UNIVERSITÀ DI PARMA

UNIVERSITA' DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN
"Scienze Mediche e Chirurgiche Traslazionali"

CICLO XXXVII

The molecular mechanisms behind Darier's Disease: from genes to proteins

Coordinatore:
Chiar.mo Prof. Nicola Sverzellati

Tutore:
Chiar.mo Prof. Antonio Percesepe
Chiar.ma Prof.ssa Valeria Barili

Dottoranda: Erika De Sensi

Anni Accademici 2021/2022 – 2023/2024

Sommario

1. Abstract

Darier Disease (DD) (OMIM #124200) is a rare autosomal dominant genodermatosis affecting around 1 in 50,000 people. From a histopathological point of view, DD is characterized by loss of intercellular adhesion (acantholysis), premature and abnormal keratinization (dyskeratosis) and presence of rounded keratinocytes (corps ronds). Skin lesions typically appear in patients between the age of 6 and 20 years, predominantly in body areas more exposed to UV irradiation, heat and friction. DD is primarily attributed to missense, non-sense, deletion, insertion and altered splicing mutations within the ATP2A2 gene (12q23-24.1) encoding the Sarco/Endoplasmic Reticulum Calcium ATPase type 2 (SERCA2), a ubiquitously expressed cellular pump responsible for the translocation of Ca^{2+} from the cytosol to the Endoplasmic Reticulum. Altered SERCA2 function impairs the intracellular calcium homeostasis leading to ER stress response and cell apoptosis. The perturbation of calcium levels could be responsible for desmosomes assembly alteration, consequently impairing cell-to-cell adhesion. However, the molecular mechanism by which SERCA2 mutations cause disease abnormalities is not well clarified.

Considering the SERCA2 role in the modulation of the NOTCH1 pathway in several disease models, our hypothesis posits that SERCA2 loss-of-function variants could inactivate NOTCH1 activity and its signaling. The project's aims include the definition of the role of SERCA2 mutations on the NOTCH1 and other molecular pathways and the transcriptional effects of SERCA2 inhibition.

By combining *in vitro* and *in vivo* assays we identified novel signaling pathways deregulations. In particular, upregulated ones included immunological, inflammatory, and cell responses to external stimuli, while genes associated with metabolic functions such as fatty acid metabolism, ribosomal structural components, and mitochondrial activity resulted to be downregulated. Additionally, NOTCH1 signaling target proteins were found to be dysregulated, according to our starting hypothesis.

Our findings provide important insights into the molecular mechanisms underlying Darier disease, which may offer potential targets for therapeutic interventions.

1.1 Abstract (italiano)

La malattia di Darier (OMIM #124200) è una rara genodermatosi autosomica dominante che colpisce circa 1 persona su 50.000. Da un punto di vista istopatologico, la DD è caratterizzata da perdita di adesione intercellulare (acantolisi), cheratinizzazione prematura e anomala (discheratosi) e presenza di cheratinociti tondeggianti (corps ronds). Le lesioni cutanee compaiono tipicamente in pazienti di età compresa tra i 6 e i 20 anni, prevalentemente nelle aree corporee più esposte ai raggi UV, al calore e allo sfregamento. La DD è principalmente attribuita a mutazioni missenso, non-senso, di delezione, di inserzione e di splicing alterato all'interno del gene ATP2A2 (12q23-24.1) che codifica per la Sarco/ATPasi del calcio del reticolo endoplasmatico di tipo 2 (SERCA2), una pompa cellulare ubiquitariamente espressa responsabile della traslocazione degli ioni Ca^{2+} dal citosol al lume del reticolo endoplasmatico. L'alterata funzione della proteina SERCA2 compromette l'omeostasi intracellulare del calcio portando all'attivazione della risposta allo stress ER e all'apoptosi cellulare. La perturbazione dei livelli di calcio potrebbe essere responsabile dell'alterazione dell'assemblaggio dei desmosomi, compromettendo di conseguenza l'adesione cellula-cellula. Tuttavia, il meccanismo molecolare con cui le mutazioni di SERCA2 causano anomalie della malattia non è ben chiarito. Considerando il ruolo di SERCA2 nella modulazione della via di segnalazione di NOTCH1 in diversi modelli di malattia, la nostra ipotesi postula che le varianti con perdita di funzione di SERCA2 potrebbero compromettere la maturazione NOTCH1 e di conseguenza la trascrizione dei suoi geni target. Gli obiettivi del progetto includono la definizione del ruolo delle mutazioni di SERCA2 sulla via di segnalazione di NOTCH1 e di altre vie molecolari e gli effetti trascrizionali dell'inibizione di SERCA2.

Combinando saggi *in vitro* ed *in vivo* abbiamo identificato la deregolazione di diverse vie di segnalazione. In particolare, quelle sovra regolate includono processi immunologici, infiammatori e risposte cellulari a stimoli esterni, mentre i geni associati alle funzioni metaboliche come il metabolismo degli acidi grassi, le componenti strutturali ribosomiali e l'attività mitocondriale risultano sotto regolati. Inoltre, è stato riscontrato che la segnalazione NOTCH1 è deregolata. I nostri risultati forniscono importanti informazioni sui meccanismi molecolari alla base della malattia di Darier, che possono offrire potenziali bersagli per interventi terapeutici.

2. Introduction

2.1 Role and structure of skin

Being the largest organ in the body, the skin acts as a vital defense against various environmental threats such as toxins, viruses, UV radiation, trauma, and high temperatures. In a similar manner, it acts as a barrier from the inside of the body to prevent excessive electrolyte and water loss. In addition, the skin senses and responds to both internal and external stimuli. (P. M. Elias, 2005; Proksch et al., 2008; Nestle et al., 2009; Di Meglio et al., 2011; Simpson et al., 2011).

For these reasons, preserving the integrity and control of skin function is critical to human survival.

Anatomically, the human skin comprises three distinct layers: hypodermis, dermis, and epidermis, which in turn includes various layers and cell types (**Figure 1**).

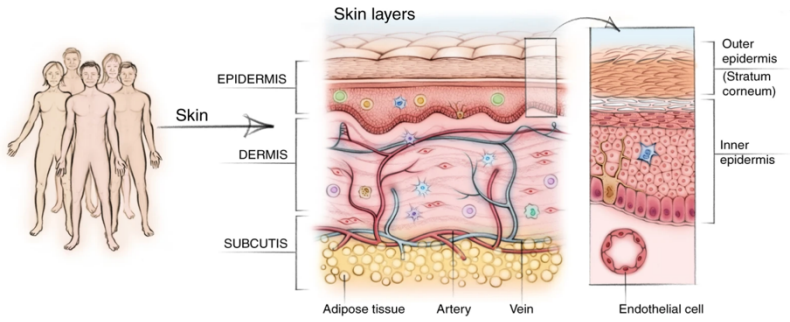


Figure 1. Human skin layers (Dyring-Andersen et al., 2020)

2.1.1 Hypodermis

The hypodermis, also known as subcutaneous stratum or subcutis, is the skin's deepest layer, predominantly composed of adipose tissue. This layer is highly vascularized and provides skin thermal insulation and shock absorption. Adipocytes and fibroblasts are the main cell types found in this layer, along with immune-related cells such as macrophages and monocytes (Desruisseaux et al., 2007; Driskell et al., 2014). It also functions as an endocrine organ since

it secretes different types of hormones involved in inflammation, hair growth, and glucose homeostasis (Kershaw & Flier, 2004).

2.1.2 Dermis

The dermis, situated above the subcutis and below the epidermis, is the skin's middle layer. It includes two sublayers: the papillary dermis (superficial) and the reticular dermis, which is deeper, thicker, and denser. Collagen and elastin, two extracellular matrix (ECM) proteins released by fibroblasts and responsible for the skin's elasticity and strength, are abundant in this layer. Neural mechanoreceptors and thermoreceptors, which allow for the sensory transmission of touch and temperature, respectively, are found in the dermis. It also includes hair follicles, blood vessels, sweat and sebaceous glands. From a cellular perspective, the main constituents of this layer of skin include fibroblasts, mast cells, macrophages, and stem cells, among other cell types (Prost-Squarcioni et al., 2008; Pasparakis et al., 2014).

2.1.3 Epidermis

The epidermis, the outermost skin layer, represents an effective barrier against external threats (Wertz, 2013). Over 90% of its cells are keratinocytes, whose name derives from keratin, representing its main protein and conferring stability to the epidermis, nails, and hair (Steinert, 1993). Other cell types found in the epidermis include melanocytes, Langerhans cells and T lymphocytes, involved in the immune response, and Merkel cells, related to touch perception.

From deep to superficial, the structure of this stratified squamous epithelium contains the following layers:

- The Stratum basale (or stratum germinativum) is the deepest one where cells actively divide. It consists of a single layer of cube-shaped stem cells, attached to the basal membrane, generating transit-amplifying cells that divide and differentiate (Rousselle et al., 2019). Here are also found melanocytes, responsible for producing melanosomes containing melanin, the pigment that gives skin its color (Cichorek et al., 2013). The expression of keratins (KRT) 5 and

14, which anchor cells to the basal membrane below, is a characteristic of basal layer keratinocytes (Barbieri et al., 2014; Srivastava et al., 2018).

- The Stratum spinosum is made up of 8-10 cell layers. Because of their spiky appearance, the cells of this layer are called “prickle cells”, which differentiate, express keratins 1 and 10, and are implicated in immunological responses.

- The Stratum granulosum is the skin layer where the keratinocytes assume a flattened aspect. Cells express late-differentiation markers including loricrin, involucrin, and filaggrin. The latter is contained in keratohyalin granules which confer the typical aspect to granular keratinocytes.

- The Stratum lucidum (only in thick skin, like hands and feet palms) is a clear layer consisting of densely packed keratinocytes that are flattened, filled with keratin, and containing a lipid-rich protein, the eleidin.

- The Stratum corneum is the outermost layer of the epidermis and is made up of dead keratinocytes, or corneocytes, which are continually shed in the desquamation process and replaced by new cells. The epidermal differentiation culminates in the generation of the cornified envelope, which replaces the plasma membrane in this specific type of keratinocytes. Its structure, which acts as a barrier against external influences and stops the body from losing water, is made up of crosslinked, protein-embedded keratin wrapped in a lipid envelope mainly composed of ceramides and cholesterol (Fuchs & Byrne, 1994; Kalinin et al., 2002; J. Segre, 2003; Candi et al., 2005; Blanpain & Fuchs, 2009; Di Meglio et al., 2011; P. M. Elias, 2012; Iwai et al., 2012; Gonzales & Fuchs, 2017) (**Figure 2**).

Each epidermal layer with its specific proteins plays a crucial role in preserving skin integrity, protection, and functionality (Goleva et al., 2019) and the stratification process of the epidermis is highly regulated. Basal keratinocytes are involved in epidermal homeostasis maintenance since they have an active proliferative activity. These mitotic keratinocytes differentiate towards the epidermis surface to form the spinous, granular, and corneum strata, which together constitute the suprabasal layers and generate an impermeable body surface (Gonzales & Fuchs, 2017).

Skin homeostasis depends on the preservation of cellular organization and composition throughout cell turnover. Two models have been evaluated to explain stem cells maintenance in the skin. In the classic model, every cell division leads to the generation of two daughter cells with different fates: one remains in the stem cell niche, while the other commits to

differentiation and is called “transit-amplifying progenitor cell” (Watt et al., 2006). In the second model, one of the daughter cells differentiates, without any other divisions, while the other continues to proliferate (Clayton et al., 2007). Keratinocytes that reach the outermost epidermal layer are shed from the skin surface following apoptosis. In contrast, the basal keratinocytes remain tightly attached to the below basal membrane, thereby preserving the stability of the epidermis by continually replenishing the suprabasal cells that are undergoing terminal differentiation (Plikus et al., 2012).

During the transition from the basal towards the skin surface, each epidermal layer is defined by distinctive morphological features and the expression of specific proteins, indicating its differentiation stage (Fuchs, 1990; Candi et al., 2005; Bikle et al., 2012). Basal keratinocytes are characterized by KRT5 and KRT14, while the early differentiation markers KRT1 and KRT10 are predominant in the spinous layer, which contains also precursors of the cornified envelope, including involucrin (IVL) and transglutaminase-1 (TGM1). Granular layer keratinocytes express late differentiation markers such as the cornified envelope protein loricrin (LOR) and filaggrin (FLG). The cross-linking of TGM1 with IVL, LOR, and other structural proteins leads to the cornified envelope formation (Goleva et al., 2019) (**Figure 3**).

Moreover, the basal membrane is rich in extracellular matrix (ECM), with laminin being its key component and basal keratinocytes interact with the underlying membrane through integrins α6β4 and α3β1 (L. Yap et al., 2019). Following the differentiation of basal keratinocytes, there is a reduction in their integrins and laminin levels, while the expression of specific keratins increases. Keratin intermediate filaments (IFs) are key structural components in the epidermis, linking to hemidesmosomes and connecting desmosomes to establish intercellular junctions. The KRT1/KRT10 intermediate filament network in spinous cells is more stable than the KRT5/KRT14 one in the basal layer (Singh, 2022). The transition of keratinocytes from the basal layer to the stratum corneum in the human epidermis typically takes 40 to 56 days (Liu et al., 2019).

Several evolutionarily conserved pathways, such as NOTCH, c-Myc, Wnt/β-catenin and p63, have been demonstrated to play key roles in the embryonic skin development, the maintenance of epidermal stem cells, as well as their differentiation and lineage commitment (Fuchs et al., 2004; Senoo et al., 2007; Watt, Frye, et al., 2008) and emerging evidence suggests

interactions between them. The perturbation of this organized architecture has been observed in the case of different human genetic skin disorders and cancers.

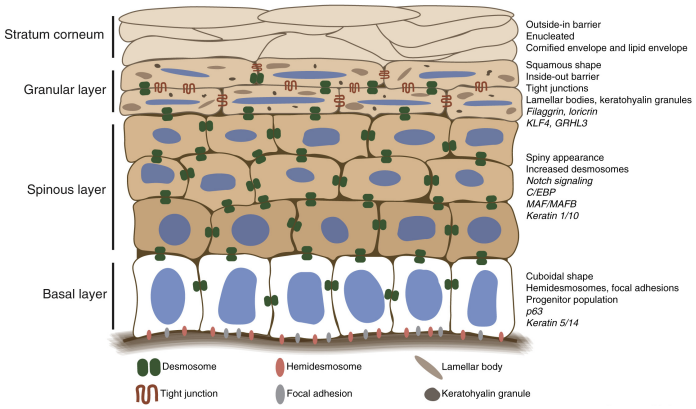


Figure 2. Basic epidermal structure. Cells from the proliferative basal layer transit towards the cornified envelope (stratum corneum). Physical characteristics are indicated in the legend, whereas each layer-specific genes and proteins are listed on the right side (Moreci & Lechler, 2020).

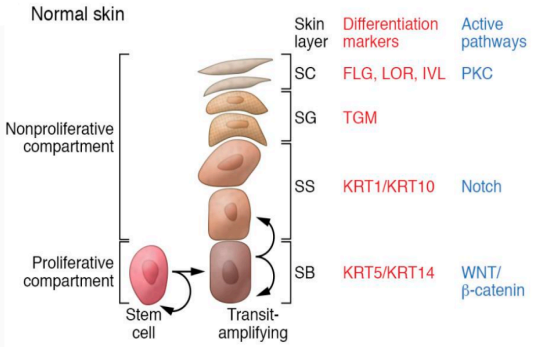


Figure 3. Epidermal differentiation pattern. The epidermal differentiation process is tightly regulated. When basal cells detach from the basement membrane and migrate in the spinous layer, they exit the cell cycle and express KRT1/KRT10 instead of KRT5/KRT14 typical of the basal layer. Subsequently, they switch from KRT1/KRT10 to granular layer differentiation markers FLG, LOR, and TGM1. The basal layer is characterized by the Wnt/β-catenin pathway, whereas in the spinous cells, the NOTCH pathway is active (Goleva et al., 2019).

