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Darier disease: first molecular study of a Portuguese family

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ABSTRACT

Background: Darier disease (DD) is a rare autosomal dominant condition characterized by skin lesions. Additionally, a wide range of neuropsychiatric symptoms is frequently reported in DD patients. This genodermatosis relies on mutations in the ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (*ATP2A2*) gene, which encodes an ATPase responsible for pumping Ca^{2+} from the cytosol to the lumen of the ER.
Objective: Herein we studied the molecular aspect of a two-generation Portuguese family with DD history with clinical variability.
Methods: All exons and intron-exon borders of genomic *ATP2A2*, as well as coding *ATP2A2*, were sequenced. Relative levels of *SERCA2* mRNA and protein were quantified by qPCR and western blotting, respectively.
Results: The c.1287+1G > T variant was identified in all affected individuals, whereas the unaffected individual was shown to carry the wild-type *ATP2A2* sequence in both alleles. This variant leads to the skipping of full exon 10, which consequently generates a frameshift originating a premature STOP codon in exon 11 (p.V395 = fs*19). Although the mutant mRNA seems to partially escape degradation, results suggest synthesis inhibition or immediate degradation of the mutant protein. Neuropsychiatric and other occurrences affecting certain patients are also reported.
Conclusion: This is the first study of DD in Portugal, the variant identified, previously described in a single Japanese patient, may be considered a pathogenic mutation, and haploinsufficiency the mechanism underlying DD pathology in these patients. This study also highlights the co-occurrence of neuropsychiatric features in DD.

1. Introduction

Darier Disease (DD, OMIM #124200) is a rare autosomal dominant genodermatosis, with complete penetrance and variable expressivity. It is caused by heterozygous mutation in the ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (*ATP2A2*) gene, which encodes the Sarco/Endoplasmic Reticulum Ca^{2+} ATPase 2 (SERCA2), responsible for pumping Ca^{2+} from cytosol to the lumen of the ER. According to Leiden Open Variation Database v3.0 (http://www.lovd.nl/3.0/home), 420 public variants have been reported in the *ATP2A2* gene, of which 295 are unique.

ATP2A2 is transcribed into 21 exons and alternative splicing of the pre-mRNA generates multiple isoforms [1, 2]. These isoforms vary in their tissue expression pattern: SERCA2a has been found to be restrained to cardiac slow-twitch muscle, skeletal and smooth muscle [3], whereas SERCA2b is the ubiquitous isoform, expressed in non-muscle cells, including several areas of the brain [4], and highly expressed in the epidermis [1, 5].

At a molecular level, *ATP2A2* mutations lead to depletion of ER Ca^{2+} stores and ER stress, particularly in the skin [1]. Haploinsufficiency or dominant-negative effect have been the mechanisms postulated for DD pathology [6, 7, 8]. Histologically, acantholysis and dyskeratosis are the two major hallmarks observed in lesional epidermis [1]. Interestingly, both lesional and non-lesional DD keratinocytes appear to suffer ER Ca^{2+} depletion and ER stress, but only lesional keratinocytes exhibit changes in trafficking and maturation of desmosomal proteins [9, 10], suggesting the need for an interaction between secondary factors and the pathogenic variant.

DD runs a chronic and relapsing course after its onset, which usually occurs during the first or second decade of life, and may aggravate with age [3, 11]. Clinical manifestations include nail abnormalities and focal skin lesions, characterized as redish-brownish crusted keratotic papules,

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distributed in seborrheic areas of the body [1]. Skin lesions can be exacerbated by environmental factors, such as UV-B radiation, and are more susceptible to infection by bacterial, fungal, and viral microorganisms [3].

Additionally, a range of neuropsychiatric features such as depression, suicidal behavior, bipolar disorder, schizophrenia, intellectual disability, epilepsy, cognitive deficit, mental retardation, and learning disability have been reported in DD patients [12, 13, 14, 15]. Gordon-Smith et al. have found higher lifetime prevalence rates of mood disorders (50%), especially major depression (30%) and epilepsy (3%), to be present within a sample of one hundred unrelated DD patients compared with that in the general population [16].

