

Autosomal-Dominant Calcium ATPase Disorders

Réka Szigeti¹ and Richard Kellermayer²

Darier disease (DD) and Hailey–Hailey disease (HHD) are the only known autosomal-dominant Ca^{2+} ATPase disorders. Epidermal symptoms selectively occur in the affected individuals, the precise reason for which is still not fully understood. Here, we review the clinical, epidermal, and molecular features of the two genodermatoses. It is concluded that epidermal Ca^{2+} regulation disturbances and epigenetic factors may play an even more prominent role in the pathogenesis of DD and HHD than earlier appreciated.

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INTRODUCTION

Major advancements have been made in our understanding of skin disorders with epidermal cell adhesion defects around the turn of the millennium. One such step was the identification of pathogenic *ATP2A2* mutations encoding SERCA2 (sarco/endoplasmic reticulum Ca^{2+} -transport ATPase isoform 2) in Darier- or Darier–White disease (DD, also known as keratosis follicularis, OMIM#124200) (Sakuntabhai *et al.*, 1999b). Shortly after, two separate research groups have identified *ATP2C1* (the gene encoding the human secretory pathway $\text{Ca}^{2+}/\text{Mn}^{2+}$ ATPase (hSPCA1)) as the pathogenic gene in Hailey–Hailey disease (HHD, OMIM#169600), or chronic benign familial pemphigus (Hu *et al.*, 2000; Sudbrak *et al.*, 2000). These observations underscored the pivotal role of epidermal Ca^{2+} regulation in promoting proper cell-to-cell adhesion. DD and HHD represent the only known autosomal dominantly inherited Ca^{2+} ATPase defects in humans. Except for a subpopulation of DD patients – in whom psychiatric symptoms develop likely as a result of neighboring gene co-segregation – only epidermal symptoms occur. This peculiar feature of these disorders is still not fully understood despite advancements in elucidating their pathogenesis. Here, we focus on the most

recent literature and highlight the possible explanations for the selective epidermal defects in DD and HHD.

CLINICAL AND HISTOLOGIC FEATURES OF AUTOSOMAL-DOMINANT Ca^{2+} ATPASE DISORDERS

Autosomal-dominant mutations of *ATP2A2* lead to DD. Acantholytic, dyskeratotic epidermal nevi appear to be unilateral segmental presentations of DD (Sakuntabhai *et al.*, 2000; Reese *et al.*, 2005) and acrokeratosis verruciformis of Hopf is also allelic to DD (Dhitavat *et al.*, 2003c). On the other hand, autosomal mutations in *ATP2C1* lead to HHD. Both diseases selectively affect the skin, more precisely the stratified squamous epithelium, as the occasional, uncommon involvement of the mucosa has been noted in DD (Al Robae *et al.*, 2004), more rarely in HHD (Heinze, 1979; Vaclavinkova and Neumann, 1982). Mood disorders have also been described in association with DD, but current data suggest that this is due to a susceptibility locus co-segregating with *ATP2A2* rather than pleiotropy (Green *et al.*, 2005).

The incidence of the two disorders is similar, 1:25,000–1:100,000 (Svendsen and Albrechtsen, 1959; Miljkovic *et al.*, 2005) for DD and 1:50,000 for HHD (Burge, 1992). This is true for the onset and the triggering factors of the symptoms also, as both skin defects present after age 10, usually around puberty, but before the third to fourth decades of life. Heat, sweating, mechanical trauma, infection, and UVB exposure can cause the exacerbations. Remissions and relapses characterize the course of the disorders (Burge, 1992; Burge and Wilkinson, 1992). As far as distribution and the appearance of the rash, there may be considerable differences, with DD usually affecting the seborrheic and HHD the flexural areas. Greasy, warty papules, and plaques characterize the rash of DD that are frequently malodorous. Punctate keratoses of the palms and the soles with a central depression are also pathognomic features. The nails are thin with white lines and/or longitudinal ridges and tend to break at the distal end (Zaias and Ackerman, 1973; Burge, 1994; Cooper and Burge, 2003; Hovnanian, 2004). The morphology of HHD varies. Vesicular lesions, crusted erosions with vesiculopustules, and small erythematous scaly plaques can occur. Longitudinal white lines on the nails are also frequent findings (Burge, 1992).

