Mutations in the Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} ATPase Isoform Cause Darier's Disease

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Darier's disease is an autosomal dominantly inherited skin disorder, characterized by loss of adhesion between epidermal cells and abnormal keratinization. *ATP2A2* encoding the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA)2 has been identified as the defective gene in Darier's disease. All mutations previously reported occur in the region of *ATP2A2* encoding both SERCA2a and SERCA2b isoforms. These isoforms result from alternative splicing of exon 20, with SERCA2b being the major isoform expressed in the epidermis. In this report, we studied a family affected with Darier's disease and identified a deletion (2993delTG) in a region of exon 20 of *ATP2A2*, which is specific for SERCA2b. This heterozygous mutation predicts a frameshift with a premature termination codon (PTC + 32aa) in the eleventh transmembrane domain of SERCA2b. It segregates with the disease phenotype in the family members tested, and functional analysis shows a drastic reduction of the expression of the mutated protein in comparison with the wild-type SERCA2b. Our result suggests that the mutated allele causes the disease phenotype through loss of function of SERCA2b isoform. This finding indicates that SERCA2b plays a key role in the biology of the epidermis, and its defects are sufficient to cause Darier's disease. ***Key words: ATP2A2/SERCA2. J Invest Dermatol 121:486–489, 2003***

Darier's disease (DD; OMIM 124200) is an autosomal dominant skin disorder characterized by warty papules and plaques in the seborrheic area (central trunk, flexures, scalp, and forehead), palmoplantar pits, and distinctive nail abnormalities (Burge and Wilkinson, 1992). Onset is usual before the third decade, and penetrance is complete in adults, although expressivity is variable (Munro, 1992). Mildly affected patients have scattered keratotic papules, whereas those with more severe disease present with verrucous plaques or malodorous hypertrophic flexural disease. The typical histopathology includes focal areas of separation between suprabasal epidermal cells and abnormal keratinization. Immunohistopathology and electron microscopy reveal loss of desmosomal attachments and perinuclear aggregates of keratin filaments (Burge and Garrod, 1991).

ATP2A2, which encodes the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2 (SERCA2) pump, has been identified as the defective gene in DD (Sakuntabhai *et al*, 1999a). SERCA2 belongs to a family of P-type membrane bound ATPase, which pump Ca^{2+} from the cytoplasm into reservoirs in the endoplasmic reticulum (ER) lumen, using energy from ATP hydrolysis. Alternative splicing of exon 20 of *ATP2A2* (**Fig 1**) produces two isoforms, SERCA2a and SERCA2b, which differ only in their C-termini (Lytton and MacLennan, 1988). SERCA2b is the major isoform expressed in the epidermis from skin sections, although both isoforms are detectable in cultured keratinocytes and fibroblasts (Ruiz-Perez *et al*, 1999).

Mutation analysis in at least 100 families with DD has shown a variety of missense, non-sense, frameshift, and splicing mutations in *ATP2A2*, and considerable interfamilial and intrafamilial phenotypic variability of the disease (Jacobsen *et al*, 1999; Ruiz-Perez *et al*, 1999; Sakuntabhai *et al*, 1999a,b; Ringpfeil *et al*, 2001; Takahashi *et al*, 2001; Yang *et al*, 2001). Recently, we have shown that somatic mosaicism for *ATP2A2* causes segmental DD (Sakuntabhai *et al*, 2000). All these mutations occur within the first 20 exons of *ATP2A2* encoding both SERCA2 isoforms, predicting the loss of function of both isoforms.

In this report, we studied a family affected with classical DD and identified a mutation in a region of *ATP2A2* molecule, which is specific for SERCA2b. We also describe the effect of this mutation on the expression of the corresponding mutant SERCA2b in COS-1 cells.

MATERIALS AND METHODS

Clinical characteristics We studied a family of French extraction, affected with classical DD through three generations (**Fig 2**). The proband is a 54 y old male who presented with severe extensive hyperkeratotic papules and verrucous plaques of the neck, axillae, extremities, buttocks, and perineum. He developed extended erosions of the scalp and face. He also had oral lesion and nail involvement. The condition is exacerbated by sunlight, heat, sweating, and has been complicated by episodes of cutaneous herpetic and bacteria infection. Skin lesions developed when he was 17 y old and worsen with age. He also has learning difficulties and depression. He has been admitted to the hospital because of DD at least 20 times in the past 10 y. The histopathology of a skin biopsy confirmed the diagnosis of DD. Treatment with oral retinoid makes his disease

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Abbreviations: CSGE, conformation sensitive gel electrophoresis; DD, Darier's disease; SERCA2, sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase isoform 2.