Current treatments used to manage DD lack efficacy and safety: oral or systemic retinoids are the most frequently prescribed drugs, which nevertheless harbor a large number of noxious side effects and cannot be prescribed to women of child-bearing age [17].

In this study we aim to describe the molecular aspects, the clinical features and the neuropsychiatric occurrences within this two-generation Portuguese family carrying Darier disease.

2. Materials and methods

2.1. Participants

Five individuals from a two-generation Portuguese family carrying Darier disease agreed to enroll in this study: four affected relatives and one unaffected relative. This study was approved by the local Ethics Committee, and written informed consents were obtained from each participant in this study. The dermatologists who follow-up the patients were informed about the study and gave their consent.

Data concerning the clinical manifestations and the neuropsychiatric features of the affected individuals were gathered by medical note assessment and interview.

2.2. PCR and RT-PCR amplification

Nucleic acids were extracted from whole blood peripheral lymphocytes, collected from each individual. All exons and flanking intronic boundaries of genomic *ATP2A2* were amplified by PCR using 17 sets of primers designed with Primer3 program (http://primer3.ut.ee) (Table 1). PCR conditions were the following: initial denaturation at 98 °C for 30 s; 35 cycles of 98 °C for 10 s; annealing at 55 °C or 58 °C for 10 s; extension at 72 °C for 20 s; a final extension step for 5 min. Each PCR reaction was carried out in a total volume of 25 μL using the Phusion® High-Fidelity DNA Polymerase (New England Biolabs) protocol, and approximately 200 ng of genomic DNA. First strand cDNA was synthesized from total RNA (1μg) using Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The entire coding region of *SERCA2b* cDNA was amplified by PCR using six overlapping sets of primers, specifically designed with Primer3 program (Table 1). Each PCR was performed according to protocol for Phusion® High-Fidelity DNA Polymerase, with annealing temperatures of 60 or 61 °C, a final $MgCl_2$ concentration of 1.5 mM or 3.0 mM, in a total volume of 25 μL. RT-PCR products were run on 2% agarose gels for detection of splicing errors.

2.3. Sequence analysis

PCR and RT-PCR products were purified from solution or directly from agarose gel, using Isolate II PCR and Gel Kit (Bioline). PCR forward and/or reverse primers were added to the purified products from each individual sample and submitted to bi-directional Sanger sequencing (GATC Biotech). All chromatograms corresponding to PCR and RT-PCR fragments were carefully analyzed using the BLAST program (NCBI).

2.4. Quantitative PCR

Quantitative PCR was performed using Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific) and the CFX96 Touch™ Real-Time System (Bio-Rad). Two sets of primers were designed to amplify both mutant and wild-type transcripts (GGTGACAAGTTCCTGCTGA forward and AGTGTGCTTGATGACAGAGACA reverse), or the wild-type transcripts only (TGTGCTCTTTGTAATGACTCTGC forward and AGAAAGACCCTTCAATTCGGTATCA reverse). The set of primers designed strictly for wild-type transcripts allows the amplification of exon 10, which is missing in mutant mRNA, while the set of primers for both wild-type and mutant anneals in exons 6 and 7. Target complementary DNA sequences were normalized to beta-actin.

2.5. Western blotting

Whole blood peripheral lymphocytes were homogenized and proteins were isolated using RIPA buffer (Pierce, Thermo Fisher Scientific), containing protease and phosphatase inhibitors (Thermo Fisher Scientific). An amount of 25 μg, evaluated by bicinchoninic acid method of total

Table 1
PCR and RT-PCR primers and related parameters for amplification of genomic and coding *ATP2A2*, respectively.