Penetrance is complete but expressivity varies significantly in both disorders. No clear correlations have been established between phenotype and genotype in either disease. The onset around puberty and the fact that allergens and drugs can exacerbate the symptoms argues that hormonal status and epigenetic factors greatly influence the clinical appearance of DD and HHD (Dhitavat *et al.*, 2004; Foggia and Hovnanian, 2004; Sehgal and Srivastava, 2005).

¹Department of Dermatology, University of Pécs, Pécs, Hungary and

²Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary

Correspondence: Dr Richard Kellermayer, Department of Medical Genetics, University of Pécs, József A. u. 7., 7623 Pécs, Hungary.
E-mail: richard.kellermayer@aok.pte.hu

Abbreviations: DD, Darier disease; ER, endoplasmic reticulum; HHD, Hailey–Hailey disease; hSPCA, human secretory pathway $\text{Ca}^{2+}/\text{Mn}^{2+}$ ATPase; SERCA2, sarco/endoplasmic reticulum Ca^{2+} -transport ATPase isoform 2

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The most prominent common epidermal histologic feature of DD and HHD is suprabasal acantholysis, resulting from desmosomal disintegration. DD epidermis on light microscopy also shows premature and abnormal keratinization (dyskeratosis, hyperkeratosis), corps ronds (these structures contain multiple lamellar bodies resembling apoptotic cells), and eosinophilic dyskeratotic cells (Burge and Schomberg, 1992). Electron microscopy reveals basal-cell vacuolization, decreased lateral membrane desmosomes, separation of tonofilaments from their insertion sites on the cell membrane, and large circular aggregates around the nucleus (Caulfield and Wilgram, 1963; Tada and Hashimoto, 1998). Histopathology of HHD skin lesions shows a widespread loss of cell-to-cell adhesion (acantholysis) in the suprabasal layer of the epidermis. Ultrastructural studies demonstrate perinuclear aggregation of keratin intermediate filaments, which have retracted from the desmosomal plaques in the acantholytic cells (Hashimoto *et al.*, 1995; Metze *et al.*, 1996).

MOLECULAR ASPECTS OF AUTOSOMAL-DOMINANT CA²⁺ ATPASE DISORDERS

Currently known Ca²⁺ ATPase-related human disorders are Brody disease, type II diabetes mellitus, DD, and HHD. Brody disease is an autosomal recessive myopathy where considerable genetic heterogeneity has been detected. However, a subset of patients carry mutations in both alleles of *ATP2A1*, the gene encoding SERCA1 (Odermatt *et al.*, 1996). Polymorphisms in the *SERCA3* gene (*ATP2A3*) have been implicated to contribute to type II diabetes genetic susceptibility (Varadi *et al.*, 1999). Yet, it is only DD and HHD that are related to dominant mutations in Ca²⁺ ATPases.

ATP2A2

ATP2A2, the gene mutated in DD, is located in the 12q23–24.1 chromosomal region. The gene spans 76 kb with 21 exons. It encodes a 4.5 kb transcript that is alternatively spliced into three variants *ATP2A2a*, *ATP2A2b*, and *ATP2A2c*, which are translated into SERCA2a, SERCA2b, and SERCA2c, respectively (Gelebart *et al.*, 2003). These proteins belong to the SERCA subfamily of the P-type Ca²⁺ ATPase family catalyzing the hydrolysis of ATP coupled with the translocation of two cytoplasmic Ca²⁺ ions into the endoplasmic reticulum (ER) lumen per cycle (Wuytack *et al.*, 2002). Based on the key architectural features of SERCA1, the translated SERCA2a and SERCA2c proteins are composed of three cytoplasmic domains and 10 hydrophobic transmembrane helices (Xu *et al.*, 2002). SERCA2b appears to contain an additional transmembrane domain with the extreme C terminus protruding into the ER lumen (Bayle *et al.*, 1995). Observations that among the variants, epidermal expression of SERCA2b is most preponderant and that some pathogenic *ATP2A2* mutations selectively affect SERCA2b indicated that loss of isoform b expression only is sufficient to cause DD (Ikeda *et al.*, 2003; Dhitavat *et al.*, 2003b). This isoform has a higher affinity but a lower

transport capacity for Ca²⁺ than SERCA2a and SERCA1 (Dode *et al.*, 2003).