2.2 Skin cell-to-cell adhesion

The adhesion between keratinocytes is essential for the skin to function effectively as a human body protective barrier. Indeed, basal keratinocytes' intercellular junctions have a polarized organization and are involved in spindle orientation during skin stratification (C. Niessen,

2010). In the epidermis, cell-to-cell adhesive complexes include desmosomes, adherens junctions, and tight junctions.

2.2.1 Desmosomes

Desmosomes form complexes providing strong mechanical intercellular adhesion between keratinocytes. These complexes are generated by transmembrane glycoproteins belonging to the Ca^{2+}-dependent cell adhesion protein family called cadherins. Desmogleins (DSG) and desmocollins (DSC), two cadherins that attach to each other extracellularly, are specifically the implicated ones. The keratin intermediate filament cytoskeleton is intracellularly linked to the DSG and DSC complexes by an intricate protein network that includes plakoglobin, desmoplakin, and plakophilin. This arrangement provides significant adhesive strength (Getsios et al., 2004; Green & Simpson, 2007; Schmidt & Koch, 2007; Garrod & Chidgey, 2008; Franke, 2009) (**Figure 4**).

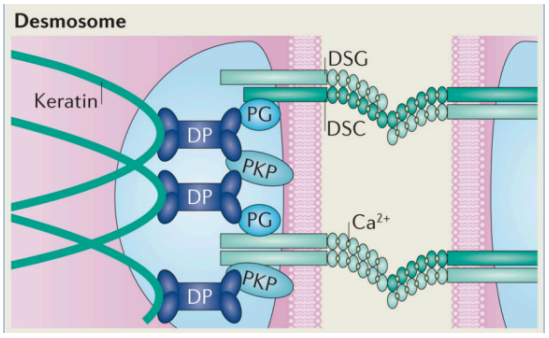


Figure 4. Desmosome structure. Intracellularly, desmosomes link to the keratin intermediate filaments network and are connected with neighboring cells by the cadherins DSG and DSC (Simpson et al., 2011).

2.2.2 Adherens junctions

Adherens junctions consist of proteins belonging to the cadherin family, particularly E-cadherin, which establish connections in the intercellular space. E-cadherin ablation causes hyperproliferation, altered differentiation, and defects in barrier formation (Tinkle et al., 2004). They are intracellularly interconnected to the actin microfilament cytoskeleton through a protein complex comprising α-catenin, β-catenin, p120, and plakoglobin (also known as γ-catenin in adherens junctions). These junctions are essential for maintaining the mechanical

stability and barrier integrity of the epidermis, and they may also be involved in controlling inflammatory responses that occur within the epidermis (Perez-Moreno et al., 2003; C. M. Niessen, 2007; C. M. Niessen & Gottardi, 2008; Franke, 2009) (**Figure 5**).

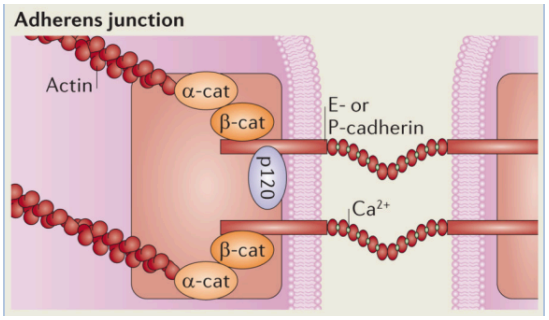


Figure 5. Adherens junction structure. Adherens junctions are involved in the assembly and organization of cortical actin cytoskeleton in the epidermis. They are constituted by E-cadherin and P-cadherin, an extracellular homology domain with a conformation regulated by calcium and a cytoplasmic tail which recruits p120 catenin, beta-catenin and alpha-catenin (Simpson et al., 2011).

2.2.3 Tight junctions

Tight junctions are known also as “zonula occludens” and are primarily found in stratum granulosum keratinocytes. They form junctions that effectively seal the intercellular space, generating a crucial barrier against the passive diffusion of water, molecules, and ions, while also providing adhesive functions. Unlike desmosomes and adherens junctions, the proteins in the intercellular space of tight junctions are not members of the cadherin family. Instead, they are made up of claudins and occludins, which intracellularly link to the actin cytoskeleton (Yuki et al., 2007; C. M. Niessen, 2007; Franke, 2009; Anderson & Van Itallie, 2009) (**Figure 6**).

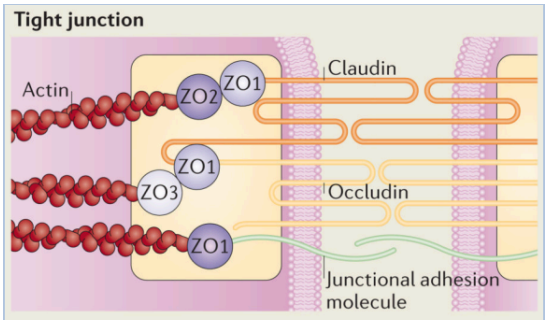


Figure 6. Tight junction structure. Tight junctions are typical of the granulosum layer and are involved in a barrier formation below the stratum corneum. They are made up of claudins, occludins, and junction adhesion molecules. These junctions scaffold is mediated by zona occludens proteins ZO1, ZO2, and ZO3, which also links them with the actin cytoskeleton (Simpson et al., 2011).

2.3 Role of calcium in the skin

Calcium ions are necessary for the maintenance of skin health and function because of their effects on keratinocyte differentiation, the establishment of the skin barrier, and wound healing (Lee & Lee, 2018; Subramaniam et al., 2021; Nopriyati et al., 2022). The Endoplasmic Reticulum (ER), the main intracellular Ca^{2+} storage compartment, maintains calcium homeostasis via several channels and pump activities. In order to support a variety of biological functions, such as cell survival, signaling, and protein folding, ER stores and releases calcium ions. In epidermal keratinocytes, ER calcium levels are tightly regulated to support cell development, barrier formation, and skin homeostasis maintenance.

In these cells, the binding of extracellular calcium to the plasma membrane G-protein coupled Ca^{2+} receptor (CaR) induces the phospholipase C (PLC) activation. This enzyme catalyzes the hydrolysis of phosphatidylinositol bisphosphate (PIP2) in inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). The second messenger IP3 binds to its receptors localized to the ER and Golgi membranes leading to the Ca^{2+} release from these intracellular compartments into the cytoplasm. The decrease of intracellular calcium stores results in the influx of extracellular Ca^{2+} across plasma membrane channels with a store-operated calcium entry function (SOCE). The consequent rise of intracellular calcium levels activates calmodulin, a main Ca^{2+}-binding protein, triggering the cytosolic calmodulin-dependent phosphatase calcineurin, which in turn dephosphorylates NFAT, a calcium-dependent transcription factor. NFAT translocates into the nucleus and induces downstream target genes. After the increase of cytosolic calcium, different transporters remove it from this compartment to restore basal levels. In particular, the plasma membrane Ca^{2+}-ATPase (PMCA) and Na^{+}/Ca^{2+} exchangers (NCX) transport Ca^{2+} from the cytosol to the extracellular medium (Savignac et al., 2011) (**Figure 7**).

ER calcium levels are essential for the proper functioning of calcium-dependent proteins and enzymes involved in maintaining the skin barrier and responding to external stimuli (Nopriyati et al., 2022). These mechanisms are sustained by epidermal calcium gradient, endoplasmic reticulum calcium homeostasis, and Ca^{2+} influx through different channels (S. E. Lee & Lee, 2018). When disruption of the skin barrier homeostasis occurs, it leads to significant changes in Ca^{2+} levels as an immediate response, which subsequently return to normal as barrier homeostasis is restored (Menon et al., 1992; Mauro et al., 1998). Calcium gradient is involved

in barrier recovery following its perturbation by stimulating lipid release and promoting keratinocytes migration during wound healing (Tu & Bikle, 2013; Tu et al., 2019).

Prior research has shown that skin injury-induced calcium waves are essential for fostering intercellular coordination and communication in the epidermis. These waves dramatically increase the skin's ability to react to inflammation and injury (Donati et al., 2022). Furthermore, STIM and ORAI protein-based systems of store-operated calcium entry (SOCE) are essential to optimal skin physiology. Melanogenesis (synthesis of melanin), keratinocyte differentiation, and sweat gland secretion are all influenced by these pathways (Manning et al., 2022).

Understanding the multifaceted role of calcium in skin biology is essential for developing strategies that effectively promote skin health and enhance wound healing processes.

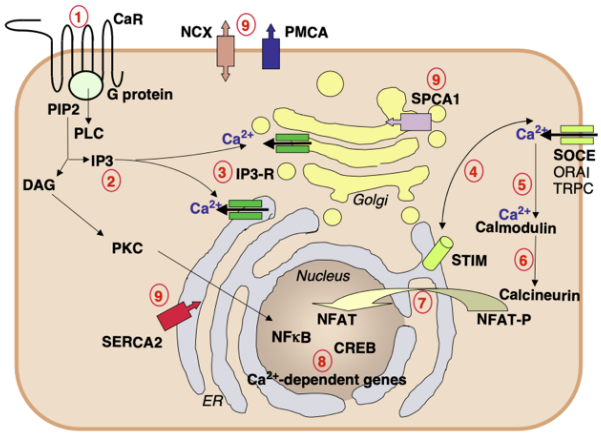


Figure 7. Calcium signaling in keratinocytes. 1) Ca2+ binding to CaR; 2, IP3 synthesis; **3**) IP3 binding to IP3-R and Ca^{2+} stock release; **4**) Ca^{2+} entry by SOCE; **5**) Ca^{2+} is transported by Calmodulin; **6**) Calcineurin activation; **7**) NFAT dephosphorylation; **8**) Ca^{2+}-dependent gene transcription; **9**) Clearance systems (Savignac et al., 2011).

2.3.1 Calcium and cell adhesion

As mentioned before, keratinocytes adhere to each other through different types of structures (desmosomes, adherens, and tight junctions), determining the skin's integrity and function. Also in this case, calcium plays a pivotal role in the formation of adhesion complexes, for example by stimulating E-cadherins dimerization and clustering, leading to the interaction of cadherins cytoplasmic tail to the actin network of the cytoskeleton, essential for the stabilization and formation of adherens junctions (A. S. Yap et al., 1997). Calcium is also

necessary for the stimulation of the DSG and DSC cadherins to assemble desmosomes (Kowalczyk et al., 1999). On the contrary, it has been observed that a reduced level of extracellular Ca^{2+} in keratinocyte cell cultures impedes the formation of cell-to-cell adhesion molecules and maintains this inhibition over time. The impact of calcium on the desmosomes and adherens junctions development is intricately linked to the epidermal Ca^{2+} gradient and the keratinocytes terminal differentiation (Jamora & Fuchs, 2002; Garrod & Chidgey, 2008). Indeed, in Darier and Hailey-Hailey diseases, two skin conditions characterized by suprabasal cells loss of adhesion, altered regulation of calcium, and increased transepidermal water loss have been observed (Lavrijsen et al., 1993).

2.3.2 Calcium regulation of keratinocytes differentiation

Calcium is a major modulator of keratinocytes differentiation (Elsholz et al., 2014) by regulating their commitment in the basal and spinous layers and the terminal differentiation process in the granular one (Bikle et al., 2012). Indeed, the skin maintains a distinct extracellular gradient of Ca^{2+}, starting with low levels in the basal layers, gradually increasing to a peak in the stratum granulosum, and then rapidly decreasing in the outermost stratum corneum (Menon & Elias, 1991; P. Elias et al., 2002). Calcium is involved in the transcription regulation of keratinocytes differentiation-specific genes. For example, transcription factor AP-1 (activator protein-1) controls the expression of several differentiation markers expressed in keratinocytes, including loricrin, involucrin, profilaggrin, and keratins (Eckert et al., 1997). The promoter region of the involucrin gene contains an AP-1 responsive element site, which is crucial for the calcium-mediated response in regulating the synthesis of differentiation-specific proteins (Ng et al., 2000).

Calcium levels required for stage-specific expression of proteins involved in differentiation vary across different epidermal layers. For instance, the extracellular calcium concentration necessary for the expression of profilaggrin, a late differentiation marker, is higher than that needed for keratin 1 and keratin 10, in the spinous layer (Yuspa et al., 1989). These observations denote the importance of the epidermal calcium gradient for proper epidermal differentiation and barrier formation. Additionally, calcium plays a significant role in posttranslational modification processes, including the conversion of profilaggrin into filaggrin.

Profilaggrin contains an N-terminal domain with Ca^{2+}-binding motifs and when calcium binds to its head domain, it induces conformational changes that reveal essential cleavage sites, thus initiating the modification mechanism (Presland et al., 1995). Moreover, calcium is also involved in the terminal differentiation stage, regulating the cornified envelope formation. Indeed, transglutaminases 1, 3, and 5 determine the crosslinking between late differentiation proteins such as loricrin, involucrin, filaggrin, keratin, and small proline-rich proteins (SPRRs) in a calcium-dependent manner (Hitomi, 2005). Additionally, the calcium-sensitive protein kinase C (PKC) plays an important role in keratinocytes differentiation, determining the cell transition from the stratum spinosum to the stratum granulosum, by decreasing the expression of keratin 1 and 10 genes while promoting the ones associated with late-stage differentiation (Denning, 2004). Calcium gradient perturbation within the epidermis and the consequent alterations of the differentiation process are involved in dermatological diseases pathogenesis, including atopic dermatitis, psoriasis, Hailey-Hailey, and Darier diseases (S. E. Lee & Lee, 2018). Indeed, in Darier Disease patients' keratinocytes dysregulated differentiation and cell adhesion are observed due to SERCA2 alterations and the consequent ER inability to store calcium (Foggia & Hovnanian, 2004).

2.4 Darier Disease

Darier Disease (DD) (OMIM: # 124200), also known as keratosis follicularis, is a rare autosomal dominant inherited skin disease characterized by hyperkeratotic papules appearing in folds and seborrheic body areas, including trunk, scalp, neck, and face, and by nail abnormalities. The estimated prevalence of the disease is around 1 in 50,000, and individuals with DD typically develop cutaneous lesions between the ages of 6 and 20 (S. M. Burge & Wilkinson, 1992; Hovnanian, 2019).

From the histological point of view, DD skin lesions exhibit loss of cohesion between suprabasal epidermal cells (acantholysis), abnormal and premature keratinization (dyskeratosis) and thickened epidermis (acanthosis) (Brini & Carafoli, 2009). Acantholytic lesions are mainly caused by the loss or the altered function of desmosomes, responsible for intercellular adhesion of keratinocytes (Burge & Garrod, 1991; Dhitavat et al., 2003; Li et al., 2017). Dyskeratosis is caused by keratinocytes apoptosis, which determines the formation of ''corps

ronds", characterized by a dark color with contracted and fragmented nuclei surrounded by a clear cytoplasm, and "grains", smaller than "corps ronds" and typically located in the stratum corneum. Their cytoplasm appears eosinophilic due to the presence of keratin clumps. These two histopathological signs are distinctive features of the DD diagnosis (Pels & Goodman, 1939) (**Figure 8**).