	Exon	Forward Primer	Reverse Primer	Product size (bp)	Annealing Temperature (°C)
PCR amplification	1	gcaagaggaggaggggaga	ccatcttccctggctctccc	307	58
	2 + 3	cctccctcttgacacattgct	agacacagcttgcaactcca	354	
	4	catgttgggcaggttggtct	ccattgcactccagcctg	480	
	5	tgtccttgtgtctgttgcct	tgacaggaagggaggtgcta	490	
	6	ctcatttcagccgcctttt	aagggacagtgaggcaagag	202	
	7	ggtgggcatgaatgagaggt	agtgatggttggcagtgaaa	249	
	8	cagcgtcggtatttaagttggg	acaaagaaccacgacacgga	573	
	9	tgtttgcctttgtcctaagct	tgccacaccagatcctttaaa	225	
	10	ggggcgggaggaatcaatag	ctttcattccacccacccca	234	
	11	gacagattgtgcttttgtgga	gagagtaggacagtgcagaca	309	
	12 + 13	ttgccacccagtagtatcca	tgcagggtgtggacaaagaa	516	
	14	ggcaacaagagcgaaact	gaggctactatgtgcttgtg	478	
	15	tttgccaagagacctacgg	tttctgtcttgcccactccc	483	55
	16	ttttctggaggagggcg	agggcatctctgtctttttgc	426	
	17 + 18	ccggttaccatcactgtccc	tgactacacacaattccccgg	690	
	19 + 20	tactgccactgtgacacgtg	gaaatcccatcggtgcatgc	449	58
	21	tctagatgctaccctgtgtgg	tcagtcatgcacagggttgg	299	
RT-PCR amplification	1–6	aagaggaggaggggagac	ttcacctgtgagaattgactgg	676	61
	6–8	tggtgacaaagttcctgctg	tgcagactgacatctggtttg	629	60
	8–13	gctcttggaactcgcagaat	accccactctcgaatgacag	699	
	13–15	acccacattcgagttggaag	gccacaatggtggagaagtt	659	
	15–18	gtgaacgatgctcctgctct	aaagtccgggttgtcctctt	537	
	17–20	ctgctgcatggtggttcat	ttcagcagaaatagacagttgctt	663	

protein from each individual was mixed with Laemli loading buffer. Two sets of samples were studied as heated (95 °C for 5 min) and unheated samples, which were resolved in 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes (GE Healthcare Life Sciences, Amersham Hybond) at 300 mA for 100 min at 4 °C. Blots were blocked with a solution containing 5% bovine serum albumin, TBS (1x), and 0.1% Tween® 20 for 1 h at room temperature, followed by incubation with rabbit polyclonal anti-SERCA2 (dilution 1:10000; #4388 Cell Signaling). Membranes were probed with anti-rabbit IgG secondary antibody (dilution 1:2000; #7074, Cell Signaling Technologies, Inc.), followed chemiluminescent detection with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific). SERCA2 expression was normalized to beta-actin (dilution 1:4000; Sigma-Aldrich).

2.6. Genotype-phenotype correlation

Sequenced exons and flanking intronic boundaries from *ATP2A2* were analyzed closely in search for genetic variants other than the pathogenic mutation. Additionally, *ATP2A2* and SERCA2 expression levels were also considered for genotype-phenotype correlation.

3. Results

3.1. Clinical characterization of the DD patients

The pedigree structure of the two-generation family under study is depicted in Fig. 1. All affected family members were diagnosed by dermopathology during their first decade of life. These individuals shared a monomorphic dermatosis, notably symmetrical, containing multiple non-follicular hyperkeratotic papules with 2–4 mm in diameter, brownish, rounded and occasionally crusted, on skin usually healthy or erythematous skin during periods of inflammatory exacerbation. The lesions were distributed along the entire lateral cervical region, anterior thorax and lower abdominal region (especially flanks), appearing also in the scalp, and predominant in retroauricular region. Palms of the hands presented multiple millimetric depressions filled by keratin (palmar pits). Nails of the hands and feet presented discrete longitudinal leukonychia, fragility, fissure and dyskeratosis with V-shaped notches on the distal end of the nail plate. Relapses of greater clinical severity usually led to skin eruptions or infections by bacterial agents. Patients' clinical course was in line with the worsening of the disease over the years. Moreover, patients agreed that skin lesions were exacerbated by sweat, friction, and sunlight, particularly during the summer.

However, the clinical severity of the disease varied from patient to patient. At the skin level, patient I.2 had more relapses than his brother (I.3) whereas patient II.1's relapses were more frequent and more severe than those of his father (I.2). In addition to their skin phenotype, viral, immunologic, and neuropsychiatric occurrences were mainly observed in the younger patients (II.1 and II.3) (Table 2).