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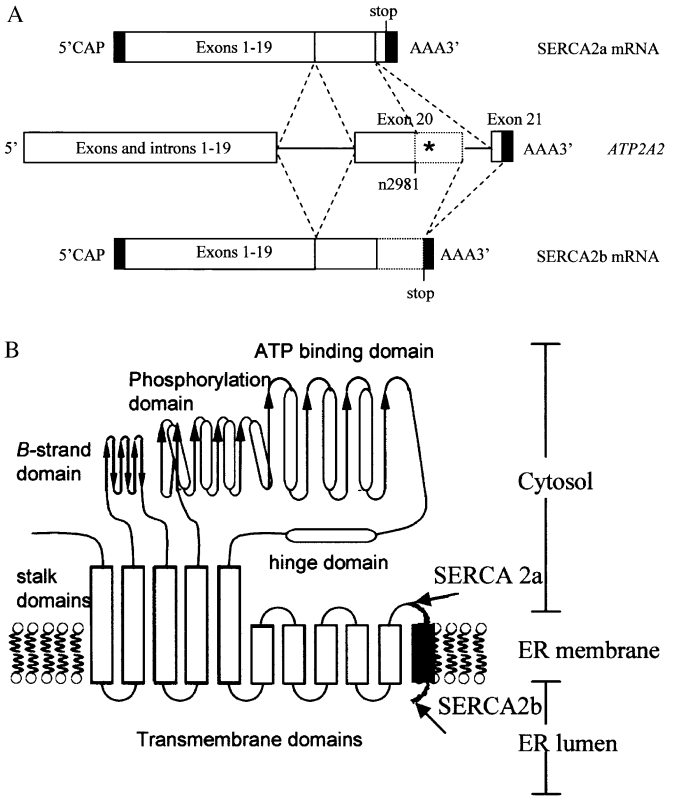

Figure 1. Splicing of *ATP2A2*. (*A*) Alternate splicing of *ATP2A2* transcripts at nucleotide position 2981 in exon 20 gives rise to two distinct isoforms, SERCA2a and SERCA2b. The mutation identified in this family (2993delTG) is indicated (*). Black boxes represent untranslated regions. (*B*) Model of the human SERCA2 polypeptide. The predicted secondary structure of SERCA2a consists of 10 transmembrane domains and a tetrapeptide tail located in the cytoplasm, whereas the SERCA2b tail comprises 49 amino acids extending into the ER lumen.

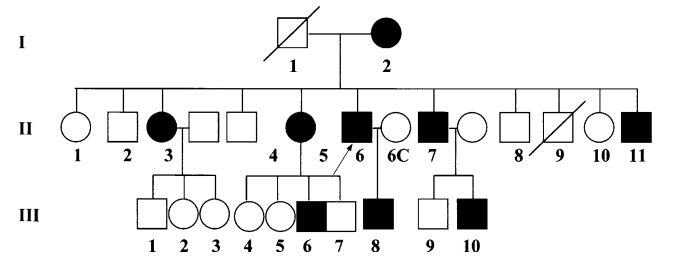

Figure 2. Pedigree of the family. Men are represented by squares and women by circles. The solid symbols denote the affected individuals.

stable. Other members of the family were affected with DD of varying severity. This study was approved by the Central Oxford Committee (C98–177).

Mutation analysis Blood samples were obtained from patients I.2, II.6, II.11, III.8, and unaffected individuals II.2, II.4, II.6C, II.8, and II.10 from the family (**Fig 2**). Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. Polymerase chain reaction (PCR) amplification of the 21 exons and flanking intronic splice sites of *ATP2A2* from a proband (II.6) was performed using previous published primers and conditions (Sakuntabhai *et al*, 1999b). *ATP2A2* was screened for mutations by conformation sensitive gel electrophoresis (CSGE), as described (Ganguly *et al*, 1993). PCR products showing abnormal electrophoretic mobility were gel purified using QIAquick PCR purification columns (Qiagen, Crawley, UK) and sequenced directly using the ABI PRISM AmpliTaq reaction dye terminator cycle-sequencing kit (PE Applied Biosystems, Warrington, UK) and an Applied Biosystems model 377 automated sequencer. All products were sequenced in forward and reverse orientations.