More than 100 pathogenic *ATP2A2* mutations, scattered throughout the gene, have been reported to date. These affect the Ca²⁺ transport activity and/or the expression level of SERCA2b (Dode *et al.*, 2003; Sato *et al.*, 2004). Most mutant SERCA2 display decreased expression and/or stability, suggesting that haploinsufficiency is the common mechanism for the dominant inheritance of DD. Yet, some findings indicate that dimerization between SERCA2b proteins may occur consequently leading to a dominant-negative mechanism compounding haploinsufficiency (Ahn *et al.*, 2003). This may be the reason in part why some missense mutations cause severe forms of DD (Sakuntabhai *et al.*, 1999a).

ATP2C1

The *ATP2C1* gene associated with HHD comprises 28 exons in an approximately 30 kb segment of chromosome 3q21. It encodes a 4.5 kb transcript that is alternatively spliced into four variants, *ATP2C1a–d* (Hu *et al.*, 2000; Sudbrak *et al.*, 2000; Ikeda *et al.*, 2001; Dobson-Stone *et al.*, 2002; Yokota *et al.*, 2002). *ATP2C1d* is the largest variant containing the entire exons 27 and 28 (Fairclough *et al.*, 2003). The isoforms are translated into hSPCA1a–d, respectively. These proteins belong to the SPCA subfamily of P-type ion motive ATPases. SPCA pumps possess only one of the two high-affinity Ca²⁺ binding sites characteristic of SERCA. However, these can transport not only a single Ca²⁺ ion but also a Mn²⁺ ion per cycle. Such characteristics separate SPCA from the P-type SERCA and plasmamembrane Ca²⁺ pumps (Wuytack *et al.*, 2002, 2003). Recent observations indicate that hSPCA1c is a rapidly degradable, non-functional protein. The other isoforms appear to be expressed similarly, with hSPCA1d transporting Ca²⁺ most effectively. Nevertheless, hSPCA1 isoforms are also high-affinity, low-capacity Ca²⁺ ATPases in comparison to SERCA1 (Dode *et al.*, 2005).

To date, more than 80 different *ATP2C1* mutations have been reported in HHD. Among these, 20% are nonsense mutations, 19% are splice site mutations, 30% are frameshift mutations leading to premature termination codons, 28% are missense mutations, and ~3% are in-frame deletions or insertions (Foggia and Hovnanian, 2004; Missiaen *et al.*, 2004; Majore *et al.*, 2005). The high number of mutations leading to premature termination codons pointed to haploinsufficiency as the prevalent mechanism for the dominant inheritance of HHD. Studies on missense mutations affecting protein stability and function have shown that although mutant *ATP2C1* mRNA levels are normal, the level of the defective protein is lower than that of the wild type despite correct targeting to the Golgi. These observations lent further support for haploinsufficiency as the primary mechanism for the dominant inheritance of HHD (Fairclough *et al.*, 2003, 2004). Experiments in a yeast model system were also consistent with this notion. Namely, overexpression of mutant SPCA1 did not lead to the suppression of PMR1 (the structural and functional homologue of SPCA1 in yeast) function (Ton and Rao, 2004).

Expression, regulation, and function of ATP2A2 and ATP2C1. SERCA2b and hSPCA1 are most abundantly expressed in keratinocytes and contribute to cellular Ca²⁺ homeostasis most prominently in these cells (Sakuntabhai *et al.*, 1999b; Hu *et al.*, 2000; Behne *et al.*, 2003; Callewaert *et al.*, 2003; Van Baelen *et al.*, 2003; Tavadia *et al.*, 2004). An increase in extracellular Ca²⁺ induces the transcription of both ATP2A2 and ATP2C1 (Mayuzumi *et al.*, 2005a). Ca²⁺ appears to exert this effect on the latter gene by promoting the nuclear accumulation of the transcription factor Sp1 that binds to *cis*-enhancing elements of the ATP2C1 promoter. This regulation was found to be absent in HHD keratinocytes (Kawada *et al.*, 2005). Nevertheless, extracellular Ca²⁺ stimulation positively affects the amount of SPCA1 in HHD keratinocytes compared to normal cells (Behne *et al.*, 2003). Similarly, the yeast homologue of hSPCA1, PMR1, is positively regulated by Ca²⁺ both at the transcriptional and translational level (Cunningham and Fink, 1996), suggesting that Ca²⁺-induced secretory pathway Ca²⁺/Mn²⁺ ATPase upregulation may be conserved throughout evolution. However, it should be noted that all the above observations have been made in cultured keratinocytes and have not been confirmed *in vivo* in the epidermis.