Cutaneous lesions often appear during puberty and have a chronic relapsing course. Symptoms include pain, malodor, and itch, and are exacerbated by sunlight, ultraviolet irradiation, heat, sweating, infections, and friction (Munro, 1992; S. Burge, 1994). Moreover, DD patients often suffer from itch (mild to moderate) and manifest malodor due to lesional skin infections with bacteria, yeast, and fungi. For these reasons affected subjects have a reduced quality of life depending on disease severity (S. M. Burge & Wilkinson, 1992; S. Burge, 1994; Ringpfeil et al., 2001; Dodiuk-Gad et al., 2013; Yeshurun et al., 2021).

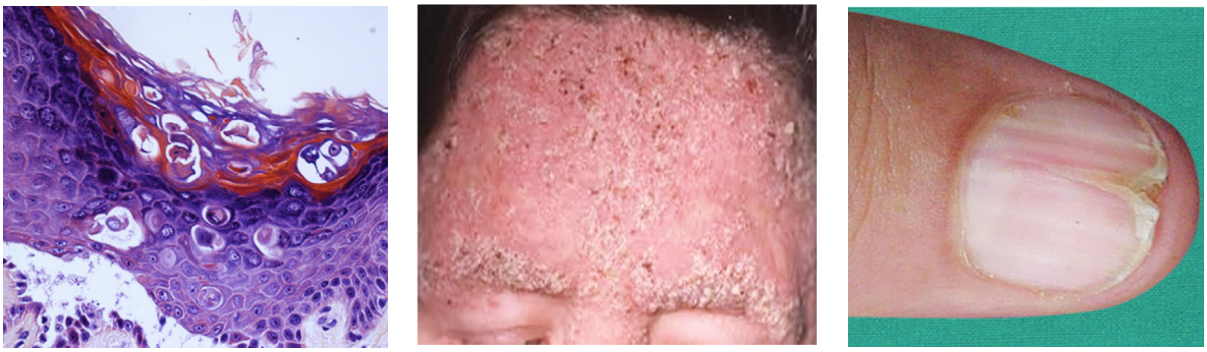


Figure 8. Darier Disease typical lesions. A) DD skin biopsy with an epidermal suprabasal cleft containing acantholytic cells. Hyperkeratosis and "corps ronds" are also shown (Savignac et al., 2011). **B**) Keratotic papules in the forehead. **C**) V-shaped nails (Deepika, 2020).

2.4.1 ATP2A2 gene dysfunction and SERCA2 protein alteration

Darier Disease is inherited in an autosomal dominant fashion (Berg & Bassett, 1993) and has been linked to ATP2A2 gene variants in chromosome region 12q23-q24.1 (Sakuntabhai, Ruiz-Perez, et al., 1999). Currently, a total of 454 *ATP2A2* variants have been reported of which 311 are unique [Leiden Open Variation Database; https://databases.lovd.nl/shared/genes/ATP2A2, accessed 2024-08-04]. The majority of *ATP2A2* variants are missense/nonsense, deletions,

splicing mutations, and insertions and have been found in all exons without a mutational hotspot (Ringpfeil et al., 2001; Nellen et al., 2017).

ATP2A2 encodes four isoforms (a-d) of the sarco-endoplasmic reticulum (ER) Ca^{2+} ATPase protein 2 (SERCA2), a calcium pump located on the ER membrane transporting Ca^{2+} ions from the cytosol to the ER lumen (Hovnanian, 2007; Brini & Carafoli, 2009; Gorski et al., 2017). The main isoforms generated from ATP2A2 gene transcript through alternative splicing are SERCA2a and SERCA2b, consisting of 21 and 20 exons, respectively (MacLennan et al., 1985; Lytton & MacLennan, 1988). SERCA2b is expressed ubiquitously, however, it is predominant in the human epidermis (Tavadia et al., 2004). Perturbation of SERCA2 Ca^{2+} transport determines the depletion of epidermal keratinocytes ER Ca^{2+} stores which could be responsible for desmosomal instability (Leinonen et al., 2005; Foggia et al., 2006; Müller et al., 2006). The skin of Darier disease patients is defective in desmosomes, involved in cell-to-cell adhesion, and components of these structures have been observed to accumulate in the cytoplasm of the acantholytic cells (S. M. Burge & Garrod, 1991). Additionally, also Stuart and colleagues observed that the perturbation of intracellular calcium stores alters the biogenesis of desmosomes and tight junctions (Stuart et al., 1996) (**Figure 9**).

ATP2A2 haploinsufficiency has been postulated to explain DD development (Prasad et al., 2005), however, the employment of SERCA-specific inhibitors, siRNA, and site-directed mutagenesis in several *in vitro* studies on epidermal and other cell types revealed that this mechanism may not sufficiently explain DD features (Ahn et al., 2003; Wang et al., 2011; Li et al., 2017). Previous studies on SERCA2 variants in epidermal cell lines found protein mutants to be insoluble or partially soluble due to their misfolded state. Insoluble SERCA2 mutants are not degraded by proteasomes and form aggregates in *aggresomes* causing ER stress, cells rounding up, and apoptosis (Y. Wang et al., 2011). Co-immunoprecipitation assays showed the interaction between SERCA2 mutants with WT monomers leading to the formation of dysfunctional dimers and the inhibition of Ca^{2+} uptake rate by the wild-type protein (Ahn et al., 2003).

Considering the late onset and the previous suggestions about the insufficiency of *ATP2A2*$^{+/-}$ to be the exclusive cause of the disease, other pathways could play a role in the pathogenesis of DD. In particular, this study is focused on the involvement of the NOTCH1 signaling pathway, since SERCA2 is one of its modulators as emerged in previous observations (Roti et al., 2013).

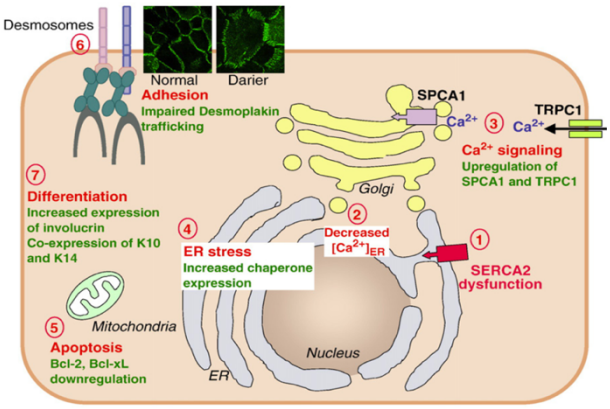


Figure 9. Consequences of SERCA2 mutations in Darier keratinocytes. **1**) SERCA2 alterations determine disruption of Ca^{2+} transport by the pump and **2**) lead to a reduction of ER calcium stores. **3**) Compensatory mechanisms in calcium signaling include the up-regulation of SPCA1 and TRPC1 expression. **4**) The decrease of ER Ca^{2+} stores results in an ER stress response with the increase of chaperone protein expression. **5**) Persistent ER stress in DD keratinocytes determines apoptosis with the decreased expression of Bcl-2 and Bcl-xL. **6**) Altered desmoplakin trafficking is associated with intercellular adhesion. **7**) DD keratinocytes differentiation is also perturbed, with enhanced and premature involucrin expression (Savignac et al., 2011).

2.4.2 SERCA structure and function

SERCA proteins are P-type ion-motive ATPases which actively transport two Ca^{2+} ions from the cytosol to the ER lumen by employing an ATP molecule (Toyoshima, 2008; Bublitz et al., 2010). The SERCA pumps family is evolutionarily conserved and is encoded by three genes: *ATP2A1*, *ATP2A2,* and *ATP2A3*. Tissue-specific alternative splicing mechanisms determine the generation of 12 isoforms (Bobe et al., 2005), consisting of single polypeptide chains of around 1000 amino acids and 110 kDa in weight (MacLennan et al., 1985; Dally et al., 2006; Toyoshima, 2008). SERCA2 protein is located on the ER membrane and is made up of 10 transmembrane helices (M1-M10), a cytosolic stalk domain (S1-S5), and three cytosolic domains: actuator (A), nucleotide adenosine triphosphate (ATP)-binding (N) and phosphorylation (P). The Ca^{2+} channel is formed by the M2, M5, M6, and M8 domains, while the Ca^{2+} transportation across the ER membrane is mediated by M4-M6 (Guerini, 1998; Zhang et al., 1998; Toyoshima et al., 2000; Møller et al., 2005; Toyoshima, 2008) (**Figure 10 A**). Through the hydrolysis of adenosine triphosphate, SERCA2 actively mediates the transport of two Ca^{2+} ions from the cytosol into the ER lumen, thereby sustaining a higher concentration of Ca^{2+} in this compartment compared to the cytosol (Lytton et al., 1992). This enzymatic reaction (E) is characterized by the

alternation of phases with high and low affinity to Ca^{2+}, $2Ca^{2+}E_1$ and E_2, respectively, which are linked to high and low energy phosphorylated states, $2Ca^{2+}E_1$~P and E_2~P, respectively: $E_2 \rightarrow 2Ca^{2+}E_1 \rightarrow 2Ca^{2+}E_1$~ P $\rightarrow 2Ca^{+}E_2$~P $\rightarrow E_2$~ P$\rightarrow E_2$ (Albers, 1967).

At the Ca^{2+} low-affinity state (E_2), SERCA Ca^{2+} binding sites are exposed to the ER lumen (Olesen et al., 2007), while ATP is located in the N domain, distant from the Asp351 residue of the P domain (Jensen et al., 2006). The exchange of 2/3 H^+ induces a conformational change in the M1-M4 helices, facilitating the rotation of the A domain and the binding of cytosolic Ca^{2+} ($2Ca^{2+}E_1$). Subsequently, the N domain moves closer to the Asp351 residue, leading to the energy-phosphorylated state $2Ca^{2+}E_1$~P (Sørensen et al., 2004; Møller, Nissen, et al., 2005) in which the Ca^{2+} binding sites are exposed to the cytosol. The transition to a lower energy state ($2Ca^{+}E_2$~P) determines the rotation of the N and A domain leading to the interaction of the M1-M2 and M3-M4 helices with the M5-M10 complex causing the exposure of Ca^{2+} binding sites to the ER lumen and protons are transported in the cytosol (Toyoshima, 2007, 2009). Following SERCA2 dephosphorylation and the release of ADP, free phosphate and Ca^{2+}, the catalytic cycle is closed (E_2~ P$\rightarrow E_2$) (Gorski et al., 2017) (**Figure 10 B**).

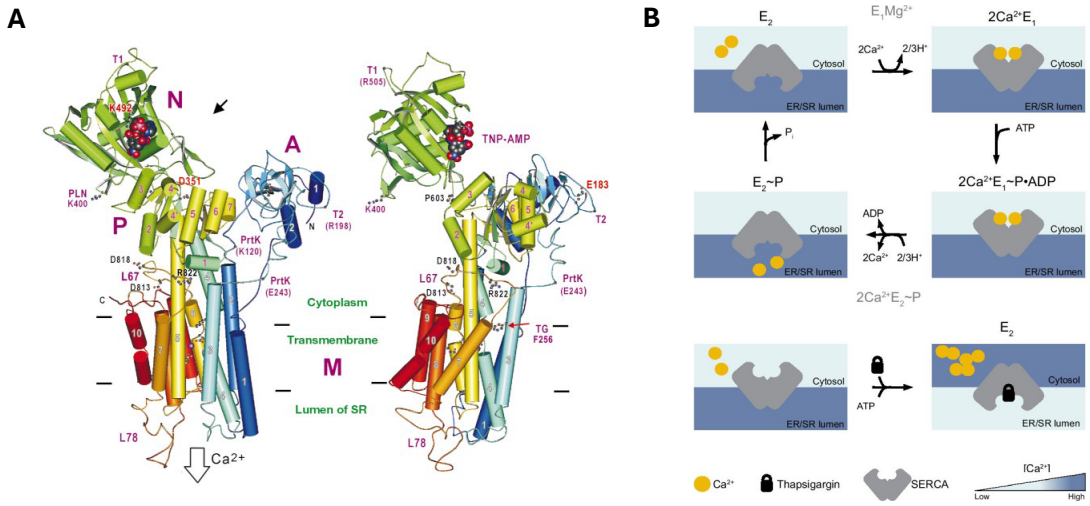


Figure 10. **A**) Architecture of the sarcoplasmic reticulum Ca^{2+}-ATPase. N terminus in blue and C terminus in red. A, N and P are the three cytoplasmic domains. Transmembrane helices (M1–M10) and those in domains A and P are numbered. The model in the right panel is rotated by 50° around M5. D351 is the residue of phosphorylation. Ca^{2+} is represented by two purple spheres in the transmembrane binding sites. PLN and TG are the binding sites for phospholamban and thapsigargin (TG), respectively (Toyoshima et al., 2000). **B**) SERCA2 protein Catalytic cycle (Pagliaro et al., 2021).

Compared to the cytosol, in the ER calcium is stored at a 1000-10,000-fold higher concentration (Meldolesi & Pozzan, 1998; Michalak et al., 2009), defining the ER as the largest deposit of calcium in the cell (Coe & Michalak, 2009). Considering that calcium intracellular stores are the main constituent of the Ca^{2+} gradient in the epidermis (Cornelissen et al., 2007; Celli et al., 2010; Adams et al., 2012) and that SERCA2 is the principal Ca^{2+} pump in the ER, this protein regulates not only the intracellular calcium but also the epidermal Ca^{2+} gradient and keratinocytes differentiation (L. Li et al., 1995b; Savignac et al., 2011; Celli et al., 2016). Reduced Ca^{2+}-ATPase function affects the trafficking of proteins with signal and/or adhesive properties towards the plasma membrane (Periz & Fortini, 1999).

2.4.3 SERCA isoforms

Alternative splicing mechanisms determine the generation of twelve tissue-specific SERCA isoforms: SERCA1a-b, SERCA2a-d, and SERCA3a-f, with similar size and weight but distinct tissue expression; also their Ca^{2+} binding affinity may differ (Brandl et al., 1987; Britzolaki et al., 2018, 2020).

2.4.3.1 SERCA1

ATP2A1 gene encodes for SERCA1 protein, distinguished in two isoforms. SERCA1a is mainly expressed in adults, while SERCA1b during the neonatal period. SERCA1 is temporally expressed and has been identified in primarily fast-twitch skeletal muscle (Brandl et al., 1987).

2.4.3.2 SERCA2

SERCA2 is encoded by the ATP2A2 gene and has four isoforms. Mutations in this gene have been associated with the onset of DD (Sakuntabhai, Ruiz-Perez, et al., 1999). SERCA2a (997 aa) is predominantly expressed in slow-twitch and cardiac muscle and in different brain cells at low levels (Lytton & MacLennan, 1988; Wuytack et al., 1989; Baba-Aissa et al., 1996).
SERCA2b (1042 aa) is ubiquitously expressed and exhibits the highest affinity for Ca^{2+} compared to other SERCA isoforms (Verboomen et al., 1995).

It is the main isoform present in the skin and is critical for cellular calcium homeostasis (Burk et al., 1989; Vandecaetsbeek et al., 2009; Chemaly et al., 2018). The SERCA2b extended C-terminus localizes to the ER membrane and determines the formation of an eleventh transmembrane helix (M11) (Verboomen et al., 1992, 1994), which seems to be involved in the determination of the highest affinity for calcium of SERCA2b compared to the SERCA2a isoform (Lytton et al., 1992; Verboomen et al., 1992; Vandecaetsbeek et al., 2009) (**Figure 11**). SERCA2c is expressed in mesenchymal, hematopoietic, and epithelial cells and at low levels in the brain (Gélébart et al., 2003; Dally et al., 2006, 2010) and has the lowest Ca^{2+} affinity compared to the other SERCA2 isoforms (Dally et al., 2006).