Construction of the *ATP2A2* mutant and expression in COS-1 cells The mutation was engineered into *ATP2A2* full-length SERCA 2b cDNA cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, Carlsbad CA) (kindly provided by Dr David MacLennan) by site-directed mutagenesis using QuickChange Site-Directed Mutagenesis Kit (Stratagene, London, UK). In order to verify that the mutation was correct and to ensure that no unwanted mutations had occurred, sequencing of mutant cDNA clone was performed using the previous published cDNA primers (Sakuntabhai *et al*, 1999b) as described above.

The wild-type and mutant cDNA clones were transiently overexpressed in the COS-1 cells by the calcium phosphate coprecipitation method (Sambrook and Russell, 2001). In brief, calcium phosphate-DNA coprecipitates were prepared by combining 100 μL of 2.5 M $CaCl_2$ with 25 μg of plasmid DNA and bringing the volume to 1 mL with sterile H_2O. This solution was mixed with equal volume of 2 × HEPES-buffered saline and the calcium phosphate-DNA suspension was added to the culture medium of COS-1 cells grown at 60% confluency in 90 mm dish. The transfection efficiency was about 60% to 70%. Cells were harvested at 72 h and microsomal fraction containing the overexpressed Ca^{2+} ATPase was isolated as described previously (Maruyama and MacLennan, 1988). Briefly, cells were washed twice with phosphate-buffered saline, swollen on ice for 10 min in a hypotonic solution of 10 mM Tris–HCl and 0.5 mM $MgCl_2$, and then homogenized in a glass Dounce homogenizer (Jencons, Bedfordshire, Leighton Buzzard, UK). The suspension was centrifuged at 9000 × *g* for 20 min to pellet nuclei and mitochondria and supernatant was centrifuged again at 100,000 × *g* for 1 h to sediment the microsome fraction. Microsomes were vesicles derived primary from the ER after cells were disrupted by homogenization.

Immunodetection analysis To determine the localization and the expression level of the mutant protein in comparison with wild-type SERCA2b, immunodetection analysis, including immunocytochemistry on intact COS-1 cells and western blotting of microsomal protein was performed as in a standard protocol using a monoclonal anti-SERCA2 antibody (CN Biosciences, Cambridge, UK), which recognizes both isoforms. We did not use SERCA2b antibody because this antibody was raised against a peptide corresponding to the last 12 amino acids (1031–1042) of the protein, which are expected to be missing in this mutant.

For immunofluorescence analysis, 48 h after transfection, COS-1 cells were fixed in methanol, and processed for indirect immunofluorescence and confocal microscopy. Briefly, cells were block in 0.2% bovine serum albumin in phosphate-buffered saline containing 0.1% Triton X-100. Bound mouse anti-SERCA2 antibody were detected using fluorescein isothiocyanate conjugated anti-mouse secondary antibodies. Cells were then viewed using a Nikon Optiphat Microscope and a 60 × oil objective operating on a Bio-Rad 1024 confocal system. Images were collected using the Bio-Rad Lasersharp software for viewing (Bio-Rad Laboratories, Hemel Hempstead Hertfordshire, UK). For western blot analysis, 10 μg of microsomal proteins were mixed with Laemli sample buffer, boiled for 5 min, resolved by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred on to PVDF membranes (Millipore, Watford Herts, UK). Blots were probed with primary antibody, and anti-mouse secondary antibodies linked to peroxidase (Amersham Pharmacia Biotech, Amersham, Buckinghamshire, UK) followed by enhanced chemiluminescent (ECL) blotting (Amersham Pharmacia Biotech).

Reverse transcription–PCR analysis Total RNA was extracted from transfected COS-1 cells using Trizol (Invitrogen, Paisley, UK). *ATP2A2* transcripts were amplified by reverse transcription–PCR using *ATP2A2* specific primers as previously described (Sakuntabhai *et al*, 1999b).

