Molecular and Genetic Studies Open the Door to Understanding the Pathogenesis of Darier's and Hailey-Hailey Diseases

Laura Raiko and Sirkku Peltonen*
Department of Dermatology, University of Turku, P.O. BOX 52, FIN- 20521 Turku, Finland
*Corresponding author.
E-mail: sirkku.peltonen@tyks.fi

Darier's and Hailey-Hailey diseases are autosomally inherited skin diseases with incidence of about 1:30 000. Hailey-Hailey disease is characterized by superficial blistering of the epidermis, resulting in erosions mostly in flexural areas. Darier's (or Darier-White disease, also known as dyskeratosis follicularis) shows a more prominent differentiation defect leading to abnormal keratinisation, in addition to blister formation. Darier's disease is localized in seborrheic areas and produces brownish red papules and plaques covered by crusty scurf (Fig. 1). Both diseases fluctuate with exacerbation by physical factors such as mechanical trauma, UV light, and warm atmospheres that cause sweating. Bacterial or yeast colonisation, or infection of the susceptible skin regions, may also be provocative or causative factors. Thus, epigenetic factors have a great impact on the expression of the disease. The severity of the disease varies between families, and between individuals of the same family. Treatments of Hailey-Hailey disease include topical antibacterials, antimycotics, corticosteroids, tacrolimus, calcitriol, dermabrasion with CO_2 laser, or cryotherapy. In Darier's disease, oral isotretinoin and etretinate have been used, on their own as well as in addition to the above mentioned topical agents; 1 % fluorouracil cream and topical retinoids have also been applied. Some studies using photodynamic therapy have also been published (1–4). The large spectrum of treatments reflects the fact that there are no treatments effective for every patient and situation.

Histology reveals superficial epidermal acantholysis in both diseases, in addition to the abnormal keratinocyte differentiation seen in Darier's disease. This could suggest that the mutations for these diseases hide in structural genes. This educated guess, however, proved to be incorrect, when the causative genes for both diseases were found in the beginning of this century. It may seem surprising, but is indeed very reasonable that both diseases are caused by mutations in genes which regulate the concentration of intracellular calcium ion (Ca^{2+}).

Intracellular calcium pumps

Calcium is an important signalling molecule for cells in general, and for keratinocytes in particular. Calcium signalling is needed, for example, for the maintenance of the epidermal structure by intercellular junctions (5). Calcium has an essential role in formation of functional adherens junctions and desmosomes (6). Calcium is also important in controlling the proliferation and differentiation of keratinocytes (7). Thus, the concentration of calcium in the cells needs to be under strict control. Calcium pumps are located in different

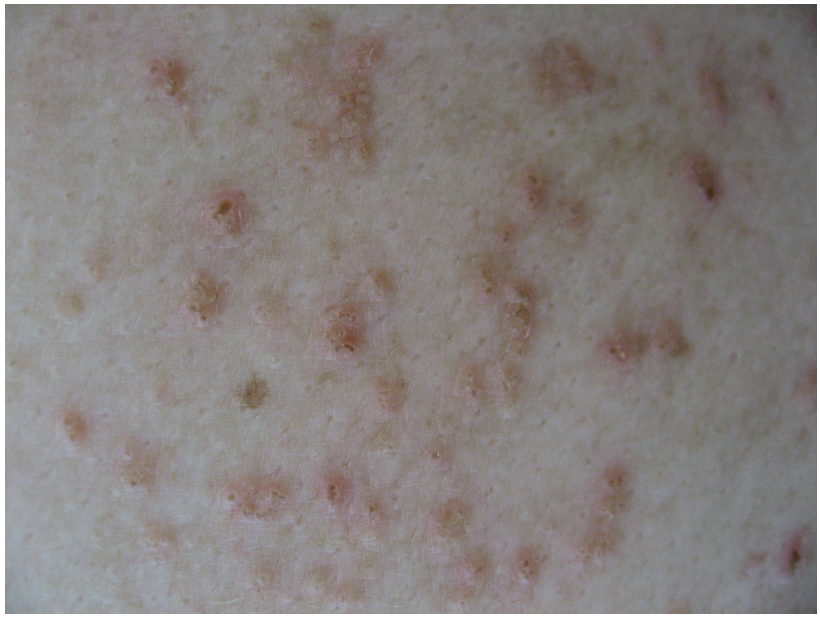

Fig.1. Keratotic papules of Darier's disease.

cellular compartments: plasma membrane, endoplasmic reticulum and sarcoplasmic reticulum (8, 9). Cell organelles contain different calcium pump molecules. These pumps are ATPases, which are phosphorylated and dephosphorylated during the pumping process (8).