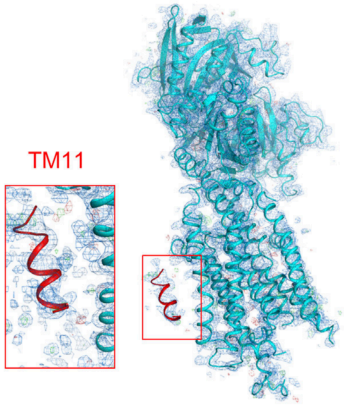


Figure 11. Electron density maps of SERCA2b (Inoue et al., 2019).

2.4.3.3 SERCA3

The ATP2A3 gene encodes for SERCA3 protein which exists in six isoforms. SERCA3a and b are expressed in different tissues including the heart, brain, immune cells, lung, pancreas, liver, and placenta (Kovács et al., 2001; Martin et al., 2002). SERCA3c has been identified in immortalized T-lymphocytes and platelets (Kovács et al., 2001). SERCA3d is expressed in the same tissues as isoforms a and b, but also in skeletal muscle, while SERCA3e is confined to the lung and pancreas (Martin et al., 2002). SERCA3f seems to be broadly expressed in human tissues and cells (Bobe et al., 2004).

2.4.4 Darier Disease treatments

Another challenging aspect of DD is the lack of curative treatments, aside from the prevention of triggers and symptoms management (Takagi et al., 2016). Current medications include steroids, retinoids, analogs of vitamin D, surgical excision, and photodynamic therapy (Cooper & Burge, 2003), and could be distinguished into three categories: systemic, topical, and procedural therapies. Systemic treatments include oral retinoids (Vieira et al., 2020), acitretin (van Dooren-Greebe et al., 1989; Blanchet-Bardon et al., 1991; X.-B. Zhang et al., 2008; Zavattaro et al., 2014), isotretinoin (Dicken et al., 1982; Bhat et al., 2010), alitretinoin (Letulé et al., 2013; Anuset et al., 2014), vitamin A analogs (Burgoon et al., 1963; Stüttgen et al., 1977; Thomas et al., 1982; Sondhi et al., 2020), systemic immunomodulators (Shahidullah et al., 1994; Legrand et al., 2020), and antibiotics (Sfecci et al., 2015; Pettit et al., 2018). However, using some of these therapies causes side effects and the relapse of the condition after their discontinuation or the decreased frequency of assumption, leading to the necessity of continuous treatment (Farb et al., 1980; Thomas et al., 1982; Dicken et al., 1982; Archer et al., 1989; Orihuela et al., 1995; Letulé et al., 2013; Anuset et al., 2014; Zavattaro et al., 2014). Among topical treatments, tretinoin (Fulton et al., 1968; Steijlen et al., 1991; Dogan et al., 2011;), isotretinoin (S. M. Burge & Buxton, 1995; McKenna et al., 1999), synthetic vitamin D analogs (Abe et al., 2011), calcineurin inhibitors (Pérez-Carmona et al., 2011), and non-steroidal anti-inflammatories (Palacios-Álvarez et al., 2017) have been reported. Vitamin D plays an important role in the differentiation process and inhibits the proliferation of epidermal keratinocytes (Kragballe et al., 1995), whereas calcineurin inhibitors, thanks to their ability to reduce T-cell proliferation, diminish inflammation (Pérez-Carmona et al., 2011). However, also for these treatments, patients experienced side effects and variability in efficiency (Steijlen et al., 1991; S. M. Burge & Buxton, 1995; Abe et al., 2011; Millán-Parrilla et al., 2014). Surgical excision and dermabrasion (Dellon et al., 1977; Wheeland & Gilmore, 1985; Ji et al., 2018), lasers (O'Brien et al., 2020), radiation (Rodriguez et al., 2018), and photodynamic therapies (Exadaktylou et al., 2003) are the procedural treatments currently available.

Recently, calcipotriol/betamethasone dipropionate, a two-compound topical unguent, has been reported to be a promising therapeutic strategy for DD. The treatment has been applied

for three months to a patient with a missense *ATP2A2* variant, leading to the reduction of the erythema and scales on the scalp, to the flattening of the papules on the neck and groin, leaving behind mild residual pigmentation. However, also in this case, the employment of the ointment is limited to short-term or intermittent usage since it could cause skin irritation (Kragballe et al., 1995; Hagino et al., 2022).

In conclusion, even though some of the above-mentioned oral, topical, and interventional treatments could be more effective compared to others, further studies on the efficacy and safety of DD therapies are required.

2.5 ER-stored calcium regulation

Calcium stored in the ER plays a crucial role in post-translational protein processing and folding, as well as their trafficking towards the plasma membrane or their secretion (Berridge et al., 2003). The calcium concentration between ER and cytosol is tightly regulated by Ca^{2+} channels, transporters, pumps, and calcium-binding proteins, and to ensure normal cellular functions, a balance between the calcium release and uptake into the ER is crucial (Krebs et al., 2015; Bahar et al., 2016; Carreras-Sureda et al., 2018).

As discussed before, maintaining a calcium gradient within the epidermis is fundamental for proper barrier homeostasis and epidermal differentiation.

The depletion of ER calcium causes the accumulation of misfolded proteins and the consequent ER stress response activation (Yoshida, 2007). For example, the reduction of ER calcium could lead to the generation of perinuclear E-cadherin aggregates, altering cell junction formation (Celli et al., 2012). Moreover, as reported in previous studies, the inactivation of ER Ca^{2+} ATPase determines the inhibition of early differentiation proteins expression (L. Li et al., 1995a, 1995b). The reduction of ER calcium levels has been observed in functional studies by employing Thapsigargin, a selective inhibitor of SERCA2 causing the formation of insoluble oligomers (Celli et al., 2011; Y. Wang et al., 2011), with a consequent alteration of E-cadherin and desmoplakin processing and localization, thus cell-to-cell adhesion perturbation and defects in the differentiation protein involucrin synthesis (Hakuno et al., 2000; Celli et al., 2012). Considering the importance of calcium in regulating epidermal

homeostasis, the skin is mainly affected in the presence of ATP2A2 gene alterations, even though SERCA2 proteins are ubiquitously expressed. This could be due to the absence of compensatory mechanisms by SERCA3 in counteracting SERCA2b dysfunction in keratinocytes compared to other tissues. Additionally, the exacerbation of the condition in the presence of external stimuli could be determined by the insufficient activity of the remaining WT SERCA2 in counteracting the stresses they trigger (Savignac et al., 2011).

2.5.1 ER stress and unfolded protein response (UPR)

As above mentioned, the ER is fundamental for protein synthesis, maturation, folding, trafficking, secretion, and localization and the alteration of its homeostasis leads to protein misfolding or unfolding and ER stress. Different external and/or internal stimuli or excessive proteins production, which leads to their accumulation, are determinants of cellular stresses (Hampton, 2000; Yoshida, 2007). Several processes are induced by UPR to restore normal ER functions, including the modulation of translation, the intensification of the degradation rate of misfolded/unfolded protein via the ubiquitin-proteasome mechanism induced by ER and the increase of ER chaperones, and folding enzymes to improve the efficiency of protein processing (Hampton, 2000; Yoshida, 2007; Almanza et al., 2019) (**Figure 12**).

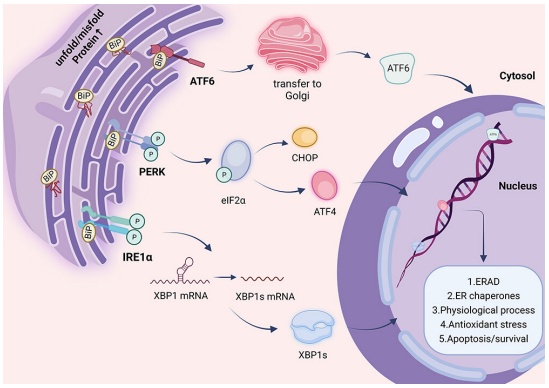


Figure 12. Overview of Unfolded Protein Response (UPR). BiP, binding immunoglobulin protein; IRE1, inositol-requiring enzyme; PERK, RNA-dependent protein kinase-like ER-resident kinase; ATF6, activating transcription factor 6; XBP1, X-box binding protein 1; elF2α, eukaryotic initiation factorα; CHOP, CCAAT-enhancer-binding protein homologous protein; ERAD, ER-associated protein degradation (Xie et al., 2024).

Three ER transmembrane sensor proteins are involved in the UPR regulation, including the inositol-requiring enzyme 1 alpha (IRE1α), the activating transcription factor 6 (ATF6), and the

double-stranded RNA-dependent protein kinase (PER)-like ER kinase (PERK). When bound by the ER-resident chaperone glucose-regulated protein 78/binding immunoglobulin protein (GRP78/BiP), the UPR is inactivated, and this occurs in the absence of stress conditions. In the presence of misfolded and/or unfolded proteins in the ER lumen, there is a dissociation of the GRP78/BiP from the above-mentioned ER sensor proteins and this leads to the UPR activation (Hampton, 2000; Yoshida, 2007; Almanza et al., 2019) .

When GRP78/BiP dissociates from IRE1α, it binds to unfolded/misfolded proteins and by its kinase activity determines the dimerization and auto-phosphorylation of IRE1α (Almanza et al., 2019; Yoshida et al., 2001). This process increases the nuclease activity of IRE1α which determines the catalyzation of the excision of 26 nucleotides intron from the X-box binding protein 1 (XBP1) mRNA leading to the formation of a spliced isoform (XBP1s) (Yoshida et al., 2001). XBP1s is involved in the transcription of several target genes related to lipid metabolism, inflammatory and immune responses, cellular differentiation, structural/functional development of ER, and processes involved in ER-mediated protein degradation to reestablish homeostasis. On the contrary, the unspliced XBP1 protein (XBP1u), lacking a transactivation domain, is not able to induce gene expression (Travers et al., 2000; Iwakoshi et al., 2003; Sriburi et al., 2004; So, 2018; Covino et al., 2018). The phosphorylation-induced IRE1α leads to the enrolment of the tumor necrosis factor receptor-associated 2 factor (TRAF2), with the consequent formation of the IRE1α-TRAF2 complex (Urano et al., 2000), which activates both JNK and NF-kB, which in turn modulate cell death (Urano et al., 2000; Kaneko et al., 2003).

Similarly, PERK activation occurs with its dimerization and auto-phosphorylation after the detachment of GRP78/BiP (Walter & Ron, 2011; Almanza et al., 2019). By its kinase activity, the activated PERK recruits and phosphorylates the eukaryotic translation initiation factor 2 alpha (eIF2α), a subunit of the eIF2 complex involved in the regulation of protein translation initiation through the promotion of the initiator tRNA binding to the ribosomal subunit 40S. In turn, eIF2α inhibits eIF2B, the eukaryotic translation initiation factor 2B, causing the reduction of protein synthesis and folding load in ER-stressed cells. The phosphorylated eIF2α also leads to the translation of the activating transcription factor 4 (ATF4), which in turn regulates the expression of genes involved in the protection mechanisms of cells against ER stress, including autophagy, protein homeostasis, and amino acid metabolism. In the presence of continuous

ER stress, the overactivation of PERK leads to pro-death mechanisms mediated by the upregulation of CAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP), which induces cell death by increasing oxidative stress and ATP depletion (Walter & Ron, 2011; Rozpedek et al., 2016).

ATF6 is expressed in the ER, whereas during ER stress it is transferred to the Golgi apparatus where undergoes a Golgi proteases-mediated cleavage and becomes functional. The activated fragment of ATF6 is translocated into the nucleus, where it triggers the expression of genes related to the restoration of ER homeostasis (Yoshida et al., 2001; Almanza et al., 2019).

A physiological level of unfolded protein response (UPR) induced by ER stress is needed for maintaining normal functions of cells, such as the epidermal keratinocytes differentiation (Sugiura et al., 2009; Celli et al., 2010, 2011;). During this process, the spliced forms of XBP1, CHOP and GRP78/BiP expression are enhanced. However, the sustained UPR activation leads to cell deleterious effects, could induce apoptosis (Sugiura et al., 2009), and is involved in the pathogenesis of different skin diseases including Darier Disease (Sakuntabhai, Ruiz-Perez, et al., 1999).

Indeed, as previously mentioned, Wang and colleagues observed the formation of insoluble aggregates of undegraded SERCA2 protein mutants around nuclei, resulting in increased ER stress and apoptosis. Additionally, mutant-induced ER stress also leads to the aggregation of WT SERCA2. On the contrary, SERCA2 KO causes cell apoptosis induction insensitivity, which could explain dyskeratosis in DD patients. Noteworthy, since SERCA2 is also expressed in neurons and considering the neuropsychiatric symptoms experienced by some DD patients, it is possible that SERCA2 mutants aggregation and accumulation could be responsible for their manifestation (Y. Wang et al., 2011).

2.6 Mitochondria's role in skin and link with other cellular organelles

Mitochondria are the main energy source of cells by generating adenosine triphosphate (ATP) through cellular respiration. In addition to energy production, these organelles play essential roles in the regulation of cellular metabolism and homeostasis including cell viability, proliferation, calcium homeostasis, reactive oxygen species production (ROS), immune

signaling, and programmed cell death (Vasileiou et al., 2019; S.-E. Lee et al., 2022; Shen et al., 2022). These processes rely on the interaction of mitochondria with the nucleus by a feedback mechanism. In the presence of stress, mitochondria transmit the signal to the nucleus, inducing the activation of stress response pathways (Picard & Shirihai, 2022).

Furthermore, mitochondria are the primary producers of reactive oxygen species (mtROS) and redox signaling (Balaban et al., 2005) important for survival mechanisms and cytokine production of immune cells involved in infection prevention and prolonged inflammation (Allaoui et al., 2009; Segal et al., 2012; van der Vliet & Janssen-Heininger, 2014; Hoffmann & Griffiths, 2018). mtROS plays an important role also in wound healing, by promoting E-cadherin trafficking, junction, and cytoskeleton polarization. After wound generation, there is the release of calcium signals which induces an increased mtROS production and leads to redox signaling-mediated cytoskeleton remodeling (Hunter et al., 2018) (**Figure 13**).

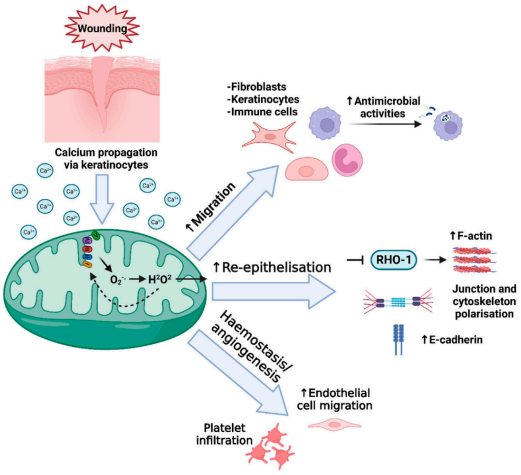


Figure 13. Mitochondrial redox signaling in wound healing (Hunt et al., 2023).

One of the main skin characteristics is its high-rate turnover, a process requiring a large amount of energy, mainly supplied by the mitochondrial electron transport chain (Sreedhar et al., 2020). This energy is also needed for other cutaneous processes, such as cell signaling, hair growth, and wound healing, for epidermal homeostasis and the preservation of the microbiological defense function (Stout & Birch-Machin, 2019).