Ca^{2+} transport assay Ca^{2+} transport was measured at 37°C by a rapid Millipore filtration technique as described previously (Vilsen *et al*, 1989). Briefly, Ca^{2+} transport activity was initiated by addition of microsomes in a reaction mixture containing 20 mM 3-(N-Morpholino) propanesulfonic acid (MOPS) (pH 6.8), 100 mM KCl, 5 mM $MgCl_2$, 5 mM potassium oxalate, 5 mM ATP, 0.5 mM EGTA, 10^5 Bq^{45} Ca^{2+} per mL, and 0.45 mM $CaCl_2$ to set the free Ca^{2+} concentration at 3.6 μM. The reactions were terminated at times 5, 15, and 30 min by addition of ice cold stop solution containing 150 mM KCl and 5 mM $LaCl_3$ followed by micropore filtration through 0.45 μM Millipore filters and an additional washing with stop solution. The radioactivity associated with the filters was measured using a Beckman LS 6500 multipurpose scintillation counter (Beckman, Bucks, High Wyconbe, UK).

RESULTS

Identification of a TG deletion in *ATP2A2* CSGE analysis of *ATP2A2* amplified products from genomic DNA from proband II.6 (**Fig 3*A***) showed an abnormal electrophoretic mobility of exons 19 + 20. Direct sequencing of the PCR product revealed a TG deletion at nucleotide position 2993 (2993delTG), which resulted in a frameshift and downstream premature termination codon at nucleotide position 3091 (PTC + 32aa) (**Fig 3*B***). This mutation is present at the heterozygous state and occurs in the eleventh transmembrane domain of SERCA2b molecule. This mutation was also found in the other three affected individuals tested (I2, II.11, III.8) and was absent from the unaffected members (I.2, II.4, II.6C, II.8, II.10) as confirmed by CSGE (**Fig 3*A***) and sequencing (data not shown). No other change was detected in *ATP2A2* by CSGE.

Expression of 2993delTG mutant Immunocytochemistry on intact COS-1 cells (**Fig 4*A*–*C***) and western blotting of 10 μg of microsomal proteins (**Fig 4*D***) showed that the 2993delTG mutant was expressed at much lower level than that of the wild-type SERCA2b. Control COS-1 cells transfected with the empty vector showed minimal endogenous SERCA2 expression. We tried several concentrations of DNA, different transfection method (liposome-mediated), and subcloned cDNA into another expression vector, pMT2, but could not increase protein expression levels. All these experiments were repeated at least three times. Reverse transcription–PCR analysis of COS-1 cells transfected with the mutated cDNA showed no significant reduction in the amount of *ATP2A2* mRNA in comparison with COS-1 cells transfected with the wild-type SERCA2b cDNA (data not shown).

Ca^{2+} transport activity Oxalate-stimulated Ca^{2+} uptake was measured in microsomes isolated from COS-1 cells transfected with each of the following cDNA: wild-type SERCA2b, 2993delTG mutant cDNA, and the expression vector without any insert. The wild-type SERCA2b showed a Ca^{2+} uptake rate of 5.0 nmol Ca^{2+} per mg microsomal protein per min. In contrast, the mutant 2993delTG showed very low Ca^{2+} uptake (<0.1 nmol Ca^{2+} per mg microsomal protein per min) indistinguishable from that of microsomes isolated from COS-1 cells transfected with the expression vector without insert (data not shown).