Calcium pumps are mutated in Darier's and Hailey-Hailey diseases

Darier's disease is caused by a mutation that affects the type 2 Ca-ATPase of the sarcoendoplasmic reticulum of the cell (SERCA2). SERCA2 has an important role in intracellular calcium signalation. SERCA2 transports calcium from the cytosol of the cell to the lumen of endoplasmic reticulum (10). It thus maintains calcium stores of the endoplasmic reticulum and creates calcium oscillations into the cytosol when the cell gets stimuli from its environment (11). The gene encoding SERCA2 is ATP2A2, located in chromosome12q23-24.1 (10). In Darier's disease the mutation in the ATP2A2 disturbs the function of SERCA2 and this leads to the alteration in the calcium concentration of the cytoplasm.

In Hailey–Hailey disease the mutation affects SPCA1-pump which is Ca/Mn-ATP-ase (11). SPCA1 transports manganese and calcium into the lumen of the Golgi apparatus. SPCA1 has an important role in maintaining the calcium and manganese balance in the cytoplasm and inside the Golgi apparatus (6). In the Golgi apparatus, calcium and manganese are needed for the synthesis and modification of the proteins that are going to be secreted or placed on the cell membrane (11). The "Hailey-Hailey" gene encoding SPCA1 is called ATP2C1 and it is located in the chromosome 3q21-24.

How do the calcium pump gene mutations affect keratinocytes

The calcium oscillations of the cytosol affect the gene expression and differentation of the cells (11). The Hailey-Hailey mutations in the ATP2C1 gene lead to a decreased amount of SPCA1-pumps in keratinocytes, and disturb the intracellular maintenance of calcium and manganese balance. The insufficient amount of SPCA1 leads to complications in keeping calcium and manganese balance in the Golgi apparatus (6, 11). It seems that the differentiation of keratinocytes requires high ATP2C1 expression. Active production of SPCA1-pumps contributes to transportation of cellular adhesive proteins via secretory pathways. The defects in expression may lead to the symptoms of Hailey–Hailey disease (12).

Various mutations of Darier's disease alter SERCA2 synthesis, activity and degradation. Most of the mutations accelerate the proteasome mediated degradation of the SERCA2-pumps (10). Mutations can also cause complete loss of the synthesis of the SERCA2-pump, or synthesis of defective pumps leading to reduced or abnormal function (11). The defective function of the pumps can affect the calcium signaling of the cell, post-translational protein modification and chaperon mediated transportation (13). How the altered calcium concentration leads to acantholysis and dyskeratosis of the skin is not exactly known. One explanation for skin symptoms may be abnormal protein synthesis and modification of calcium dependent cadherins which in turn affects the epidermal cell junctions and leads to acantholysis and apoptosis of the epidermis (6, 11, 14).

It has been estimated that in normal conditions the epidermis can compensate the missing SERCA2-pumps by adding the expression of ATP2A2-gene or using other mechanisms (15). Without harmful epigenetic factors the reduced amount of functional calcium pumps may be sufficient, while in stressful situations (for example mechanical irritation, sweat or UV-light) one functional ATP2A2-gene or ATP2C1 is not able to maintain a calcium concentration that is high enough for normal formation of desmosomes. This results in exacerbation of the skin symptoms of the disease. Another mechanism might also be that the stress downregulates the expression of the calcium pump genes (11, 15).