Basal keratinocytes mainly use glycolysis for energy production. As they differentiate and move towards the upper layers of the epidermis, mitochondrial oxidative phosphorylation, and ATP production through the TCA cycle and electron transport chain increase (Hamanaka

& Chandel, 2013). Since mitochondria are the primary sources of ROS, oxidative stress is most pronounced in the deepest layer of the epidermis, especially in the stem cells of the basal layer, where keratinocytes heavily depend on mitochondrial function (Vidali et al., 2023). Moreover, keratinocytes proliferation and differentiation from the basal to the suprabasal layer and the migration towards the outer stratum requires mitochondrial ROS generation, which exerts a regulatory role through different downstream transcriptional factors, including C/EBP, NOTCH, and β-catenin (Hamanaka & Chandel, 2013). Additionally, mitochondria play a crucial role also in skin photo-aging since exposure to UV leads to mtDNA damage, altering the flow of electrons and hampering energy production, and to skin cells pathogenic oxidative stress. Skin cancer cells also present altered mitochondria, characterized by deletions in mtDNA and excessive ROS production (Sreedhar et al., 2020). Many dermatologic diseases have defective mitochondrial structure and function. For example, in psoriasis mitochondria upregulate ROS production, and increase downstream inflammatory cytokines, and dendritic cell activation (Alalaiwe et al., 2021; Mizuguchi et al., 2021).

During epidermal keratinocytes differentiation mitochondria show a fragmented phenotype according to the reduced energetic requirements of these cells, depolarization and acidification mediated by the upregulation of the mitochondrion-tethered autophagy receptor BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like (BNIP3L/NIX) (Mellem et al., 2017; Ipponjima et al., 2020) supporting the mitochondria degradation required in this process. Indeed, BNIP3- (Moriyama et al., 2014, 2017) and NIX-mediated (Simpson et al., 2021) mitophagy as well as autophagy is required for the epidermal keratinocytes differentiation (Aymard et al., 2011; Yoshihara et al., 2015; Akinduro et al., 2016). NIX depletion impairs epidermal maturation and interferes with mitochondrial clearance, while its overexpression accelerates keratinocyte differentiation and results in premature mitochondrial fragmentation (Simpson et al., 2021). In the stratum granulosum cells, there is an increased quantity of mitochondria which are structured in a more compact network compared to stratum spinosum and the mitochondria connectivity is reduced in keratinocytes from older subjects compared to younger ones (Mellem et al., 2017). UV stress is responsible for mtDNA damage in keratinocytes and fibroblasts (Pang et al., 1994; Birch-Machin et al., 1998). Noteworthy, sun exposure is one of the external stimuli determining the exacerbation of DD. UVs induce the

fragmentation of the mitochondrial network and deletions in mtDNA leading to the disruption of electron flow. This results in cellular dysfunction and premature senescence (Stout & Birch-Machin, 2019). Oblong and colleagues reported that mitochondrial damages occur mainly in dermal fibroblasts, leading to the reduction of oxidative phosphorylation genes expression and mitochondrial complexes subunits. These alterations could be attributed to decreased mitophagy which might be restored by nicotinamide treatment (Oblong et al., 2020).

ER and mitochondria interact with each other and these organelles' contact has been well described (ER-mito) (Elbaz & Schuldiner, 2011; Friedman & Voeltz, 2011; Rowland & Voeltz, 2012; Marchi et al., 2018) (**Figure 14**). The ER-mito communication involves different factors, including the transfer of ions and lipids, calcium signaling, intracellular trafficking, and mitochondrial membrane dynamics (Phillips & Voeltz, 2016), thus each organelle plays its own role and cooperates with other ones. The contacts between ER and mitochondria have also been associated with ER-stress-mediated cell death and the UPR. Actually, different ER co-factors and chaperons are enriched at mitochondria-associated membranes (MAM) (Paillusson et al., 2016). In the presence of ER stress, stress signals can be transferred to the mitochondria via the contact sites (Aboufares El Alaoui et al., 2023).
It has been observed that the depletion of ER Ca^{2+} stores is followed by the accumulation of these ions in the mitochondrial matrix via the uniporter system (Marchi et al., 2018). The calcium regulation impairment can lead to a Ca^{2+} overload in the matrix and trigger the opening of a high-conductance pore, known as the "mitochondrial permeability transition pore" (mPTP) (Baumgartner et al., 2009; Bonora et al., 2015). Mitochondrial Ca^{2+} overload is a critical triggering signal of the intrinsic apoptosis pathways (Danese et al., 2017) with alterations in the organelle functions such as reduction of ATP production and increase in ROS generation (Rimessi et al., 2016). Considering that the endoplasmic reticulum and mitochondria interact, reactive oxygen species might be transferred between the two organelles and could have effects on DD. Indeed, SERCA inhibition mediated by thapsigargin determines the release of a considerable amount of Ca^{2+} from ER and its intracellular increase, causing cell death (Marchi et al., 2018).

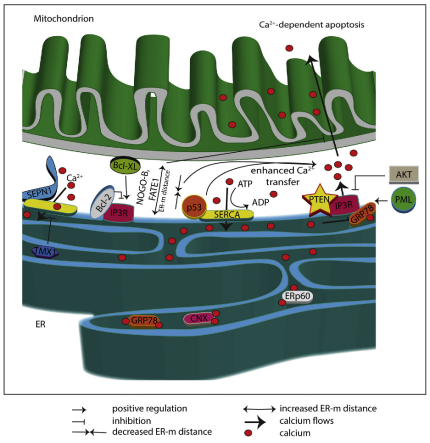


Figure 14. Ca^{2+} handling at the ER-mitochondria interface: focus on the ER Ca^{2+} homeostasis (Marchi et al., 2018).

Recently, selenoprotein N (SEPN1), an ER redox-sensitive protein, has been observed to play a role in SERCA activity modulation and calcium homeostasis. The active form of SEPN1 interacts with SERCA at the ER-Mito contact sites and regulates ER Ca^{2+} levels. Additionally, during ER stress, it reduces oxidative damage and activates the UPR. The increase of the ER oxidoreductin 1 (ERO1) activity and ROS amount after UPR induction leads to SERCA inactivation. In this context, SEPN1 reduces oxidized molecules generated by ROS activity and restores SERCA2 function (Marino et al., 2015).

2.7 The NOTCH signaling

The Notch signaling pathway is an evolutionary conserved molecular network, that depending on the tissue and cellular context and on the crosstalk with other pathways, is involved in several processes including cell proliferation, migration, differentiation, and apoptosis (Artavanis-Tsakonas et al., 1999; Lai, 2004; Bray, 2006). Mammalian NOTCH genes encode single-pass, heterodimeric type I transmembrane receptors: NOTCH1, NOTCH2, NOTCH3, and NOTCH4 (Kopan & Ilagan, 2009).

NOTCH proteins are made up of an extracellular domain (NECD) which is involved in the interaction with ligands, and an intracellular domain (NICD) responsible for the signal transduction and a connector between them.

The extracellular domain contains several epidermal growth factor (EGF)-like repeats, some of which are required for the interaction with ligands. Therefore, many of them bind Ca^{2+} ions needed for NOTCH structure and affinity in ligand binding (Kopan & Ilagan, 2009). The presence of three cysteine-rich Lin/Notch repeats (LNR) and the heterodimerization domain (HD) form the negative regulatory region (NRR) which prevents the signaling activation in the absence of ligands (Massi & Panelos, 2012). In particular, the NECD domain of NOTCH1 and NOTCH2 in humans contains 36 EGF-like tandem repeats and 3 LNR, whereas NOTCH3 and NOTCH4 NECD comprise 34 and 29 repeats, respectively (Sanchez-Irizarry et al., 2004).

The intracellular region of NOTCH is involved in the signal transfer to the nucleus and contains a membrane-proximal RBPJ-associated molecule (RAM) domain, seven ankyrin repeats (ANK), a nuclear localization sequence (NLS) which links RAM to ANKs, a transactivation domain (TAD), and a proline-glutamate-serine-threonine-rich (PEST) sequence which acts as a degradation signal to regulate NOTCH stability (Kopan & Ilagan, 2009) (**Figure 15**).

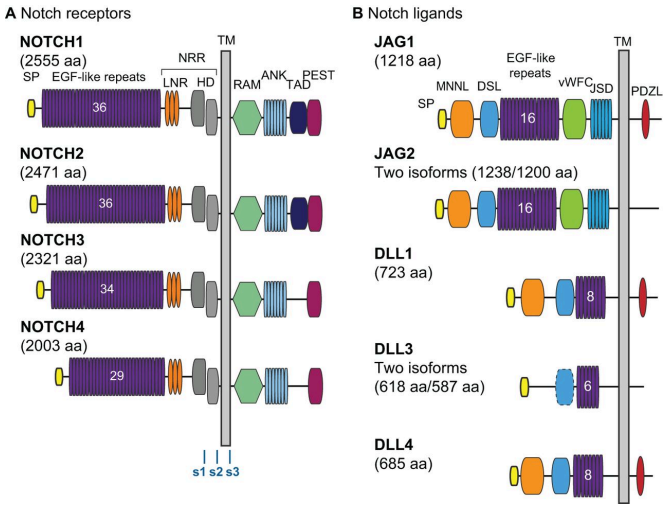


Figure 15. The human Notch repertoire. ANK, ankyrin repeats; DLL, Delta-like protein; DSL, Delta/Serrate/LAG-2 domain; EGF, epidermal growth factor; HD, heterodimerization domain; JAG, jagged; JSD, Jagged Serrate domain; LNR, Lin-Notch repeats; MNNL, Notch ligand N-terminal domain; NRR, negative regulatory region; PDZL, PDZ ligand domain [PDZ, post synaptic density protein (PSD95)]; PEST, proline (P), glutamic acid (E), serine (S) and threonine (T) degradation domain; RAM, Rbp-associated molecule domain; s, cleavage site; SP, signal peptide; TAD, transactivation domain; TM, transmembrane domain; vWFC, von Willebrand factor type C domain (Mašek & Andersson, 2017).

The NOTCH receptor maturation and trafficking towards the cell surface includes several extracellular domain's post-translational modifications. In the beginning, NOTCH proteins are processed in the endoplasmic reticulum by the O-glucosyltransferase 1 protein (POGLUT1) or

the O-fucosyltransferase 1 protein (POFUT1), which adds an O-glucose or an O-fucose, respectively, at the level of the EGF-like repeats, promoting the receptor clustering to the surface, thus the interaction with its ligands (Rebay et al., 1991; Wang et al., 2001; Okajima & Irvine, 2002; Haines & Irvine, 2003; Luo & Haltiwanger, 2005; Takeuchi et al., 2012). Subsequently, the NOTCH precursor is cleaved within the NRR by a furin-like convertase at the S1 site during the transfer through the Golgi apparatus. At this point, the heterodimerization domain (HD) converts NOTCH into a heterodimeric receptor containing the extracellular domain (NECD), the trans-membrane domain (NTM), and the intracellular domain (NICD) (Blaumueller et al., 1997). The resulting mature receptor is transported to the cell membrane where non-covalent interactions hold it and receive an inhibitory (cis) or an activation (trans) signal after the interaction with its ligand (Ntziachristos et al., 2014).

The canonical NOTCH signaling activation involves the interaction between the receptor expressed on signal-receiving cells and the specific ligands located on neighboring signal-sending cells (Bray, 2006; Hori et al., 2013). Ligands are type I transmembrane glycoproteins, including three delta-like (Dll1, 3 and 4) and two jagged (Jag1 and Jag2) (Lai, 2004).

The binding determines a conformational change in the NOTCH receptor rendering it predisposed to two consecutive proteolytic cleavages. The first one (S2) is mediated by the zinc-dependent disintegrin and metalloprotease ADAM10 (in the canonical pathway) and occurs within the protein extracellular domain, between Ala1710 and Val1711 residues (Brou et al., 2000; Mumm et al., 2000). The second cleavage (S3) is an intramembrane one between the Gly1743 and Val1744 mediated by the presenilin γ-secretase activity. This enzymatic cleavage determines the release of the NOTCH intracellular domain (NICD) and its translocation from the plasma membrane to the nucleus, where it forms a multiprotein transactivation complex with the DNA-binding protein RBP-Jk (known also as CSL proteins) (Schroeter et al., 1998). NICD binds RBP-Jk first with the RAM domain, then with the Ank one, and derepresses CSL proteins by displacing corepressors SMRT (silencing mediator of retinoid and thyroid receptors), NCoR (nuclear repressor corepressor), and CIR (CFB-1-interacting repressor) which bind CSL proteins when the NOTCH signaling is turned off. This binding converts the RBP-Jk repressor to a transcription activator (Nam et al., 2006).

In order to initiate the transcription of its target genes, including Hes and Hey families members, NOTCH requires the recruitment of Mastermind-like (MAML) protein (Wu et al.,

2000) and other co-activators (Bray, 2006; Kopan & Ilagan, 2009). MAML binds NICD and CSL through its conserved N-terminal domain, which fits in a molecular groove generated by the NOTCH Ank domain and specific residues of CSL protein. In turn, MAML recruits co-factors, including the histone acetyl-transferase p300/CBP, which determine posttranslational modifications such as acetylation. These processes mediate the binding affinity and stability of the NOTCH transcriptional machinery on the target genes promoter (Kopan & Ilagan, 2009; Gerhardt et al., 2014) (**Figure 16**).

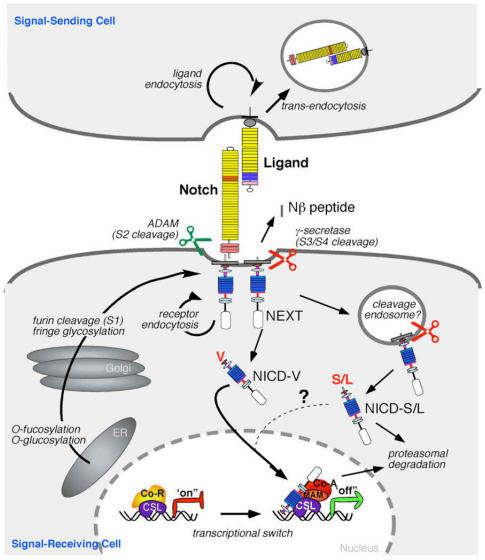


Figure 16. The Core Notch Signaling Pathway is mediated by regulated proteolysis (Kopan & Ilagan, 2009).

2.7.1 Ca^{2+} ATPase protein and NOTCH

As discussed before, calcium plays a pivotal role in the NOTCH receptor maturation process and trafficking. Indeed, several EGF repeats and the three Lin12/Notch repeats in the NOTCH extracellular domain require Ca^{2+} for correct folding (Rand et al., 1997; Aster et al., 1999; Hambleton et al., 2004; Gordon et al., 2007). For this reason, alteration in genes encoding Calcium ATPase proteins might affect these mechanisms. Periz and Fortini transfected the Drosophila S2 cell line with a *Notch* expression construct and treated them with two SERCA inhibitors, Thapsigargin and Cyclopiazonic acid, to verify how the reduced Ca^{2+} ATPase activity affects NOTCH processing. By employing these inhibitors, they observed a reduction in the NOTCH cell surface immunoreactivity, suggesting that they do not significantly alter the

intracellular NOTCH levels but predominantly reduce the amount of receptor that reaches the cell membrane due to its accumulation in ER/Golgi compartment. Additionally, it has been observed a Ca^{2+}-ATPase inhibitors dose-dependent reduction of the ~120 kDa NOTCH proteolytic fragment production but not of the unprocessed ~300 kDa full-length NOTCH precursor (Periz & Fortini, 1999; Roti et al., 2013). Previous studies on NOTCH1-associated T-ALL revealed SERCA2 inhibition as a potential therapeutical approach since it determines the downregulation of the receptor target genes and induces a G0/G1 arrest and a cell size reduction. In particular, SERCA2 inhibition determines the disruption of mutated NOTCH1 trafficking with the retainment of unprocessed and misfolded receptors in the ER/Golgi compartment resulting in the reduced expression of NOTCH1 target genes including HES1 and MYC (Roti et al., 2013; Roti & Stegmaier, 2014). Prior observations regarding the role of *Ca-P60A* deficiency, a *Drosophila* SERCA homolog, in Notch maturation defect support Roti and colleagues' results (Periz & Fortini, 1999). This evidence suggests that NOTCH signaling could be dysfunctional in several genetic disorders associated with SERCA2 mutations, including DD.