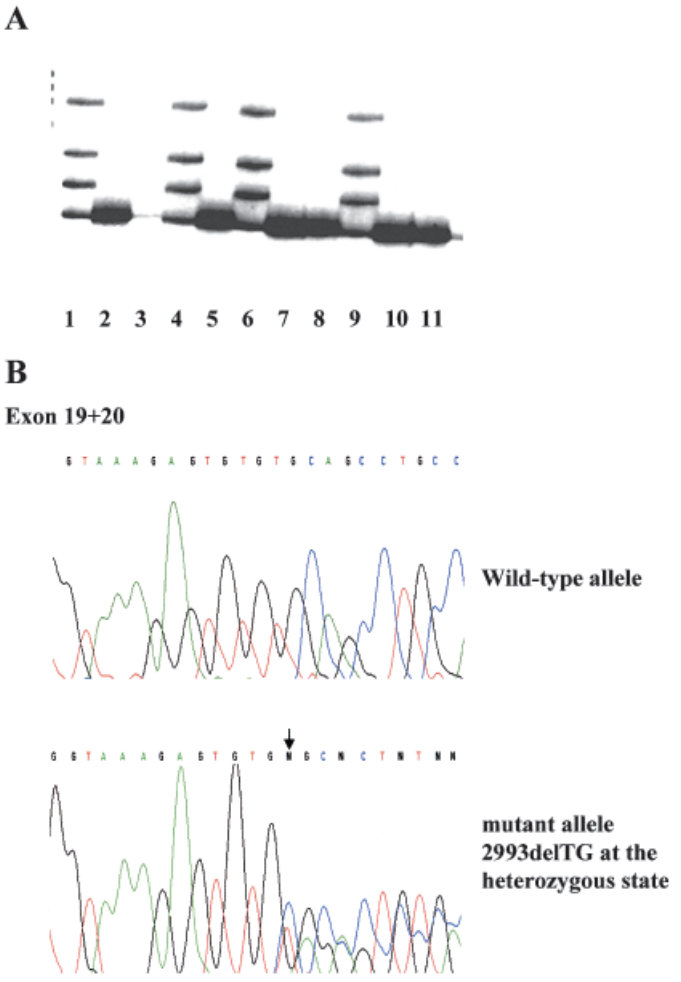

Figure 3. Identification of *ATP2A2* mutations. (*A*) CSGE showed abnormal electrophoretic mobility of exons 19 + 20 in affected individuals; I.2, II.6, II.11, and III.8 (*lanes 1, 4, 6, 9*) and normal electrophoretic mobility in unaffected individuals II.2, II.4, II.6C, II.8, and II.10 (*lanes 2, 3, 5, 7, and 8*). *Lanes 10* and *11* are from normal unrelated controls. (*B*) Direct sequencing of PCR products of exons 19 + 20 revealed a TG deletion at nucleotide position 2993 (2993delTG) at a heterozygous state. This mutation resulted in a frameshift and downstream premature termination codon at nucleotide position 3091 (PTC + 32aa).

DISCUSSION

We have shown for the first time that DD can arise from a heterozygous mutation that is specific for SERCA2b isoform. This result indicates that this isoform plays an important part in epidermal biology and that loss of its function is sufficient to cause DD.

SERCA enzymes belong to P-type class ATPase, which form a phosphoprotein intermediate and undergo conformation changes during the course of ATP hydrolysis. Three known genes encode for these SERCA pumps and differential splicing of primary transcripts gives rise to at least nine SERCA isoforms (MacLennan *et al*, 1997; Dode *et al*, 1998; Martin *et al*, 2002). Tissue-specific alternative processing of the *ATP2A2* transcripts results in the expression of the cardiac and slow-twitch skeletal muscle SERCA2a isoform and of the ubiquitous SERCA2b isoform in every other cell type. Immunocytochemistry of adult skin sections showed clear expression of SERCA2b in epidermal structures. Although SERCA2a was expressed at much lower levels in the epidermis, both isoforms were identified in cultured keratinocytes and fibroblasts (Ruiz-Perez *et al*, 1999). SERCA2a and SERCA2b isoforms share a common stretch of 993 amino acids, including 10 transmembrane segments and the catalytic site. The C-terminal of SERCA2a, however, consists of a tetrapeptide tail located in the cytoplasm, whereas the SERCA2b tail comprises 49 residues protruding into the ER lumen. The extended tail in SERCA2b contains a hydrophobic stretch that is suggested to be a possible eleventh transmembrane segment, and an asparagine residue at position 1036 forming part of a glycosylation consensus signal where it apparently interacts with, and is modulated by, calreticulin (John *et al*, 1998).

The *ATP2A2* mutation that we describe predicts a frameshift with a premature termination codon in the potential eleventh transmembrane molecule of SERCA2b. The mutation segregates with the disease phenotype in the family. The functional analysis of this 2993delTG SERCA2b mutant showed that the expression level of the mutant protein was markedly reduced in comparison with that of the wild type. This may be secondary to instability of the truncated protein harboring an abnormal C-terminal tail resulting in a marked reduction of Ca^{2+} uptake. We suggest that in the epidermis, compensatory mechanisms by other Ca^{2+} pumps are insufficient to normalize Ca^{2+} signaling.