Abnormal calcium regulation manifests itself in intercellular junctions

Desmosomes and adherens junctions are the adhering cell junction types in the epidermis while tight junctions participate in diffusion

barrier. Acantholysis is caused by desmosomal dispersion which is a consequence of faulty action of desmosomal proteins, plasminogen activation or reduced intercellular interaction of junction proteins. The reduced intercellular interaction is a result of either reduced calcium concentration or inhibition of a protein kinase C (16). Acantholytic cells of epidermis in Darier's and Hailey-Hailey diseases show several abnormalities of junction proteins. It is possible that the abnormal expression of cadherins is a result of altered calcium concentration which is caused by mutations in the calcium pumps. The abnormal calcium concentration can cause defects in cadherin function, or abnormal cadherin expression because that is dependent on calcium ions (14).

E-cadherin is expressed on the cell surfaces of all epidermal layers. It is situated in the intercellular space of desmosomes and adherens junctions (17, 18). E-cadherin is also needed in the tight junctions because it has an important role in localization of key tight junction proteins (18). The expression of E-cadherin on the surface of the acantholytic cell is significantly decreased or completely lost on the surface of some of the acantholytic cells (Fig. 2).

The expression of P-cadherin in a damaged skin has also been emphasized (20). P-cadherin has a role in forming adherens junctions. In normal skin P-cadherin is only expressed in the basal layer of the epidermis while in lesional skin P-cadherin is also expressed in suprabasal cells. The increase of P-cadherin expression is not as remarkable in a lesional skin of Darier's disease than in a lesional skin of Hailey-Hailey disease (14). One explanation may be higher proliferative activity in lesional skin, or that the differentation of suprabasal cells is delayed which results in expression of P-cadherin in suprabasal cells.

Intracellular effects of the mutations in the calcium pumps – lessons learned from experimental models

Many research groups have studied the wide effects of the mutations of SERCA2 and SPCA1 using different cell types or mouse models. The studies have revealed detailed information of the effects of certain mutations in the expression of the calcium pumps. For example, it has been shown that SERCA2b has a very significant role in calcium signalling. Thus, 50% decrease in its function leads to acantholysis (21). The analysis of the protein expression revealed that the mutation causing Darier's disease has wide effects on protein expression.

Inactivation of one SERCA2 allele in mice causes exocytosis to become ten times more sensitive to calcium than in normal mice. However, there is considerable adaptation in calcium signaling and calcium dependant cell

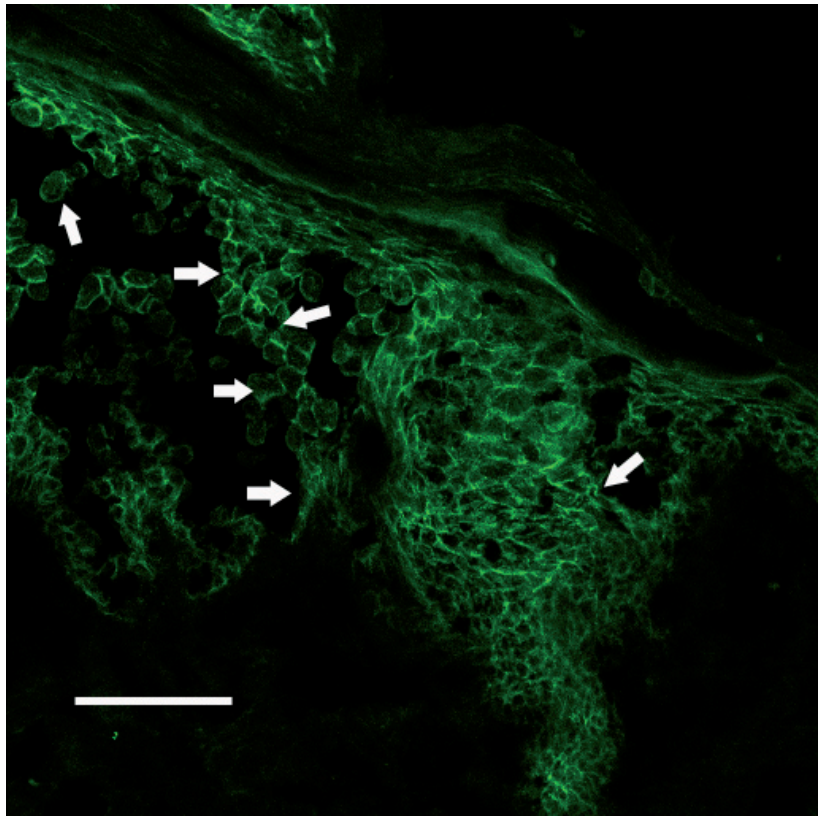

Fig. 2. E-cadherin in epidermis with Hailey-Hailey disease. Clefts formed by acantholysis are marked by red arrows. Scale bar 50 µm.

functions (22). The mouse model helps explaining why most of the organ systems of people with Darier's disease function quite normally.