As opposed to Ca²⁺, UVB irradiation (an exacerbating factor for both DD and HHD) causes decreased ATP2A2 and ATP2C1 transcription (Mayuzumi *et al.*, 2005a). Drugs that have proved useful in the treatment of these disorders inhibit the transcriptional effects of UVB, indicating that even anti-inflammatory medications may have a direct cellular (beneficial) effect in these disorders (Mayuzumi *et al.*, 2005b). Observations in a yeast model system also support this possibility (Szigeti and Kellermayer, 2004).

An earlier study showed that SERCA2b expression is somewhat more prominent in the basal epidermis (Sheridan *et al.*, 2002). However, this has not been observed in other experiments later (Tavadia *et al.*, 2004). On the other hand, the epidermal distribution of hSPCA1 has not been addressed to date. Nevertheless, one would expect – based on the positive regulatory effects of Ca²⁺ on ATP2A2 and ATP2C1 expression – that SERCA2b and hSPCA1 are most abundant in the granular layer of the skin where Ca²⁺ levels are the highest. The fact that the granular layer's Ca²⁺ content decreased in HHD indicates that this layer is affected most severely with regard to total cellular calcium by the haploinsufficiency of hSPCA1. The conflicting results on epidermal SERCA2b distribution raise the possibility that the reliability of immunochemistry may be hampered by epitope masking through Ca²⁺-dependent conformational changes and dynamically altering protein–protein interactions in the epidermal layers. Consequently, the epidermal expression pattern of SERCA2b and hSPCA1 will have to be addressed by methods other than immunochemistry in the future. However, irrespective of the expression pattern, it has been found that the amount of SERCA2b is similar in the unaffected skin of DD patients as in normal individuals and that the amount of hSPCA1 in unaffected HHD skin is only mildly decreased. On the contrary, the expression of both proteins significantly decreases in the affected areas of the skin (Tavadia *et al.*,

2004; Porgpermdée *et al.*, 2005). Interestingly, hSPCA1 expression is even decreased in the affected areas of DD skin, showing that epidermal stress induces ATP2A2 and ATP2C1 downregulation in these disorders, which could not be observed in other acantholytic dermatoses (Porgpermdée *et al.*, 2005). This latter observation is in contrast with cell culture results where an upregulation of SPCA1 was detected in DD keratinocytes (Foggia *et al.*, 2006).

Both SERCA2 and HSPCA1 are involved in the regulation of cytoplasmic Ca²⁺ oscillations upon external stimulation of the cells (Missiaen *et al.*, 2000; Berridge *et al.*, 2003; Mitchell *et al.*, 2004). Some studies showed that HHD keratinocytes possess a higher cytoplasmic resting Ca²⁺ level (Hu *et al.*, 2000). Others have failed to detect this increase but demonstrated it in DD keratinocytes, suggesting that SERCA2b has a more prominent role in maintaining resting levels of cytosolic Ca²⁺ than hSPCA1 (Leinonen *et al.*, 2005). On the contrary, a recent report found that DD keratinocytes rather possess a lower resting cytosolic calcium level, but show a more robust calcium response than normal controls (Foggia *et al.*, 2006). Despite the conflicting results, these studies all demonstrate a disturbed intracellular calcium regulation in both aberrant cell types compared to normal keratinocytes. This disturbance likely leads to the decreased ability of HHD keratinocytes to accumulate Ca²⁺ in increasing levels of extracellular Ca²⁺ concentration compared to wild-type cells (Hu *et al.*, 2000). Observations in a PMR1-deficient yeast model system suggest that this disability may be a general consequence of SPCA dysfunction (Szigeti *et al.*, 2005).