$SERCA2^{+/-}$ mice have revealed major differences in the effect of SERCA2 dysfunction in human and mouse. These heterozygous mice do not develop Darier-like lesions but display mild impairment of cardiac contractility and relaxation due to the reduction of SERCA2 mRNA, protein, and activity levels

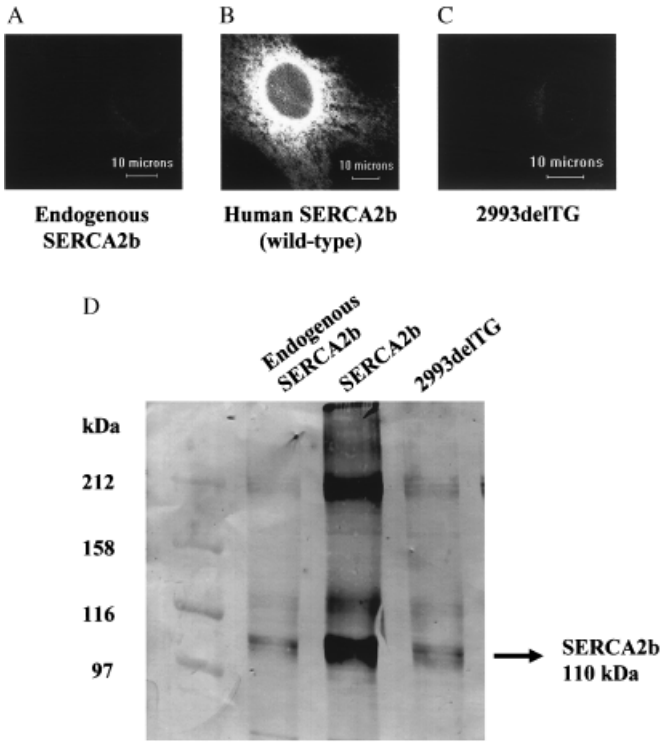

Figure 4. Immunodetection of the wild-type and the mutant SERCA2b proteins. (*A–C*) Immunofluorescence analysis: COS-1 cells transfected with wild-type SERCA2b (*B*), the mutant 2993delTG (*C*), or the empty vector (*A*) were processed for indirect immunofluorescence. Bound mouse anti-SERCA2 (*A–C*) was detected using fluorescein isothiocyanate-conjugated anti-mouse secondary antibody. (*D*) Western blot analysis: microsomal proteins extracted from COS-1 cells transfected with wild-type SERCA2b, the mutant 2993delTG or the empty vector were analyzed by western blot using mouse anti-SERCA2 antibody followed by anti-mouse secondary antibodies linked to peroxidase, and detected by enhanced chemiluminescent (ECL) blotting. The 212 kDa band corresponds to SERCA2b dimers (Verboomen *et al*, 1994).

(Periasamy *et al*, 1999; Zhao *et al*, 2001). The aged heterozygous mutant mice develop squamous epithelial papillomas and carcinomas (Liu *et al*, 2001), a complication that is not reported in DD. These observations suggest that there are species differences in susceptibility to DD and squamous cell tumors.

In conclusion, we provide the first evidence that a heterozygous mutation in *ATP2A2*, specific for the SERCA2b isoform, is sufficient to cause DD. This result suggests that expression of the normal SERCA2a isoform cannot compensate for the dysfunction of SERCA2b to maintain Ca^{2+} homeostasis in the epidermis, emphasizing a key role of SERCA2b in skin biology.

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REFERENCES

Burge SM, Garrod DR: An immunohistological study of desmosomes in Darier's disease and Hailey–Hailey disease. *Br J Dermatol* 124:242–251, 1991

Burge SM, Wilkinson JD: Darier-White disease: A review of the clinical features in 163 patients. *J Am Acad Dermatol* 27:40–50, 1992

Dode L, Greef CD, Mountian I, *et al*: Structure of the human sarco/endoplasmic reticulum Ca^{2+}-ATPase 3 gene. Promoter analysis and alternative splicing of the SERCA3 pre-mRNA. *J Biol Chem* 273:13982–13994, 1998

Ganguly A, Rock MJ, Prockop DJ: Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: Evidence for solvent-induced bends in DNA heteroduplexes. *Proc Natl Acad Sci USA* 90:10325–10329, 1993

Jacobsen NJ, Lyons I, Hoogendoorn B, *et al*: *ATP2A2* mutations in Darier's disease and their relationship to neuropsychiatric phenotypes. *Hum Mol Genet* 8:1631–1636, 1999