Based on previous studies showing the co-localization of SERCA2b and presenilin in the ER (K. N. Green et al., 2008) and the ability of presenilin to recover the full-length NOTCH1 precursor before the cleavage of NOTCH1 in the Golgi apparatus (Ray et al., 1999), Roti and colleagues put forward the hypothesis of a complex consisting of NOTCH1, SERCA, and presenilin (Roti et al., 2013).

2.7.2 NOTCH signaling in epidermis

In normal human skin, NOTCH receptors and ligands are widely expressed with a specific localization on keratinocytes membranes throughout the epidermis. NOTCH1 is primarily found in the lower and mid-epidermal layers, while NOTCH2, 3, and 4 are expressed predominantly in suprabasal ones. The ligand Jagged1 is mainly expressed in the suprabasal cells membrane, whereas Jagged2 and DLL1 are located in the basal layer (Gratton et al., 2020) (**Figure 17**).

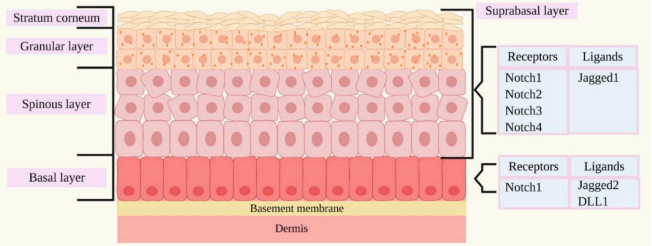


Figure 17. Distribution of NOTCH ligands and receptors in human skin (Gratton et al., 2020).

In the epidermis Notch plays a tumor-suppressive role, as evidenced by studies on its ablation, which have shown hyperproliferation of the epidermal basal layers (Rangarajan et al., 2001; Nicolas et al., 2003). Mutations determining NOTCH loss-of-function are commonly identified in epithelial tumors, such as skin squamous cell carcinoma (SCC) (Pickering et al., 2014; South et al., 2014), esophageal (Song et al., 2014), head and neck carcinoma (Agrawal et al., 2011; Stransky et al., 2011). Moreover, Blanpain and colleagues observed that NOTCH1 signaling perturbation causes defects in epidermal barrier function and hyperproliferation, which could be a basal layer compensation mechanism with the consequent thickening of the epidermis. It has also been demonstrated that NOTCH acts at the basal–suprabasal boundary to define spinous layers and inhibit basal epidermal genes through a canonical RBP-J-dependent mechanism. Furthermore, they found that NICD/RBP-J promotes spinous cell differentiation via an HES1-dependent pathway while inhibiting basal cell differentiation through an HES1-independent mechanism (Blanpain et al., 2006).

Considering the structural conformation and the function of the skin, epidermal stratification is a crucial process for its development. NOTCH signaling pathway plays a pivotal role in the early stage of epidermal development, balancing proliferative and differentiating signals. It has been demonstrated that the NOTCH signaling loss during epidermal development determines the missing of the spinous and granular layers caused by the hyperproliferation of the basal epidermis. This observation indicates the requirement of the NOTCH signaling for the commitment of basal keratinocytes to differentiation in the spinous layer during the initiation of epidermal development (Rangarajan et al., 2001; Nicolas et al., 2003; Blanpain et al., 2006; Moriyama et al., 2008). The activation of the NOTCH pathway determines the induction of genes involved in both the suppression and the promotion of differentiation

depending on cell type and context (Artavanis-Tsakonas et al., 1999). During the commitment to terminal differentiation, cells exit the cell cycle, and the expression of basal integrins, extracellular matrix proteins (ECM), p63 and keratins 5 and 14 is suppressed (Fuchs & Raghavan, 2002). When cells move from basal towards the spinous layer, they express the "early differentiation markers" keratins 1/10 and transcriptional factors which participate in the terminal differentiation process (Dai & Segre, 2004). As cells enter the granular layer, "late differentiation markers" filaggrin, proteins of the cornified envelope including loricrin, and different hydrophobic molecules are expressed (J. A. Segre, 2006). At the end of the transit in the stratum corneum, cells become metabolically inactive and lose cytoplasmic organelles and lipid bilayers. These cells are terminally differentiated and shed from the skin surface, being substituted by transiting inner cells.

Moreover, as mentioned before, extracellular and intracellular calcium level regulation is involved in keratinocytes growth and differentiation (Dotto, 1999). Calcium-dependent differentiation is further enhanced by the influence of this ion on the NOTCH1 receptor activity (Lefort & Dotto, 2004). NOTCH synthesis, maturation, trafficking, and consequently its function depends on the intracellular Ca^{2+} levels. The NOTCH precursor is a large polypeptide whose maturation requires extensive processing during the transition through the ER/Golgi before reaching the cell surface. The function of this organelle system depends on the Ca^{2+}-ATPase activity. Indeed, EGF and LIN-12/Notch repeats of the receptor extracellular region are involved in calcium binding (Rand et al., 1997; Aster et al., 1999), supporting the Ca^{2+} requirement for the binding of the receptor extracellular region to Delta and Serrate ligands with high-affinity (Fehon et al., 1990). Indeed, elevated calcium levels induce NOTCH1 activation by promoting its interaction with ligands and thereby facilitating the conformational changes required for receptor activation (Cordle et al., 2008). Additionally, the furin family of serine proteases located in the Golgi requires calcium for its autoactivation and processing of substrate proteins, including NOTCH (Seidah & Chrétien, 1997; Logeat et al., 1998). Even though the specific pathways involved in the epidermal differentiation process are not completely described, NOTCH could be a potential candidate, considering its role in determining cell fate and acting in both cell differentiation and morphogenesis in a context-dependent manner (**Figure 18**).

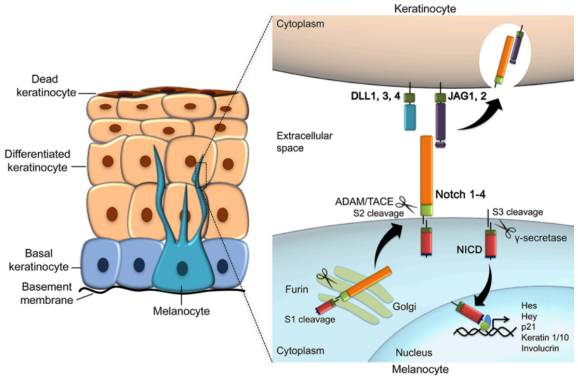


Figure 18. Overview of the NOTCH signaling pathway in keratinocytes (Dantonio et al., 2018).

To summarize, NOTCH1 plays a triple role in the spinous layer during epidermal development, including the induction of basal cells commitment, the promotion of the following differentiation into granular cells mediated by a HES1-independent mechanism, and the HES1-dependent prevention of the spinous cells' premature differentiation (Moriyama et al., 2008).

2.8 NOTCH target genes

2.8.1 HES1

HES1 (hairy/enhancer of split) is the main NOTCH1 signaling pathway target, expressed in the epidermal spinous layer. The HES1 protein belongs to the bHLH transcriptional factor family, characterized by a distinctive structural bHLH domain composed of a basic region and two alpha helices connected by a loop structure necessary for dimerization. The HES1 protein contains three structures: the N-terminal basic Helix-Loop-Helix (bHLH), the central Orange domain, and the C-terminal WRPW tetrapeptide (Kageyama et al., 2007) (**Figure 19**).

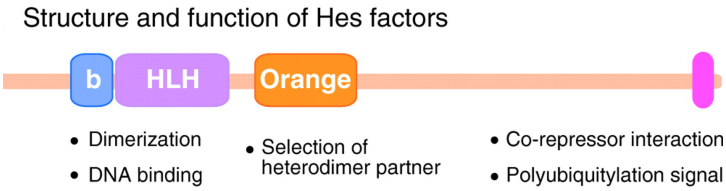


Figure 19. Structure and function of HES factors (Kageyama et al., 2007).

The Helix-Loop-Helix domain is involved in the binding to DNA and the formation of bHLH protein dimers, necessary for their regulatory activity. The majority of bHLH factors bind to

the E-box consensus sequence (CANNTG) located in the promoter region of their target genes. On the contrary, the HES1 protein shows a major binding affinity for two consensus sequences known as the class C DNA binding site (CACG C/A G) or N box (CACNAG) (Akazawa et al., 1992; Sasai et al., 1992; Ohsako et al., 1994). The Orange domain consists of two amphipathic helices (helix 3 and helix 4) responsible for the regulation of bHLH proteins with which HES1 dimerizes (Knust et al., 1992; Dawson et al., 1995; Taelman et al., 2004). The C-terminal WRPW tetrapeptide (Trp-Arg-Pro-Trp) represents a transcriptional repressor domain and a polyubiquitylation signal (S. A. Kang et al., 2005).

Three different mechanisms through which the transcriptional repressor activity of HES1 is exerted have been proposed.

The first one (DNA-binding-dependent) is characterized by an active repression through which HES1 directly binds to its target genes promoter region (Kageyama & Nakanishi, 1997; Kageyama et al., 2000). It is mediated by the C-terminal WRPW tetrapeptide which recognizes the class C DNA binding site or N box sequence and recruits the co-repressor Transducin-like Enhancer of Split (TLE) (Groucho in *Drosophila*) (Paroush et al., 1994; Fisher et al., 1996; Grbavec & Stifani, 1996). When HES1 forms heterodimers with the members of the HES-related repressor protein (HERP) family, the repression is more efficient compared to the homodimer-mediated one (Iso et al., 2001) (**Figure 20**).

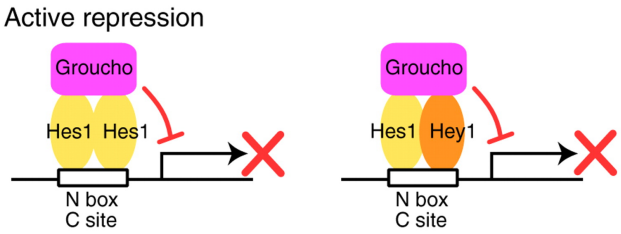


Figure 20. Structure and function of HES factors. Active repression (Kageyama et al., 2007).

The second mechanism consists of a passive transcriptional repression, in which HES1 forms nonfunctional heterodimers with other bHLH family members, including E47 preventing their binding to the consensus sequence E box (Sasai et al., 1992; Hirata et al., 2000; Iso et al., 2003) (**Figure 21**).

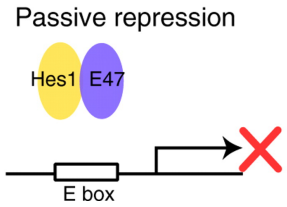


Figure 21. Structure and function of HES factors. Passive repression (Kageyama et al., 2007).

The third mechanism is involved in the self-inhibition of *HES1* expression mediated by the Orange/helix3-helix4 in the presence of a bHLH domain. Castella and colleagues proposed that Orange recruits a corepressor and/or stabilizes or regulates the active repression mediated by WRPW (Castella et al., 2000).

HES1 plays a pivotal role in maintaining the spinous cell layer's typical phenotype and preventing premature terminal differentiation in granular cells. NOTCH1 and HES1 cooperate to finely regulate the expression gene pattern and the timing of the cellular differentiation. In the first steps of epithelial stratification, NOTCH1 mediates the repression of genes involved in the transition to epidermal granular cells via an HES1-dependent manner to prevent spinous cells from prematurely progressing toward late differentiation. HES1 determines the differentiation timing and regulates its own expression by a negative feedback mechanism. When the right intracellular HES1 protein level has been reached, HES1 acts as its own transcriptional repressor. Moriyama and colleagues observed that *Hes1* KO in E15.5 mice epidermis leads to the premature differentiation of spinous layer cells into granular cells and to the reduction of proliferative potential of both cell types with the decrease of p63 mRNA (Moriyama et al., 2008).

2.8.2 HEY1

HEY1 (HES-related family bHLH transcription factor with YRPW motif 1) is another NOTCH1 signaling target belonging to the HERP (HES-related repressor protein) family.
Like HES1, HEY1 can bind to its target genes promoter region as a homodimer to repress their

expression by recruiting the N-CoR/mSin3A/HDAC complex via the bHLH domain. When HEY1 and HES1 are co-expressed in the skin, they create heterodimers that bind to the promoter area more strongly than when they form homodimers. Because the heterodimer can recruit a wider variety of transcriptional co-repressors, such as TLE and the N-CoR/mSin3A/HDAC complex, it guarantees enhanced efficacy in gene suppression (Iso et al., 2003) (**Figure 22**).

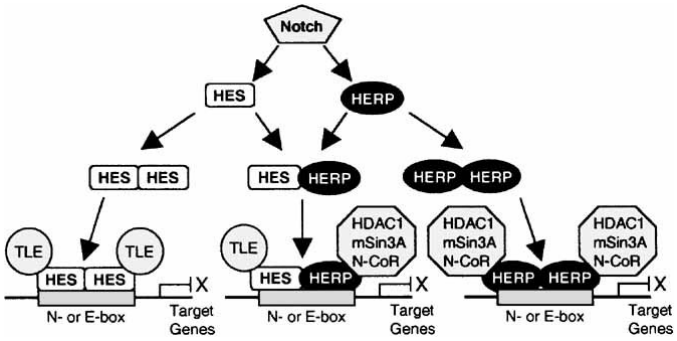


Figure 22. Model for HES and HERP cooperation in Notch signaling.