To manage DD, the patients took medicines prescribed by dermatologists such emollients, topical antibiotics, and retinoids such as acitretin, a derivative of vitamin A. They also avoided being exposed to the sun during the summer in order to prevent focal lesions to emerge or exacerbate. Patients with other occurrences, referred in Table 2, took additional medicines to manage these.

3.2. Splice-site mutation in ATP2A2 gene leads to exon skipping followed by frameshift and premature nonsense codon

In order to identify the pathogenic variant, we analyzed the genomic and messenger sequences of *ATP2A2* gene. Bi-directional Sanger sequence analysis of all exons and respective intronic boundaries revealed a substitution in the canonical splice donor site of the guanine (+1) for a thymine in intron 10 (c.1287+1G > T or IVS10+1G > T) (Fig. 2); no additional sequence alterations were detected in any

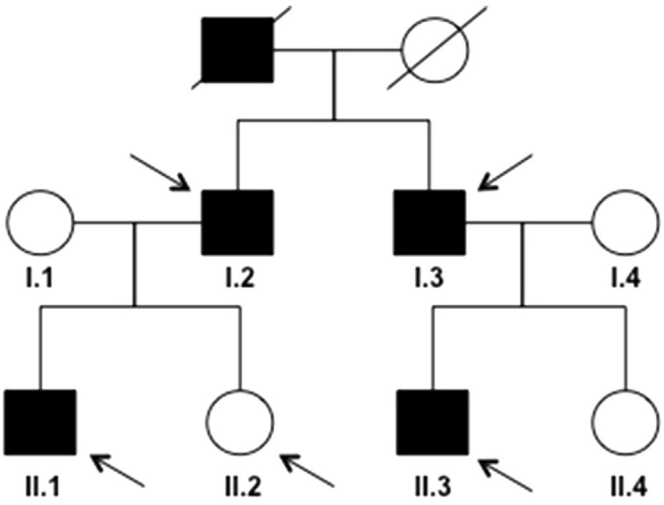

Fig. 1. Pedigree of the two-generation Portuguese family where Darier Disease segregates. The affected individuals are denoted by solid black symbols whereas unaffected individuals are denoted by open symbols. Arrows point to the individuals who participated in this study.

individual. Disruption of the mutant pre-mRNA splicing process was proposed by several public bioinformatic programs, namely Human Splicing Finder (http://www.umd.be/HSF3/) and NetGene2 (http://www.cbs.dtu.dk/services/NetGene2).

By analyzing RT-PCR products spanning exons 8 to 13 from *ATP2A2* gene, we confirmed that the splicing process is affected, leading to a shorter transcript (596 bp) in each affected relative (I.2, I.3, II.1 and II.3), whereas the unaffected relative (II.2) showed a normal RT-PCR fragment (699 bp) equivalent to two copies of the wild-type *ATP2A2* gene (Fig. 3a). Bi-directional sequence analysis of these RT-PCR products, whose normal and shorter fragments were directly purified from the gel, revealed the skipping of full exon 10 in the shorter transcript of each affected relative (Fig. 3b), whereas the normal fragment corresponded to the sequence derived from the wild-type *ATP2A2*. Therefore, all four patients were shown to carry the same mutation in heterozygosity, which, leading to the skipping of exon 10, consequently originates a frameshift, which putatively introduces a downstream premature stop codon (p.V395 = fs*19) in exon 11.

Furthermore, it was proved that the unaffected individual carries two normal alleles, what allowed us to use her as a control in subsequent analyses since she shares the same genetic background of all the patients.

3.3. Wild-type SERCA2 mRNA expression is significantly decreased while mutant mRNA appears to partially escape Nonsense-Mediated mRNA Decay (NMD)

We decided to examine the expression levels of both wild-type and mutant *SERCA2* mRNA by quantitative PCR. Results revealed a reduction of approximately 50–55% of wild-type plus mutant *SERCA2* mRNA expression when compared to the healthy subject, whose expression was considered 100 % (Fig. 4a). Relative expression levels of wild-type mRNA alone corresponded to approximately 36% in the patients, whereas the mutant mRNA expression corresponded to 18% (Fig. 4b). These findings were in agreement with what is seen in Fig. 3a, highly suggesting that a small amount of mutant *SERCA2* mRNA fails to be recognized by NMD, which could potentially lead to the generation of abnormal SERCA2 protein.

