ExAC, gnomAD, 1000 Genomes Project, and NSEuroNet) and was predicted to affect splicing by in silico tools (Alamut version 2.10.0) (Supp. Figure S3). Concordantly to the prediction, analysis of the *ATP2A2* cDNA by real-time quantitative PCR showed that c.118G>A caused 36% to 40% reduced expression of *ATP2A2* mRNA in peripheral blood leukocytes (Figure 3, panel B), consistent with *ATP2A2* c.118G>A variant being a splicing defect associated with a decreased expression of *ATP2A2* gene. Based on these evidences, the variant was classified as pathogenic.

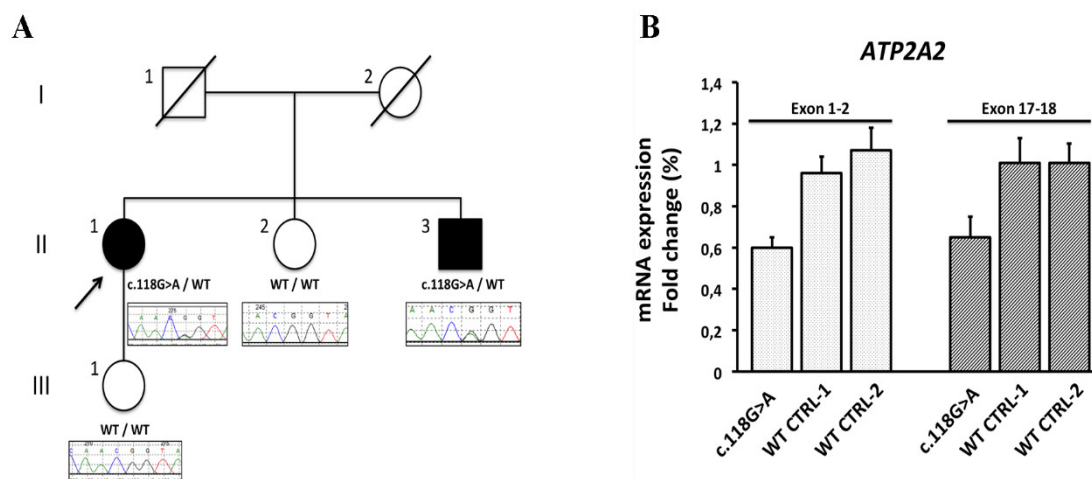


Figure 3. Panel A Pedigree of DD family (proband highlighted with arrow) along with partial sequence chromatograms showing segregation of *ATP2A2* mutation. The affected individuals are indicated by the solid symbol and unaffected individuals by open symbols. **Panel B** RT-qPCR *ATP2A2* mRNA level quantification. Histograms show the percentages of *ATP2A2* transcripts using two sets of primers designed to span the exon-exon junction between *ATP2A2* exons 1-2 and 17-18, calibrated on the actin gene ($\Delta\Delta C_t$ method) and calculated in the patient with c.118G>A mutation with respect to two wild-type healthy controls (CTRL-1 and CTRL-2). The *ATP2A2* mRNA product was detected in peripheral blood at percentages ranging from 60% to 65% in the patient with c.118G>A mutation (dotted bars) and from 96% to 107% in the wild-type healthy controls (hatched bars). Error bars represent mean \pm SD.

Treatment

Phosphodiesterase inhibitor aminophylline at the dosage of 200 mg bid was introduced to reduce degradation of cyclic AMP and improve phosphorylation and activity of ATP2A2.

After 2 weeks, the patient manifested a disappearance of chest pain, improvement in muscle function and reduction of cardiac arrhythmias (SV ectopic beats 280 and 180 V ectopic beats /24 hs without evidence of repetitive phenomena). At control stress ECG the patient was able to remarkably increase (2.3 times, heart rate.117/bts min., double product 14000) physical activity without the occurrence of precordial pain while ST segment remained at isoelectric line (Figure 2 panel H). Cardiac contractility at 2D-echo remained unchanged.

Discussion

Although Darier disease is known as a rare disorder limited to the skin, it is likely it may affect additional systems. Indeed, incidence of psychiatric manifestations is so high [5] that has been suggested that central nervous system might be involved as well. The present study identifies a novel *ATP2A2* (c.118G>A) gene mutation affecting both skin and myocardium. These tissues exhibit similar histological and ultrastructural changes consisting in alterations of keratinocyte and cardiomyocyte cell-to-cell interactions and suggest a pathway common to both tissues. To this regard it is known that function of proteins (such as connexins and tight junction proteins) responsible of lateral cell bonds are strictly Ca⁺⁺dependent and that disruption of Ca⁺⁺ homeostasis can compromise cell adhesion. Mutations in *ATP2A2* gene have been shown to cause severe disruption of CA⁺⁺ homeostasis by altering protein expression and/or the transport function in vitro [9,10] and lead to moderate depletion of calcium stores in keratinocytes of patients [11]. In DD, symptoms occur independently of *ATP2A2* mutation type or which aspect of SERCA2 functionality is compromised, suggesting that *ATP2A2* mutations exert their deleterious effect through haploinsufficiency, although also a dominant negative effect of the *ATP2A2* mutants has been proposed to underlie the DD phenotype [12]. In our patient, despite the cardiac involvement, we cannot infer which specific aspect of SERCA2 functionality is compromised. We proved that the *ATP2A2* c.118G>A mutation is associated to reduced mRNA expression at real-time quantitative PCR in peripheral blood, while the SERCA2 protein was normally expressed at western blot assessment, suggesting a sort of posttranscriptional adaptation that upregulate expression of the residual SERCA2 protein. At cardiac level, mutation consequences are derangement of cardiomyocyte organization (disarray) and remarkable intracellular changes. Specifically, at ultrastructural examination, sarcoplasmic reticulum appeared dilated and perinuclear areas of myofibrillogenesis were commonly seen. Noteworthy, structure of intercalated disks was preserved confirming cell detachment to occur at lateral cell junction. It may be argued that in mouse model of Darier disease [13] there was no histological report of cardiomyocyte disconnection as well as of physiological derangements. However, it must be relieved that there was no previous endomyocardial biopsy study in human with Darier disease and that our patient developed cardiac manifestations several decades after the appearance of skin lesions. This may suggest that cardiac involvement may occur later compared with skin damage.

Clinical implications from morphological analysis are that cardiac manifestations may occur in DD. These mimic ischemic heart disease with precordial pain worsened by effort, abnormal ECG changes and polymorphic, repetitive ventricular ectopic beats.

Interestingly, phosphodiesterase inhibitors like aminophylline, reducing degradation of cyclic AMP may abolish chest pain and improve vital capacity through enhancement of SERCA2 protein phosphorylation. In our patient, in addition to disappearance of precordial pain, double product increased by 2.5 times without abnormal deflection of ST segment at on therapy stress ECG.

In conclusion, specific mutations of *ATP2A2* may cause cardiac involvement in patients with DD. Cardiac pathologic changes are similar to those documented in the skin including cardiomyocyte disconnection that promotes precordial pain and cardiac arrhythmias.

Phosphodiesterase inhibitors, enhancing SERCA2 protein phosphorylation may substantially attenuate the symptoms.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the locally appointed ethics committee (opinion number 6/2019 and 2016-003014-28 (FARM12JCXN) and informed consent was obtained from all subjects.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: All authors

Conflicts of Interest: The authors declare that they have no competing interests.

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