2.8.3 c-MYC

c-MYC is a transcriptional regulator belonging to the basic helix-loop helix leucine zipper (bHLH-LZ) transcription factors family, also including Max and Mad subfamilies (Chin et al., 1996; Ryan & Birnie, 1996; E. V. Schmidt, 1999) involved in the modulation of different cellular processes. It is a NOTCH1 receptor transcriptional target and plays a role in adult skin homeostasis by balancing epidermal proliferation and differentiation through cell cycle regulation. c-MYC level is fundamental for skin regeneration and wound healing and to maintain cutaneous integrity. It is mainly expressed in the basal layer (Osterland et al., 1990; Nestle et al., 2009) promoting stem cell proliferation and allowing them to become transient amplifying cells committed for terminal differentiation when highly expressed (Watt, Frye, et al., 2008).

c-MYC protein consists of an N-terminal transcriptional activator domain and a bHLHZ domain, involved in the formation of heterodimers with other factors, in particular Max (Sakamuro & Prendergast, 1999). c-MYC can act both as a gene transcriptional repressor and activator, based on the co-factor with which it interacts. When it binds to the MAX co-factor, c-MYC is a

transcriptional activator and the MAX-cMYC heterodimer interacts with the E-box sequence of target genes activating their transcription (Honeycutt & Roop, 2004). On the contrary, the interaction with the co-factor MIZ1 determines the transcriptional inactivation by recruiting co-repressors, including the DNA CpG methyltransferase DNMT3a, to the gene promoter region (Brenner et al., 2005). In the absence of c-MYC, the zinc-finger protein MIZ1 is a transcriptional activator bound to the initiator element sequence (INR) of the target genes promoter region and interacts with the co-activator p300. c-MYC interacts with MIZ1 by displacing p300 and recruiting the co-repressor DNMT3a (Staller et al., 2001). Together with MIZ1, c-MYC promotes cell cycle progression by regulating the repression of genes codifying for the inhibitors of cyclin/CDK complexes, including p21 and p27, which in turn are activated by p53 during the commitment towards cell cycle arrest. MIZ1-MYC heterodimer inhibits the expression of genes codifying cellular adhesion, actin cytoskeleton, and extracellular matrix adhesion proteins, including integrins, leading to loss of polarity and premature differentiation of epidermal basal cells (Gebhardt et al., 2006). Epidermal proliferating keratinocytes are in direct contact with the underlying basal membrane, and their detachment from it reduces proliferation while promoting the differentiation process. c-MYC may play a dual role by promoting the proliferation of basal cells, preventing them from prematurely undergoing terminal differentiation, on the other hand, once a sufficient level of proliferation has been reached, it can inhibit the expression of integrins responsible for cell adhesion, allowing the cells to detach from the basal membrane and become transient amplifying cells (Watt, 2002).

c-MYC-MAX complex is involved in the cell proliferation genes activation by inducing the transition from the G1 to the S phase of the cell cycle. It has been observed that the increase of c-MYC gene expression and the formation of heterodimers with MAX correlates with the increase of genes codifying for cyclins, cyclin-dependent kinases, and E2F transcription factors (Bretones et al., 2015). Previous studies indicated that cyclin D2 and the cyclin-dependent kinase CDK4 are c-MYC targets (Bouchard et al., 1999; Coller et al., 2000; Hermeking et al., 2000). The Cyclin D2/CDK4 complex binds the p27 factor to avoid the inhibition of the E/CDK2 cyclins complex, which in turn is activated by cyclin-activating kinase (CAK). CDK2 and CDK4 mediate the hyperphosphorylation of the cell cycle negative regulator Retinoblastoma protein

(RB) leading to the release of the E2F transcriptional factor. E2F activates genes involved in the progression toward the S phase of the cell cycle (Honeycutt & Roop, 2004) (**Figure 23**).

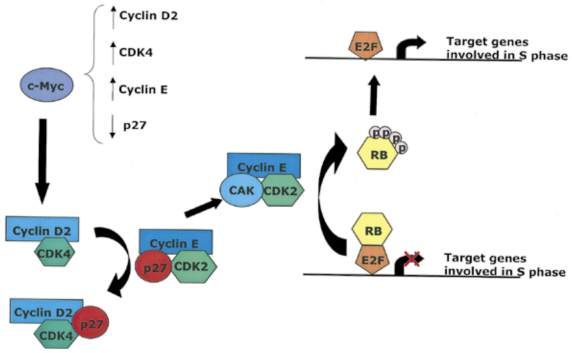


Figure 23. Promotion of the cell cycle by c-Myc.

c-MYC expression deregulation leads to a premature transition from the cell cycle G1 to the S phase, increasing the risk of epidermal tumor formation. This early transition could cause cells to bypass the checkpoint regulated by p53 and RB, reducing the effectiveness of the DNA repair mechanism and increasing the risk of mutation accumulation, leading to a condition of genomic instability (Honeycutt & Roop, 2004). On the contrary, NOTCH1 signaling stimulates the p21 and p27 expression, resulting in the inhibition of the cell cycle progression and the commitment to cell differentiation (Rangarajan et al., 2001). This opposite role could be determined by cell confluency. When cells reach a certain level of confluence, they begin to establish more cell-to-cell contacts, which facilitates the interaction between the NOTCH receptor and its ligands located on the membranes of adjacent cells. The increase in NOTCH expression is associated with the transcriptional up-regulation of p21 and p27, which in turn suppress c-MYC (Kolly et al., 2005).

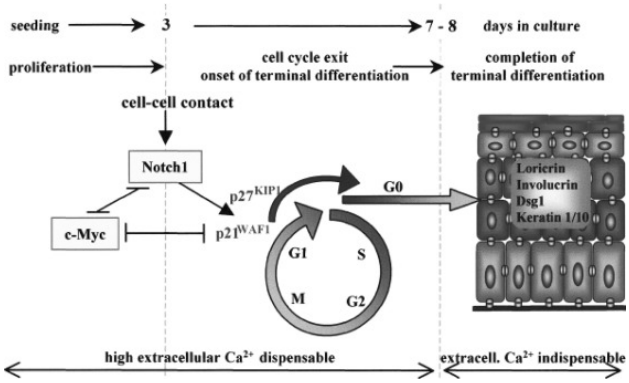


Figure 24. Involvement of high extracellular calcium, c-Myc, and Notch1 in proliferation, cell cycle exit, onset, and achievement of terminal differentiation in murine and canine keratinocyte cultures (Kolly et al.).

2.9 NOTCH1 crosstalk with other pathways

Notch signaling controls skin growth and homeostasis through complex molecular mechanisms involving interactions with other signaling pathways. For instance, both the suppression of p63 and the activation of CDKN1A/p21 are ways that conventional NOTCH signaling works. The induction of p21 expression is among the earliest cell cycle regulatory processes that determine differentiation-associated growth arrest. Studies conducted *in vitro* on mouse epidermal keratinocytes show that the induction of CDKN1A/p21 by NOTCH is dependent on RBP-J; the RBP-Jκ protein binds to the endogenous p21 promoter, whose activity is enhanced by both increased extracellular calcium and activated NOTCH1 via an RBP-Jκ-dependent mechanism (Rangarajan et al., 2001) (**Figure 25**).

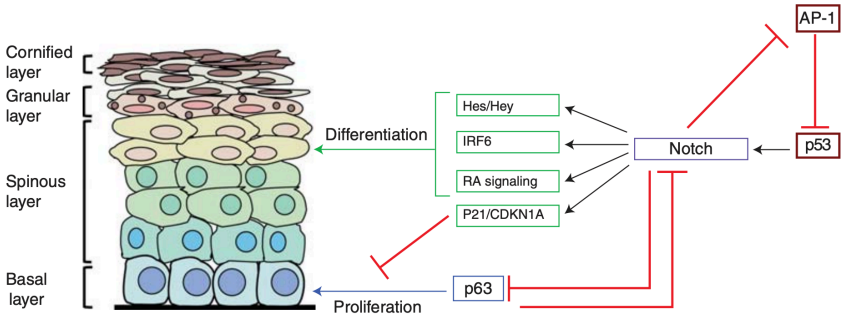


Figure 25. Molecular mechanisms of Notch-mediated epidermal homeostasis. Notch signaling in epidermal keratinocytes is promoted by p53 and antagonized by AP-1, which in turn represses p53 expression and is inhibited by Notch. Notch signaling promotes cell-cycle arrest of progenitor basal cells by repressing p63 and by inducing the expression CDKN1a/p21, a cell-cycle inhibitor. Differentiation is promoted by the induction of factors including IRF6 and the Hes/Hey family of

transcriptional repressors. Notch also promotes Retinoic Acid signaling by inducing the expression of retinol-binding proteins such as CRABP2, FABP5, and CRBP-1. The activation of this pathway promotes the differentiation of the epidermal epithelium (Nowell & Radtke, 2013).

2.9.1 NOTCH and retinoic acid

Retinoic acid (vitamin A) plays a pivotal role in the regulation of terminal differentiation programs in epithelial suprabasal layers. Low retinoic acid levels determine the formation of keratinized epithelia, such as the epidermis, whereas high levels result in nonkeratinized layers, such as cornea (Watt, Estrach, et al., 2008). NOTCH1 pathway is involved in retinoic acid metabolism by controlling cellular retinol-binding proteins CRBP1, CRABP2, FABP5 expression, which in turn regulates retinol metabolism (Vauclair et al., 2007). As reported in the Darier Disease treatments paragraph, vitamin A analogs are some of the therapies employed in the treatment of this cutaneous condition.

2.9.2 NOTCH and NF-kB

The Nuclear factor κB (NF-κB) family is composed of inducible transcription factors that play a role in the regulation of different genes involved in several mechanisms, including immune and inflammatory responses (Oeckinghaus & Ghosh, 2009). Additionally, NF-κB and peroxisome-proliferator-activated receptors (PPAR)γ take part in keratinocytes differentiation and maturation. In particular, NF-κB and PPAR activation are involved in the early and late stages of differentiation, respectively (Rivier et al., 1998; Kaufman & Fuchs, 2000). A crosstalk between NOTCH, NF-κB and PPARγ pathways in keratinocytes differentiation has been proposed, in which Jagged1-mediated NOTCH signaling activation leads to complete maturation of these cells via NF-κB and PPARγ (Nickoloff et al., 2002).

One of the main factors of the NF-κB pathway is the IKK complex, which in turn comprises the IκB kinase 1 (IKK1), playing a crucial role in epidermal development (Q. Li et al., 1999; Takeda et al., 1999). Nonetheless, the modulation of keratinocyte proliferation and differentiation by IKK1 is independent of its kinase and NF-κB activities (Hu et al., 2001). IKK1 is also involved in the regulation of NOTCH signaling. Indeed, Xin and colleagues observed a decrease in Hes1,

Hey1, and p21 genes expression in *Ikk1*$^{-/-}$ mice due to reduced transcription of NOTCH1 and 2 receptors. They also suggested crosstalk between IKK1 and p63 in the regulation of epidermal differentiation in which p63 induces IKK1 expression at the early stages of the process and in turn IKK1 suppresses p63 expression, through NOTCH pathway activation, to promote differentiation (Xin et al., 2011) (**Figure 26**).

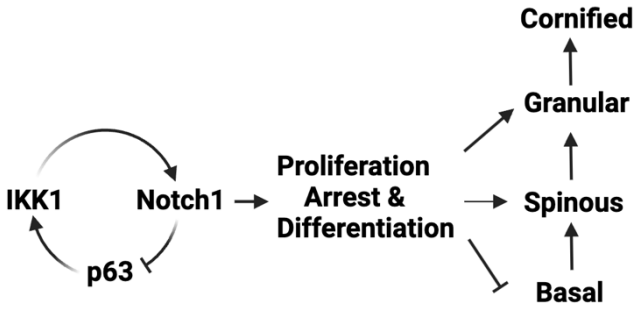


Figure 26. Schematic of molecular mechanisms in control of epidermal differentiation by IKK1.

3. Aims of the study

Although a strong relationship has been established between mutations in the ATP2A2 gene and the development of Darier disease, the exact mechanism by which these mutations lead to skin lesions remains unclear. Furthermore, no treatment has yet been identified that is both effective and free of side effects. Considering the previous evidence of SERCA2 role in NOTCH1 signaling modulation and the involvement of NOTCH in skin homeostasis, we hypothesized the negative impact of ATP2A2 gene variants on this receptor pathway leading to the exacerbation of Darier disease.

During the PhD course, my research activity has been focused on the following aims:

Gaining insight into the role of Notch1 signaling in Darier disease pathogenesis

Based on the evidence that NOTCH1 plays a pivotal role in skin homeostasis and that SERCA2 is involved in the modulation of its maturation, during my PhD course we investigated how ATP2A2 gene mutations could affect this receptor signaling, both by *in vitro* and *ex vivo* assays. By studying these interactions, we aimed to uncover how alterations in this signaling cascade might disrupt the balance of skin biology. Additionally, our work sought to evaluate the broader implications of NOTCH1 signaling dysregulation, particularly its potential contribution to the exacerbation and progression of DD, thereby providing new insights into the underlying molecular mechanisms. To reach this objective, we generated *ATP2A2* mutant plasmids through site-directed mutagenesis and employed them to transiently transfect HaCat cell line, thus overexpressing patients' gene alterations. Transfected cells were employed for *in vitro* analysis, including immunofluorescence, Luciferase assay and real-time PCR. Additionally, skin sections and primary keratinocytes were evaluated to gain insight into the NOTCH1 pathway targets level directly on patients-derived samples.

Defining a Darier disease signature

Another aim of my project was to comprehensively define a disease signature that could provide deeper insights into the biological mechanisms underlying this dermatological condition. This involved identifying the molecular pathways contributing to the onset and exacerbation of the disease, going beyond the already established gene alteration to uncover

additional factors and interactions that may play a critical role in its progression. In order to achieve this aim, we performed RNA-sequencing analysis comparing 7 patients' and 5 controls' samples by investigating differentially expressed genes and dysregulated molecular pathways, with a particular focus on the NOTCH1 signaling involvement. Results deriving from the analysis allowed us to identify pathways upregulated in Darier disease, mainly related to immunological and inflammatory response, and downregulated ones associated with metabolic mechanisms.

Identification of potential therapeutic approaches for Darier disease.

Considering the lack of effective and safe long-term treatments for Darier Disease, one of our study aims was to provide further insight into the molecular mechanisms underlying the disease, in order to contribute to the search and development of safer and more specific/effective therapies. On the basis of RNA-sequencing analyses, therapies could be developed to restore metabolic mechanisms, in particular lipidic and mitochondrial processes, which were found to be downregulated in patients compared to controls, or to intervene more effectively to reduce inflammation and the immune response, which were found to be upregulated.

4. Materials and Methods

4.1 DD patients and control subjects recruitment

For this study, patients with both sporadic and familial Darier Disease (DD) were recruited. The inclusion criteria encompassed patients aged 18 years or older with a confirmed or suspected diagnosis of DD, as well as control subjects without any genetic dermatologic diseases who had undergone surgical removal of a naevus. Non-lesional regions of the excised biopsy samples were collected at the Dermatology Unit of the University-Hospital of Parma.

The diagnosis of DD was established based on clinical features, histological examination, and/or familial history of the disease, at the Dermatology Units of the University-Hospital of Parma and Policlinico St.Orsola-Malpighi of Bologna. Patients' clinical data included affected skin areas and symptoms, type of medication, and other co-morbidities, when available.

This study was conducted according to the Declaration of Helsinki principles and was approved by the Area Vasta Emilia Nord (AVEN) Ethical Committee of the University-Hospital of Parma. All participants provided written informed consent for skin biopsy procedures, genetic testing (patients), data protection, and publication of their case details.

4.2 *ATP2A2* variants identification

Genomic DNA was extracted from 400 μL of peripheral blood samples collected in EDTA vials from DD patients using MagCore® (Applied Biosystems) following the 101 MagCore® Genomic DNA Whole Blood Kit manufacturer's protocol.

All ATP2A2b gene 20 exons were amplified by polymerase chain reaction (PCR) using the AmpliTaq Gold® DNA Polymerase (Applied Biosystems). Primer pairs annealing to the intronic regions flanking each exon were designed. The PCR reaction was carried out in a total volume of 35 μL, with around 30 ng of template DNA, under standard conditions. The protocol included an initial denaturation step at 95 °C for 10 minutes, followed by 35 amplification cycles, each consisting of a denaturation phase at 95 °C for 40 seconds, an annealing step at the primer-specific temperature for 45 seconds, and an elongation at 72 °C for 1 minute/kb. The final elongation step was carried out at 72 °C for 7 minutes. Intronic primer sets and related annealing temperatures are listed in **Table 1** (Almeida et al., 2019). PCR products were

screened using 2% Agarose gel electrophoresis and purified by AMPure XP magnetic beads (Beckman-Coulter). Sanger sequencing was performed using the Big Dye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems) and the 3500 DX Genetic Analyzers (Applied Biosystems). Results were analyzed using the SeqScape™ software and the reference Human GRCh38/hg38 *ATP2A2* sequence (GenBank accession number NM_170665). We classified the identified variants according to the American College of Medical Genetics and Genomics (ACMG) criteria on the Franklyn Genoox website. The pathogenicity and effect of missense variants were assessed using *in silico* prediction tools, including AlphaMissense, MetaDome, and DDMut. Additionally, to evaluate variants' structural effects on SERCA2 protein, we employed the ColabFold v.1.5.5-AlphaFold2 tool, whose results were visualized using the ChimeraX 1.7 software.