3.4. Wild-type SERCA2 is significantly reduced whereas mutant SERCA2 is not detected

If translated, the mutant *SERCA2* mRNA would originate an abnormal

Table 2
Additional information about participants' actual age, age of onset, and other phenotypic manifestations.

Individuals	Actual Age	Age of DD onset	Cutaneous phenotype	Neuropsychiatric co-occurrences	Other occurrences
I.2	56	1st decade of life, around six years old	++	None	Herpes simplex virus in the left eye
I.3	61		+	None	None
II.1	31		+++	Single suicide attempt Depressive moods	Membranous glomerulonephritis Left ear deafness Chronic left middle ear infection
II.3	34		+	Epilepsy	Leukemia
II.2	24	-	-	None	None

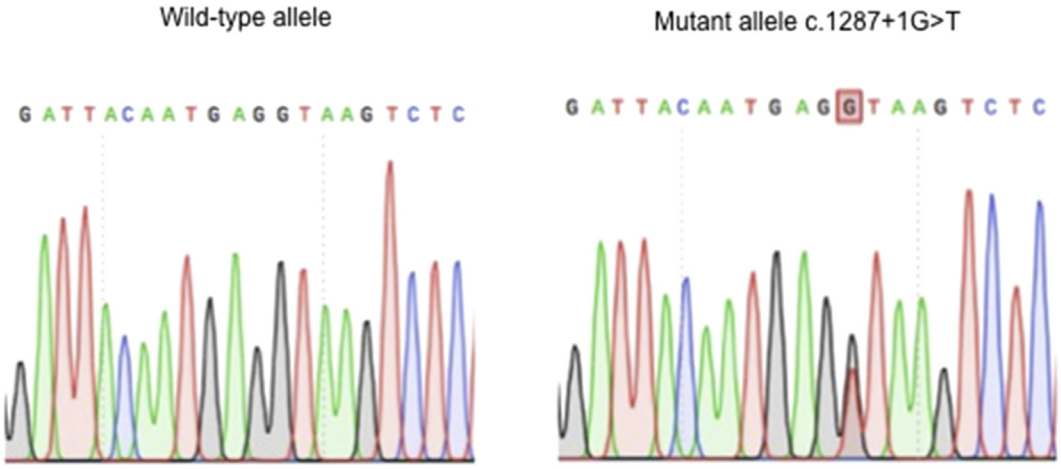

Fig. 2. Identification of ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (*ATP2A2*) pathogenic mutation. Sanger sequencing of the *ATP2A2* gene revealed the transversion c.1287+1G > T or IVS10+1G > T in intron 10.

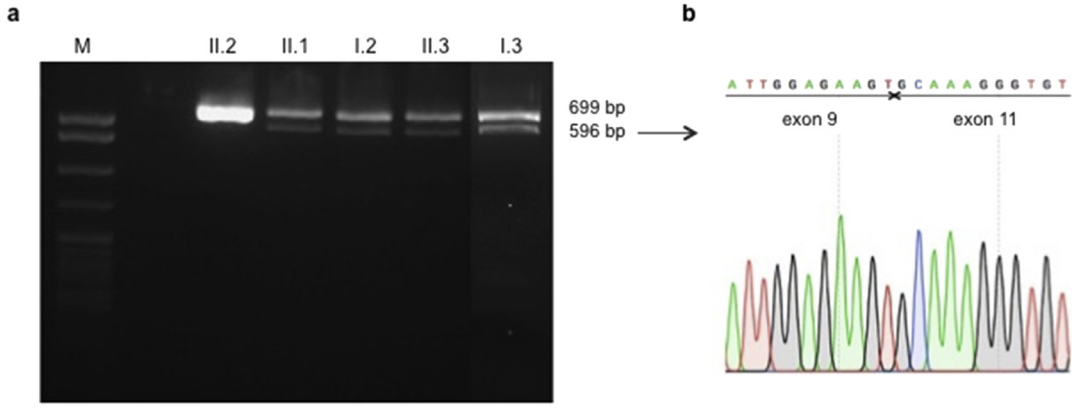

Fig. 3. The IVS10+1G > T variant leads to full skipping of exon 10. (a) Agarose gel electrophoresis of RT-PCR products, spanning exons 8 to 13, revealed the presence of two bands in the patients, the bigger one corresponding to the wild-type transcript and the shorter one to the mutant transcript, whereas the unaffected member revealed a single band corresponding to the wild-type transcripts. M- Molecular marker (pBR322 x MspI, New England Biolabs). (b) Sequence analysis of the shorter DNA fragment, purified directly from the gel, revealed the skipping of the entire region of exon 10 in every affected relative.