Genotype – phenotype correlations in Darier's and Hailey-Hailey diseases

Being able to correlate the genotype of a patient to the phenotype would be fascinating in many inherited diseases. Especially patients whose disease manifests later in life would be eager to know how their or their children's disease will develop. The same question is of course interesting from the point of view of prenatal diagnosis. However, predicting the phenotype based on genotype has proved to be complicated in many diseases. Several research groups have studied the effects of genotype on patients' phenotype in Darier's disease and in Hailey-Hailey disease. It has been shown that in Darier's disease the mutations causing neuropsychiatric symptoms were usually at the 3' end of the ATP2A2 gene. Most of the mutations were missense type. The results support the hypothesis that ATP2A2 gene has pleiotropic effects in brain and that mutations in SERCA2 have a role in the pathogenesis of neuropsychiatric disorders (23). Variant cutaneous phenotypes were caused by point mutations in which the change of one nucleotide to other caused that the codon coded different amino acid than before the mutation. Most of the mutations caused nonsense-mediated RNA decay (24).

Over 82 different mutations causing Hailey–Hailey disease are known (6). Clear genotype phenotype correlations on the classic form on Hailey-Hailey disease have not been published while post-zygotic forms of Hailey–Hailey disease which cause segmental Hailey–Hailey disease have been investigated. These segmental diseases can be divided into types 1 and 2. In the type 1 the mutation occurs during the early embryogenesis and leads to the presence of the mutation in part of the body. The skin lesions within the affected areas show similar degree of severity to that of non-segmental disease caused by a germline mutation. Outside those lesional areas the skin is both clinically and genetically normal. In the type 2 segmental disease, the embryo with segmental mutation later develops nonsegmental diffuse skin lesions. A reason for this could be a new "second hit" mutation (25).

Future perspectives

Darier's and Hailey-Hailey diseases are chronic diseases which cause pain, itch, infections, aesthetic problems, difficulties in being able to work or became employed, and extra trouble in finding comfortable clothes. The topical treatments take patience and time, and trying to find potent treatments and being ready to look for new ones asks doctor endurance. Although the diseases are not common, the individual's suffering raises the need for new treatment modalities. Understanding the molecular mechanisms behind the diseases could provide new ways to develop treatments that target the dysfunction of cells.

References

1. Cooper SM, Burge SM. Darier's disease. Epidemiology, pathophysiology and management. Am J Clin Dermaltol 2003; 4: 97–105.
2. Hulatt L, Burge S. Darier's disease: hopes and challenges. J R Soc Med 2003; 96: 439–441.
3. Exadaktylou D, Kurwa HA, Calonje E, Barlow RJ. Treatment of Darier's disease with photodynamic therapy. Br J Dermatol 2003; 149: 606–610.
4. English JC 3rd, Browne J, Halbach DP. Effective treatment of localized Darier's disease with adapalene 0,1 % gel. Cutis 1999; 63: 227–230.
5. Sudbrak R, Brown J, Dobson-Stone C, Carter S, Ramser J, White J, et al. Hailey-Hailey disease is caused by mutations in ATP2C1 encoding a novel Ca^{2+} pump. Hum Mol Genet 2000; 7: 1131–1140.
6. Missiaen L, Raeymaekers L, Dode L, Vanoevelen J, Van Baelen K, Parys JB, et al. SPCA1 pumps and Hailey-Hailey disease. Biochem Biophys Res Commun 2004; 4: 1204–1213.
7. Hu Z, Bonifas JM, Beech J, Bench G, Shigihara T, Ogawa H, et al. Mutations in ATP2C1, encoding a calcium pump, cause Hailey-Hailey disease. Nat Genet 2000; 24: 61–65.
8. Alberts B, Bray D, Hopkin K, Johnson A, Lewis J, Raff M, et al. Essential cell biology. 2. Edition. Garland Science 2004, p. 401–402.
9. Stevens A, Lowe J. Human Histology. 2. Edition. Mosby, 1997, p. 69.
10. Dhitavat J, Fairclough RJ, Hovnanian A, Burge SM. Calcium pumps and keratinocytes: lessons from Darier's disease and Hailey-Hailey disease. Br J Dermatol 2004; 5: 821–828.
11. Foggia L, Hovnanian A. Calcium pump disorders of the skin. Am J Med Genet Part C: Semin in Med Genet 2004; 1: 20–31.
12. Ramos-Castañeda J, Park Y, Liu M,