SERCA2 and SPCA1 promote the maintenance of the ER/Golgi Ca²⁺ pools that are crucial for proper protein processing through the secretory pathway. Recent studies showed an increased expression of SPCA1 in DD keratinocytes, arguing for functional overlaps between the two ATPases at the cellular level (Foggia *et al.*, 2006). Similar observations have been made earlier in PMR1-defective yeast cells with regard to other secretory pathway calcium transporters (Kellermayer *et al.*, 2003). The decreased Ca²⁺ content in the secretory pathway pools activates the unfolded protein response that may lead to apoptosis (Orrenius *et al.*, 2003), causes disturbances in protein glycosylation, and leads to defective ER-associated protein degradation (Ramos-Castaneda *et al.*, 2005). Indeed, the DD-specific corneodesmosomes resemble apoptotic keratinocytes and inhibition of all SERCAs in normal keratinocytes impairs the proper trafficking of desmosomal transmembrane and plaque proteins. Yet, glycosylation of these proteins is normal in DD keratinocytes and the desmosomal cadherins are efficiently transported to the cell surface with the exception of desmoplakin that forms insoluble aggregates (Dhitavat *et al.*, 2003a). Similarly, small interfering RNA (where SPCA1 expression decreased close to undetectable levels) studies indicated that hSPCA1 exerts significant effects on both Golgi and ER function such as post-translational glycan processing, ER-associated protein degradation (ERAD), and response to ER stress that may alter desmosomal protein processing (Mitchell *et al.*, 2004; Ramos-Castaneda *et al.*, 2005). However, HHD keratinocytes

have normal amounts of desmosomal proteins that can bind other proteins with the same efficiency as seen in normal epidermal cells. Additionally, desmosome formation and response to noxious UVB irradiation are also similar in monolayer HHD and normal keratinocyte cultures (Bernards and Korge, 2000). These observations indicate that disturbances in cellular Ca²⁺ homeostasis are present in DD and HHD keratinocytes and significant reduction of SERCA2b and SPCA1 function can adversely affect desmosomal protein processing. Yet, direct experiments on DD and HHD keratinocytes (where protein expression is usually around 50% of normal) failed to detect major disturbances in desmosomal integrity at the cellular/functional level.

WHY THE SKIN ONLY?

As highlighted earlier, autosomal-dominant Ca²⁺ ATPase disorders selectively affect the skin. The reason for this is still not well understood and is likely complex. Some of the possible explanations that may collectively lead to this organ specificity are as follows:

1. As already noted, SERCA2b and hSPCA1 are most abundantly expressed in keratinocytes and contribute to cellular Ca²⁺ homeostasis most prominently in these cells. Consequently, haploinsufficient expression of the transporters may affect keratinocytes most severely.
2. Altered intracellular Ca²⁺ homeostasis may significantly hamper the processing of desmosomal proteins causing a failure in proper cell-to-cell adhesion. However, direct cell culture studies on HHD keratinocytes oppose a major disturbance in this respect (see above).
3. Exacerbating factors of DD and HHD may affect the skin most prominently. Indeed, whereas intracellular organs are mostly protected from physical insults, the skin is exposed to the environment. Thus, friction, UV radiation, temperature changes, etc. can most significantly affect the skin and cause a decrease in the already haploinsufficient expression of SERCA2 and SPCA1 according to cell culture and immunohistochemical observations (it should be noted again that further epidermal studies will be required to address these aspects of protein expression *in vivo*). Consequently, the amount of these two proteins may reach a critically low level (enough to induce the pathologic changes of DD and HHD in the epidermis) upon noxious stimulation of the skin in the affected individuals.
4. Compensatory mechanisms for SERCA2 and SPCA1 deficiency may be less effective in the epidermis. Although SERCA3 is expressed in most tissues, it is absent (or very mildly expressed) in keratinocytes. This may indicate a decreased ability of these cells to compensate for the partial loss of SERCA2b or hSPCA1 (Martin *et al.*, 2002).
5. The skin may be special with regard to Ca²⁺ homeostasis compared to other tissues and organs. Indeed, a unique Ca²⁺ gradient is present in the healthy epidermis with lowest calcium levels in the basal layer and highest in the granular layer of the skin. This gradient appears to be a key element for the differentiation process in the epidermis through a counter-talk between keratinocytes and the extracellular space in which the calcium sensing receptor (CaR) is an important player (Tu *et al.*, 2001; Elias *et al.*, 2002). The epidermal calcium gradient dissipates upon perturbations in the permeability barrier of the skin (i.e., noxious stimuli) within minutes and is restored to normal within 6–24 hours under ideal conditions (Mao-Qiang *et al.* 1997). During this repair process, there is a small overshoot where Ca²⁺ concentrations in the spinous/suprabasal layers actually increase to a level slightly higher than the preceding resting level (Menon *et al.*, 1992; Mauro *et al.*, 1998). This increase may be critically important as extracellular Ca²⁺ can directly reinforce desmosomal connections (O'Keefe *et al.*, 1987). These processes have been shown to be important for the skin to optimize recovery following injury (Grzesiak and Pierschbacher, 1995). The fact that SERCA2b and hSPCA1 are high-affinity, low-capacity Ca²⁺ transporters argues that these pumps serve to maintain a steady, less releasable Ca²⁺ pool that has to be maintained for proper cellular functions rather than the fast replenishment of releasable Ca²⁺ pools as SERCA1 does. Consequently, it is likely that SERCA2b and hSPCA1 are the most important in building up the relatively steady epidermal Ca²⁺ gradient. Indeed, the epidermal Ca²⁺ gradient is absent in HHD epidermis even in the intact areas of the skin. This is likely due to the inability of HHD keratinocytes to accumulate calcium properly in the granular layer (Hu *et al.*, 2000). The absence of the Ca²⁺ gradient likely leads to the lack of external Ca²⁺ increase (that could reinforce desmosomal connections directly) in the suprabasal layer following physical stress. Hence, the desmosomal connections fail to be reinforced leading to the suprabasal vulnerability of HHD epidermis to noxious stimuli. Consequently, suprabasal acantholysis may ensue (Kellermayer, 2005). The fact that the rash of HHD can appear within 1 hour following physical trauma (Burge, 1992) makes it less probable that cellular mechanisms involving protein expression and transport are the primary inducers of acantholysis and supports the theory above. Interestingly, the human epidermal Ca²⁺ gradient is more distinct than that of rats and mice (Elias *et al.*, 1998), which may in part explain the observed phenotypic differences between ATP2A2 +/- mice and DD (Prasad *et al.*, 2004, 2005). However, the epidermal Ca²⁺ gradient has not been studied in DD yet.
6. Aging and hormones may differentially affect epidermal ATP2A2 and ATP2C1 expression. The peculiar presentation of DD and HHD around puberty or even later in life argues that the aging process and/or hormonal changes significantly influence the epidermal role of SERCA2 and SPCA1, which may not be the case for other tissues and organs.