SERCA2 pump truncated in the C-terminal end, containing 413 amino acids rather than the expected 1042 (SERCA2b). The amino acid sequence would be normal until Val395; it would lack the residues encoded by exon 10, which form part of the phosphorylation domain; and the sequence encoded by exon 11, from residue 396 to 413, would be completely abnormal. All mutant SERCA2 splicing variants would lose highly conserved amino acids and motifs hence it would no longer have most of phosphorylation domain, and completely lack the nucleotide-binding and hinge domains, the fifth stalk domain, the transmembrane domains 5 to 10, or 11 in case of SERCA2b.

Therefore, when assessing expression levels of SERCA2, it might be expected to observe a band around 115 kDa, corresponding to the wild-type pump, and another band of 45 kDa, corresponding to the mutant pump. Nevertheless, western blot results showed no signal of mutant protein in the affected relatives, whereas wild-type SERCA2 presented significantly reduced expression levels, between 40 and 50%, when compared to the healthy individual (Figs. 5a and 5b). Our results showed that the mutant *SERCA2* mRNA escaping to NMD is not translated or, if translated, is very unstable and immediately degraded.

3.5. Proposed pathogenic mechanism

Besides gain of function mutations, autosomal dominant disorders

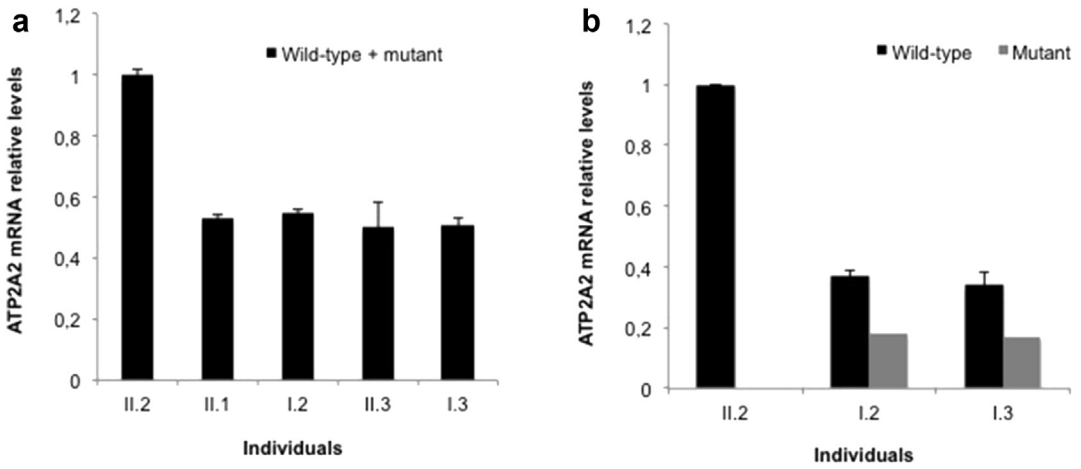

Fig. 4. All patients showed Sarco/Endoplasmic Reticulum Ca^{2+} ATPase 2 (*SERCA2*) mRNA levels lower than 50% and residual levels of mutant mRNA. Quantitative PCR analysis was performed to quantify the expression levels of (a) both wild-type and mutant transcripts and (b) wild-type transcripts alone. The data are presented as the mean ± SD of three independent experiments.