Hauser K, Rudolph H, Schull GE, et al. Defiency of ATP2C1, a Golgi ion pump, induces secretory pathway defects in endoplasmic reticulum (ER)-associated degradation and sensitivity to ER stress. J Biol Chem 2005; 10: 9467–9473.

13. Byrne CR. The focal nature of Darier's disease lesions: Calcium pumps, stress, and mutation? J Invest Dermatol 2006; 126: 702–703.
14. Hakuno M, Akiyama M, Shimizu H, Wheelock M. J, Nishikawa T. Upregulation of P-cadherin expression in the lesional skin of pemphigus, Hailey-Hailey disease and Darier's disease. J Cutan Pathol 2001; 28: 277–281.
15. Hovnanian A. Darier's disease: from dyskeratosis to endoplasmic reticulum calcium ATPase defiency. Biochem Biophys Res Commun 2004; 3: 1237–1244.
16. Hakuno M, Shimizu H, Akiyama M, Amagai M, Wahl JK, Wheelock MJ, Nishikawa T. Dissociation of intra- and extracellular domains of desmosomal cadherins and E-cadherin in Hailey-Hailey disease and Darier's disease. Br J Dermatol 2000; 142: 702–711.
17. Furukawa F, Fujii K, Horiguchi Y, Matsuyoshi N, Fujita M, Toda K, et al. Roles of E- and P-cadherin in the human skin. Microsc Res Tech 1997; 38: 343–352.
18. Tunggal J, Helfrich I, Schmitz A, Schwarz H, Günzel D, Fromm M, et al. E-cadherin is essential for in vivo epidermal barrier function by regulating tight junctions. EMBO J 2005; 24: 1146–1156.
19. Burge SM, Schomberg KH. Adhesion molecules and related proteins in Darier's disease and Hailey-Hailey disease. Br J Dermatol 1992; 127: 335–343.
20. Kovacs A, Schmidt E, Begany A, Hunyadi J, Szegedi A. Immunohistochemical examination of P-cadherin in bullous and acantholytic skin diseases. Acta Derm Venereol 2004; 84: 116–119.
21. Ahn W, Lee MG, Kim KH, Muallem S. multiple effects of SERCA2b mutations associated with Darier's disease. J Biol Chem 2003; 23: 20795–20801.
22. Zhao X, Shin DM, Liu LH, Shull GE, Muallem S. Plasticity and adaptation of Ca^{2+}-dependent exocytosis in SERCA2+/- mice. EMBO J 2001, 20: 2680–2689.
23. Jacobsen NJO, Lyons I, Hoogendoorn B, Burge S, Kwok P, O'Donovan MC, et al. ATP2A2 mutations in Darier's disease and their relationship to neuropsychiatric phenotypes. Mol Genet 1999; 9: 1631–1636.
24. Ruiz-Perez VL, Carter SA, Healy E, Todd C, Rees JL, Steijlen PM, et al. ATP2A2 mutations in Darier's disease: variant cutaneous phenotypes are associated with missense mutations, but neuropsychiatric features are independent of mutation class. Hum Mol Genet 1999; 9: 1621–1630.
25. Poblete-Gutiérrez P, Wiederholt T, König A, Jugert FK, Marquardt Y, Rübben A, et al. Allelic loss underlies type 2 segmental Hailey-Hailey disease, providing molecular confirmation of novel genetic concept. J Clin Invest 2004; 10: 1467–1474.