John LM, Lechleiter JD, Camacho P: Differential modulation of SERCA2 isoforms by calreticulin. *J Cell Biol* 142:963–973, 1998

Liu LH, Boivin GP, Prasad V, Periasamy M, Shull GE: Squamous cell tumors in mice heterozygous for a null alle of *ATP2A2*, encoding the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 Ca^{2+} pump. *J Biol Chem* 276:27637–27640, 2001

Lytton J, MacLennan DH: Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca^{2+}-ATPase gene. *J Biol Chem* 263:15024–15031, 1988

MacLennan DH, Rice WJ, Green NM: The mechanism of Ca^{2+} transport by sarco (endo) plasmic reticulum Ca^{2+}-ATPases. *J Biol Chem* 272:28815–28818, 1997

Martin V, Bredoux R, Corvazier E, *et al*: Three novel sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) 3 isoforms. Expression, regulation, and function of the membranes of the SERCA3 family. *J Biol Chem* 277:24442–24452, 2002

Maruyama K, MacLennan DH: Mutation of aspartic acid-351, lysine-352, and lysine-515 alters the Ca^{2+} transport activity of the Ca^{2+}-ATPase expressed in COS-1 cells. *Proc Natl Acad Sci USA* 85:3314–3318, 1988

Munro CS: The phenotype of Darier's disease: Penetrance and expressivity in adults and children. *Br J Dermatol* 127:126–130, 1992

Periasamy M, Reed TD, Liu LH, *et al*: Impaired cardiac performance in heterozygous mice with a null mutation in the sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoform 2 (SERCA2) gene. *J Biol Chem* 274:2556–2562, 1999

Ringpfeil F, Raus A, DiGiovanna JJ, *et al*: Darier disease—novel mutations in *ATP2A2* and genotype-phenotype correlation. *Exp Dermatol* 10:19–27, 2001

Ruiz-Perez VL, Carter SA, Healy E, *et al*: *ATP2A2* mutations in Darier's disease: Variant cutaneous phenotypes are associated with mis-sense mutations, but neuropsychiatric features are independent of mutation class. *Hum Mol Genet* 8:1621–1630, 1999

Sakuntabhai A, Ruiz-Perez V, Carter S, *et al*: Mutations in *ATP2A2*, encoding a Ca^{2+} pump, cause Darier disease. *Nat Genet* 21:271–277, 1999a

Sakuntabhai A, Burge S, Monk S, Hovnanian A: Spectrum of novel *ATP2A2* mutations in patients with Darier's disease. *Hum Mol Genet* 8:1611–1619, 1999b

Sakuntabhai A, Dhitavat J, Burge S, Hovnanian A: Mosaicism for *ATP2A2* mutations causes segmental Darier's disease. *J Invest Dermatol* 115:1144–1147, 2000

Sambrook J, Russell DW: *Molecular Cloning—Laboratory Manual*. In: C16: "Introducing cloned genes into cultured mammalian cells." (Third edition). Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2001; p 1614–1618

Takahashi H, Atsuta Y, Sato K, Ishida-Yamamoto A, Suzuki H, Iizuka H: Novel mutations of *ATP2A2* gene in Japanese patients of Darier's disease. *J Dermatol Sci* 26:169–172, 2001

Verboomen H, Wuytack F, Van Den Bosch L, *et al*: The functional importance of the extreme C-terminal tail in the gene 2 organellar Ca^{2+}-transport ATPase (SERCA2a/b). *Biochem J* 303:979–984, 1994

Vilsen B, Andersen JP, Clarke DM, MacLennan DH: Functional consequences of proline mutations in the cytoplasmic and transmembrane sectors of the Ca^{2+}-ATPase of sarcoplasmic reticulum. *J Biol Chem* 264:21024–21030, 1989

Yang Y, Li G, Bu D, Zhu X: Novel point mutations of the *ATP2A2* gene in two Chinese families with Darier disease. *J Invest Dermatol* 116:482–483, 2001

Zhao XS, Shin DM, Liu LH, Shull GE, Muallem S: Plasticity and adaptation of Ca^{2+} signaling and Ca^{2+}-dependent exocytosis in SERCA^{2+}/- mice. *EMBO* 20:2680–2689, 2001