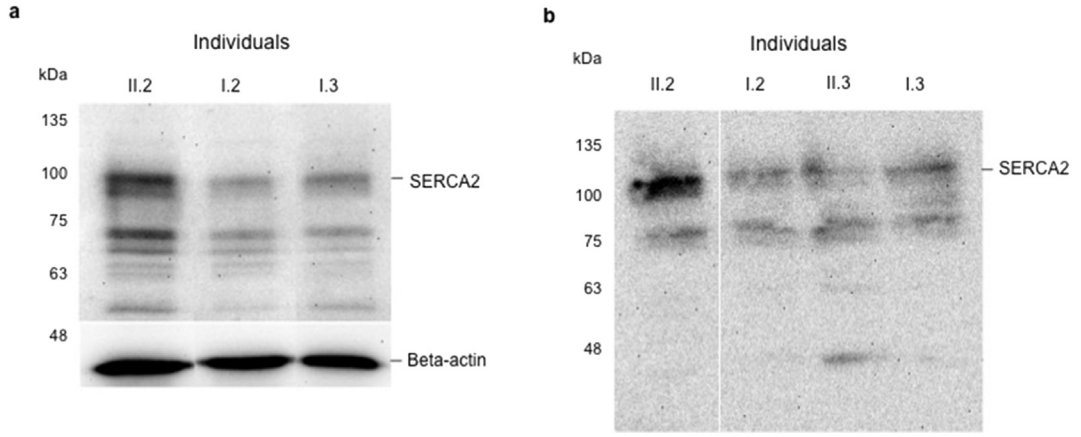

Fig. 5. SERCA2 expression was increasingly reduced in patients when compared with the unaffected relative and the presence of the putative truncated protein was not detected in Darier Disease patients. (a) Wild-type SERCA2 was significantly reduced in affected individuals. Lymphocytes were lysed with 0.1% SDS and the resulting supernatants were resolved by 7.5% SDS-PAGE loaded with reduced, unheated samples. Anti-SERCA2 antibody was used for the western blots. After being washed, the same membrane was blotted with anti-beta-actin as the loading control. (b) Putative mutant SERCA2 (45 kDa) could not be detected by western blot. Blots were incubated with anti-SERCA2 antibody only in order to detect the putative mutant SERCA2. The marker used was NZYColour Protein Marker II (Nzytech). Note: no endogenous control was used because the objective was to detect the putative presence of the mutant SERCA2 protein.

can also be caused by loss of functions mutations originating either haploinsufficiency or dominant-negative effects. In this specific family where Darier disease segregates, and which is caused by the loss of function c.1287+1G > T or IVS10+1G > T mutation, we could observe that no abnormal variant protein was produced (Fig. 5b); accordingly, a dominant-negative effect may be excluded. So, haploinsufficiency appears to be the most plausible pathogenic mechanism causing this disease condition.

3.6. No genotype-phenotype correlation was found

Since all patients revealed similar levels of SERCA2 expression, we expected to find additional mutation(s) in the *ATP2A2* gene, which might explain or contribute to the variable phenotypic spectra displayed by the four DD patients. Nevertheless, after screening by Sanger sequencing all exons and respective boundaries, no additional polymorphisms were identified.

4. Discussion

One pivotal point in this study was the identification of the disease-causing mutation in these family. The c.1287+1G > T or IVS10+1G > T transversion fulfils several criteria for being considered a pathogenic variant, namely: it is located at canonical +1 splice site originating a null variant in the *ATP2A2* gene where loss of function is a known mechanism of disease [18, 19]; it had been previously identified in a Japanese female patient presenting the same phenotype of Darier disease [20].

Another point was to elucidate how c.1287+1G > T affects splicing and the pathogenic mechanism. This transversion immediately reduces the stability of base pairing of the 5'splice site with the complementary region of U1 snRNA [21] and subsequent consequences demonstrate how crucial the highly conserved splice donor site is to the splicing process. The reason why c.1287+1G > T led to exon skipping rather than cryptic splice site usage may rely on potential spliceosome's functional response influencing factors, such as local sequence context of the affected exon-intron junction and vicinities [22]; RNA secondary structure [22]; or open reading frame conservation [22]. The mutant mRNA contains a putative PTC located 74 bp upstream the 3′-most exon-exon junction (exon11-exon12). According to the 50–55 bp rule, a transcript containing a PTC located upstream 50–55 bp from the 3′-most exon-exon junction is immediately recognized by the NMD system [23]. However, herein, the mutant SERCA2 mRNA is not entirely degraded but the putative