Possible modes of therapy in DD and HHD

Identification and avoidance of exacerbating factors is the first step in therapy for both DD and HHD. Furthermore, oral

and topical retinoids have proved to be quite useful in the treatment of DD. Topical 5-fluorouracil, tazarotene, and calcipotriol have also been effective in addition to oral cyclosporine, contraceptives, and diltiazem. Surgical methods and laser therapy has been utilized with success also (Sehgal and Srivastava, 2005). Topical steroids, antibiotics, and antiseptics have been promoted traditionally for the treatment of HHD. More recently, FK506 and vitamin D3 analogues have proved to be useful in certain cases and laser therapy has also been effective (Kruppa *et al.*, 2000; Sand and Thomsen, 2003; Bianchi *et al.*, 2004; Foggia and Hovnanian, 2004). Despite the significant variety of therapeutic modalities, the treatment of both DD and HHD can be challenging (Lorentzen and Svejgaard, 2001; Mak *et al.*, 2005). Consequently, novel approaches should be sought. We recently proposed that topically applied translational readthrough-inducing agents may be of significant value for the therapy of genodermatoses mediated by nonsense mutations, such as a subset of HHD cases (Kellermayer *et al.*, 2006).

CONCLUSION

The selective manifestation of DD and HHD in the skin indicates that both human keratinocytes and the epidermis hold unique features of Ca²⁺ regulation where the high-affinity, low-capacity Ca²⁺ ATPases play a more prominent role than in other cells and tissues of the human body. The lack of significant functional alterations at the cellular level with respect to growth and cell adhesion in DD and HHD keratinocytes highlights the potentially pivotal role of epidermal Ca²⁺ homeostasis in the autosomal-dominant Ca²⁺ ATPase disorders and proposes that the tissue differentiation of the skin may be a key element for the epidermal appearance of these genodermatoses. Additionally, the age-dependent onset and the significant variability in the expressivity of the symptoms (even within the same family) argue that epigenetic factors surpassing the cellular level of regulation greatly influence the manifestation of DD and HHD. These observations highlight the importance of future experiments at the organ (i.e., skin) and organism (i.e., clinical) level for elucidating the pathogenesis of DD and HHD.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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