abnormal protein is not detected; therefore, we predict that mutant SERCA2 is too unstable and immediately degraded by proteasomes, or that the translation of mutant mRNA is repressed by another surveillance mechanism [23]. Studies point that translational repression might depend on a putative *cis*-element residing in 3′-UTR, such as DNA transposons [23, 24]. Shen *et al.* revealed that miniature inverted-repeat transposable elements in the 3′-UTRs function as important regulators of translation, therefore these could be potential *cis*-element candidates [24]. Because putative mutant SERCA2 was not detected in the affected relatives, dominant-negative effect by SERCA2 oligomerization is unlikely to occur. Rather, although heterozygous *ATP2A2* knockout mice do not exhibit DD features [8, 25, 26], this study demonstrates that, in these patients, the identified splice-site mutation in *ATP2A2* appears to produce a dominant DD phenotype through haploinsufficiency.

The previous results, when compared with the main dermatological features of patients, strongly suggest that one single normal *ATP2A2* allele is not enough to maintain Ca^{2+} homeostasis in the skin, the organ mostly exposed to outside influence and where SERCA2b is expressed at higher levels.

Because all patients demonstrate similar levels of *ATP2A2* mRNA and protein expression, haploinsufficiency of SERCA2 by itself cannot explain the variable clinical severity observed among affected relatives. Nevertheless, the phenotype of heterozygous individuals becomes more prone to external influences, as it usually occurs in dominant inherited disorders [18]. Herein, we wanted to examine genotype-phenotype relationships by screening all exons and intronic flanking regions of *ATP2A2* gene for additional variants and try to correlate these with the patients' clinical history and different clinical severity. However, no additional polymorphisms were found. Nevertheless, *ATP2A2* contains several introns with extensive size that were not entirely sequenced. Thus, we cannot demonstrate whether additional deep-intronic variants or large insertions/deletions exist within these regions.

Although no genotype-phenotype relationship has been found, we highlight that, not only genetic factors play an important role in phenotypic variability, but also non-genetic factors. SERCA2 pleiotropy, impairment in a SERCA2 epistatic partner, epigenetic changes, or different lifestyles, could play a role in phenotypic variability.

In this two-generation family, some patients have developed non-cutaneous phenotypes that we find relevant to report. Depressive moods and epilepsy have been reported in patient II.1 and patient II.3, respectively. Disruption of intracellular Ca^{2+} homeostasis and function of adhesion molecules has been reported in both abovementioned neuropsychiatric conditions, epilepsy and depressive moods; moreover, it has already been described that the prevalence rates of these conditions are higher in DD patients than in general population [4, 17]. Because the other affected relatives do not show these symptoms, it seems that these may not be a direct consequence of the *ATP2A2* mutation alone. Rather, these phenotypes might need to occur in the presence of environmental factors and genetic variants other than the *ATP2A2* mutation, either from the same or different *loci*.

Renal complications have also been reported in DD patients [27, 28]. Herein we report a case of membranous nephropathy from patient II.1. We cannot conclude whether this disease occurs by chance or the opposite. It is well established, however, that ER stress is one of the major causes of kidney disease [29].

Additionally, a number of ocular features have been previously described in DD patients [30, 31, 32, 33]. We report a case of herpes virus simplex (HSV) infection in the cornea of the left eye of patient I.2 and alert for the relationship between possible impaired adhesion molecules in the corneal epithelium and the increased risk of HSV infection. Patient I.2 has been submitted to two transplants of the affected cornea.

Lastly, we report in patient II.1a case of chronic middle ear infection due to recurrent skin lesions in the ear, which have damaged the eardrum and reduced hearing capacity of the left ear.

In conclusion, this study identified the disease-causing mutation in this family and contributed to enlarge the worldwide mutational spectrum of DD. This study also confirmed the phenotypic variability of this pathology, namely reporting the presence of neuropsychiatric symptoms, thus highlighting the importance of a wide range of clinical occurrences deserving to be closely monitored.

Declarations

Author contribution statement

Andreia Almeida: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Maria Lurdes Lobo, Cecília Moura: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Isabel Rivera: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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