***ATP2A2* Exon**	**PRIMER SEQUENCE (5'-3')**	**Amplicon size (bp)**	**Annealing Temperature (°C)**
1	F- GCAAGAGGAGGAGGGGAGA R- CCATCTTCCCTGGCTCTCCC	307	58
2+3	F- CCTCCCTCTTGACACATTGCT R- GACACAGCTTGCAACTCCA	354	58
4	F- CATGTTGGGCAGGTTGGTCT R- CCATTGCACTCCAGCCTG	480	58
5	F- TGTCCTTGTGTCTGTTGCCT R- TGACAGGAAGGGAGGTGCTA	490	58
6	F- CTCATTTCAGCCGCCTTT R- AAGGGACAGTGAGGCAAGAG	202	58
7	F- GGTGGGCATGAATGAGAGGT R- AGTGATGGTTGGCAGTGAAA	249	58
8	F- CAGCGTCGGTATTTAAGTTGGG R- ACAAAGAACCACGACACGGA	573	58
9	F- TGTTTGCCTTTGTCCTAAGCT R- TGCCACACCAGATCCTTTAAA	225	58
10	F- GGGGCGGGAGGAATCAATAG R- CTTTCATTCCACCCACCCCA	234	58
11	F- GACAGATTGTGCTTTTGTGGA R- GAGAGTAGGACAGTGCAGACA	309	58
12+13	F- TTGCCACCCAGTAGTATCCA R- TGCAGGGTGTGGACAAAGAA	516	58
14	F- GGCAACAAGAGCGAAACT R- GAGGCTACTATGTGCTTGTG	478	58

15	F- TTTGCCAAGAGACCTACGG R- TTTCTGTCTTGCCCACTCCC	483	55
16	F- TTTTCTGGAGGAGGGCG R- AGGGCATCTCTGTCTTTTGC	426	55
17+18	F- CCGGTTACCATCACTGTCCC R- TGACTACACACAATTCCCCGG	690	55
19+20	F- TACTGCCACTGTGACACGTG R- GAAATCCCATCGGTGCATGC	449	58

Table 1. ATP2A2 gene primer sets

4.3 Site-directed mutagenesis

All plasmids used in the following experimental procedures were kindly provided by the Laboratory of Translational Hematology and Chemogenomics (THEC) led by Professor Giovanni Roti.

Site-directed mutagenesis, using the QuickChange Lightning kit (Agilent) was performed to introduce patient variants c.1060G>C, c.943A>T, c.1655dupG, c.1042A>T, c.2587A>G, c.269A>T, c.712G>T, c.1460T>C, and c.1996C>T into a plasmid containing the *ATP2A2* coding sequence followed by the *CopGFP* (Green fluorescent protein 2 from the copepod *Pontellina plumata*). Mutated primers were designed using the QuickChange Primer Design web tool according to the protocol guidelines and are listed in **Table 2**. All constructs were purified employing the HiSpeed® Plasmid Purification kit (QIAgen), according to the manufacturer's instructions, and verified by Sanger sequencing with the Big Dye Terminator Cycle Sequencing v. 3.1 kit and the 3500 DX Genetic Analyzer (Applied Biosystems).

Patient #18 cDNA was further evaluated to determine how the carried variant (c.1543-10A>G) alters the *ATP2A2* coding sequence using a specific overlapping primer set (5'-ACAATGAGGCAAAGGGTGTGT-3' forward and 5'-CCATAGCAATGCCAATCTCGG-3' reverse). Similarly, we evaluated the *.bam* file derived from RNA-sequencing of Patient #12 through the IGV_2.16 tool. PCR reaction was performed in a total volume of 35 μL using AmpliTaq Gold® DNA Polymerase (Applied Biosystems) and the following conditions: an initial denaturation step at 95 °C for 10 minutes, followed by 35 amplification cycles at 95 °C for 45 seconds, annealing temperature according to primer sequence (57 °C) for 30 seconds, and 72 °C for 50 seconds, and a final elongation step at 72° C for 7 minutes. The Patient #12 variant (c.545-3T>G) determines the loss of 23 nucleotides from the 5' end of the exon 7, while the Patient

#18 one (c.1543-10A>G) leads to the retention of 9 nucleotides from the 3' end of intron 12-13. According to these findings, we designed primers to introduce the identified sequence changes into the *ATP2A2-CopGFP* plasmid.

PATIENT ID	VARIANT	PRIMER SEQUENCE (5'-3')
#1 #5 #6	*ATP2A2*:c.1060G>C	F- ATCTGCTCAGACAAGACT**C**GTACACTTACAACAAACC R- GGTTTGTTGTAAGTGTAC**G**AGTCTTGTCTGAGCAGAT
#2 #3	*ATP2A2*:c.943A>T	F- GGTCTGCCTGCAGTC**T**TCACCACCTGCCTGG R- CCAGGCAGGTGGTGA**A**GACTGCAGGCAGACC
#7	*ATP2A2*:c.1655dupG	F- GTCATTCGAGAGTGGGG**G**TAGTGGCAGCGACACACTG R- CAGTGTGTCGCTGCCACTA**C**CCCCACTCTCGAATGAC
#8	*ATP2A2*:c.1042A>T	F- CCTTGGTTGTACTTCTGTT**T**TCTGCTCAGACAAGACTGG R- CCAGTCTTGTCTGAGCAGA**A**AACAGAAGTACAACCAAGG
#9	*ATP2A2*:c.2587A>G	F- GCTGACGGTGGTCCA**G**GAGTGTCCTTCTACC R- GGTAGAAGGACACTC**C**TGGACCACCGTCAGC
#10	*ATP2A2*:c.269A>T	F- GAAGAAACAATTACAGCCTTTGTAG**T**ACCTTTTGTAATTTTA R- TAAAATTACAAAAGGT**A**CTACAAAGGCTGTAATTGTTTCTTC
#11	*ATP2A2*:c.1675C>T	F- CAGCGACACACTG**T**GATGCCTGGCCCT R- AGGGCCAGGCATC**A**CAGTGTGTCGCTG
#12	*ATP2A2*:c.545_567del	F- CAGTCAATTCTCACAGCACACTGATCCCGTCC R- GGACGGGATCAGTGTGCTGTGAGAATTGACTG
#13	*ATP2A2*:c.712G>T	F- GGCAAGATCCGGGAT**T**AAATGGTGGCAACAGAAC R- GTTCTGTTGCCACCATTT**A**ATCCCGGATCTTGCC
#14	*ATP2A2*:c.1460T>C	F- GGAATTCACTCTAGAGT**C**TTCACGTGACAGAAAGTCAATGTCG R- CGACATTGACTTTCTGTCACGTGAA**G**ACTCTAGAGTGAATTCC
#16 #17	*ATP2A2*:c.1996C>T	F- CCCCTCCGCCCAG**T**GAGACGCCTGCCTG R- CAGGCAGGCGTCTC**A**CTGGGCGGAGGGG
#18	*ATP2A2*:c.1542_1543 insCATTTCCAG	F- GCAAGATGTTTGTGAAG**CATTTCCAG**GGTGCTCCTGAAGGTGT R- ACACCTTCAGGAGCACC**CTGGAAATG**CTTCACAAACATCTTGC
#19	*ATP2A2*:c.1A>G	F- TGTACAAAAAAGTTGGC**G**TGGAGAACGCGCACACC R- GGTGTGCGCGTTCTCCA**C**GCCAACTTTTTTGTACA

Table 2. Primer sets containing patients'-specific variants for site-directed mutagenesis

4.4 *In vitro* cell culture assays

4.4.1 *ATP2A2* variants overexpression

To evaluate the effect of SERCA2 alterations on the NOTCH1 signaling activation we recreated patient variants *in vitro*, by transfecting human immortalized keratinocytes (HaCat, AddexBio) and human embryo kidney (HEK293T) cells with plasmids containing the *ATP2A2-CopGFP* coding sequence, either in its wild-type or mutated forms, obtained through site-directed mutagenesis, and/or plx317-EGFP plasmid.

HaCat cell line was cultured in DMEM 1X (Cat#11960, Gibco) supplemented with 10% fetal bovine serum (FBS), 1% P/S (10,000 units of penicillin and 10 mg/mL of streptomycin, Merck), 1% L-glutamine 200 mM and 2% sodium pyruvate 100 mM (Gibco).

For Western Blot assays, we employed the HEK293T cell line, which is easier to transfect compared to HaCat cells, ensuring a higher cell yield. HEK293T cells were cultured in DMEM 1X (Gibco) supplemented with 10% FBS, 1% P/S, and 1% L-glutamine 200 mM.

Cells were transiently transfected using Lipofectamine LTX with Plus Reagent (Invitrogen) according to the manufacturer's protocol for different culture support sizes, as reported in **Table 3**. Briefly, we prepared a solution containing the plasmid of interest (*ATP2A2* WT-*CopGFP*, mutant *ATP2A2-CopGFP*, plx317-EGFP), Plus reagent (1:1 volume to plasmid amount) and OptiMEM®I (Gibco) to a final volume of 150 μl (6-well) or 25 μl (96-well). After a 10-minute incubation at room temperature (RT), the mixture was added to a solution of Opti-MEM®I 1X and Lipofectamine with a final volume of 150 μl (6-well) or 25 μl (96-well). The solution was gently mixed and incubated for 30 minutes at RT. Subsequently, the culture medium was removed from the plates; we added twice the volume of antibiotic-free culture medium and the transfection mix. The CopGFP signal was assessed 24 and 48 hours after transfection.

COMPONENT	**96-well** (mix final volume 50 μl)	**6-well** (mix final volume 300 μl)
Plasmid DNA/well	100 ng	2000 ng
PLUS™ reagent/well	0.1 μl	2 μl
Lipofectamine® LTX Reagent/well	0.5 μl	12.5 μl

Table 3. Transfection mixture preparation

4.4.2 Real-time PCR

A total of $500*10^3$ HaCat cells/condition were seeded 24 hours before transfection in 6-well Petri dishes. Once 80% confluency was reached, cells were transfected with *ATP2A2* WT-*CopGFP* or mutant *ATP2A2-CopGFP* plasmid, using Lipofectamine LTX and Plus Reagent as previously described.

Total RNA was extracted 24 and 48 hours after transfection using the NucleoSpin RNA kit (Macherey-Nagel) according to the manufacturer's protocol and quantified by Nanodrop spectrophotometer and the QuantiFluor RNA System High Standard kit with a Quantus™ Fluorometer (Promega). Up to 2000 ng of RNA were employed for reverse transcription using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's instructions. The resulting cDNA was diluted to a final concentration of 10 ng/μl. Real-time PCR was performed in a 10 μl final volume solution containing TaqMan® Universal PCR MasterMix 2x (Invitrogen), TaqMan® Gene Expression Assay 20x (*HES1* Hs00172878_m1, *c-MCY* Hs00153408_m1, ThermoFisher), and 3 μl of the diluted cDNA template using the QuantStudio 7 Flex instrument (Applied Biosystems) with the following amplification settings: 10 minutes at 95 °C and 40 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Human housekeeping gene *GAPDH* (Hs.PT.39a.22214836, Integrated DNA Technologies) was used as a normalization control, and relative quantification of gene expression was calculated according to the $2^{-\Delta\Delta CT}$ method.

4.4.3 Western blot

A total of $1.6*10^6$ HEK293T cells/condition were transfected 24 hours after being seeded with Lipofectamine LTX and Plus Reagent. Cells were lysed 24- and 48-hours post-transfection on ice for 30 minutes with a nondenaturing Cell Lysis Buffer (10X) (Cat#9803, Cell Signaling Technology) diluted to 1X in ddH_2O and supplemented with Protease/Phosphatase Inhibitor Cocktail (100X) diluted to 1X (Cat#5872 Cell Signaling Technology). Lysis was facilitated with 10-second sonication cycles. Protein concentration was quantified by the Bradford method using Protein Assay Dye Reagent Kit (Bio-Rad) and the Victor X4 plate reader (Perkin Elmer).

A total of 30 μg of cell protein extracts were denatured for 7 minutes at 95 °C and 300 rpm, resolved on a 7% NuPage Tris-Acetate Gel (ThermoFisher), and transferred onto a Nitrocellulose membrane (Cat#88018, ThermoFisher) for 1 hour at 4 °C. Membranes were blocked for 1 hour at RT with 5% (w/v) non-fat dry milk (for SERCA2, NOTCH1 and β-Actin) and 5% (w/v) Bovine Serum Albumin (for HES1 and c-MYC) in PBS-Tween20. Primary antibody incubation was performed overnight at 4 °C, followed by three washes of 20 minutes in PBS-Tween20 and the specific secondary antibody (Li-Cor) hybridization for 1 hour at RT (**Table 4**). Signal detection was performed using ChemiDoc System (Bio-Rad) and protein bands quantification by ImageLabs software after protein expression normalization to β-Actin.

ANTIBODIES	SOURCE	IDENTIFIER
Rabbit monoclonal anti-NOTCH1 (1:1000)	Cell Signaling Technology	Cat#3608
Rabbit anti-cMYC (1:1000)	Cell Signaling Technology	Cat#9402
Rabbit monoclonal anti-SERCA2 (1:1000)	Cell Signaling Technology	Cat#9580
Mouse monoclonal anti- β-Actin (1:1000)	Cell Signaling Technology	Cat#3700
Rabbit monoclonal anti-HES1 (1:1000)	Abcam	Cat#ab108937
IRDye 680LT Goat anti-Mouse IgG	Li-Cor	Cat#92668020
IRDye 800CW Goat anti-Rabbit IgG	Li-Cor	Cat#92632211

Table 4. Western blot antibodies

4.4.4 Luciferase gene reporter assay

A total of $15*10^3$ HaCaT cells/condition were seeded into 96-well ViewPlates (Cat# 6005181, Perkin Elmer) and co-transfected the next day (at 70–80% confluence) with a 4xCSL-*Firefly Luciferase*, a pRL-TK *Renilla-Luciferase*, an *NICD* and *ATP2A2-CopGFP* (WT and mutated) expression plasmids using Lipofectamine LTX and Plus Reagent (Invitrogen). The 4xCSL luciferase construct contains four tandem repeats of the high-affinity CSL binding sites (5'-CGTGGGAA-3'), which act as a NOTCH1-ICD inducible promoter essential for the activation of gene transcription. The coding sequence of the *Firefly* luciferase was inserted downstream of the CSL region as a reporter gene for expression. All conditions were tested in triplicate on the same plate, and experiments were repeated at least three times. The pLX317-EGFP plasmid was used as a transfection control and to ensure an equal amount of DNA for each well (135

ng). 70 ng of 4xCSL-*Firefly Luciferase* and 5 ng of pRL-TK *Renilla* plasmids (1:14) were used along with 20 ng of each *NICD* and *ATP2A2* expression plasmids. The assay was performed 48 hours after transfection using a DualGlo-Luciferase Reporter Assay System (Cat#E2920, Promega) and the Victor X4 plate reader (Perkin Elmer). Results were evaluated as the ratio of *Firefly* to *Renilla* Luciferase signal and of patients' condition compared to the wild-type one.

4.4.5 Immunofluorescence

A total of $500*10^3$ cells/condition were seeded 24 hours before transfection on poly-D-lysine-coated coverslips placed in 6-well Petri dishes. Once 80% confluence was reached, cells were co-transfected using Lipofectamine LTX and Plus Reagent (Invitrogen) with *ATP2A2*-WT or *ATP2A2*-mutant (1.5 μg) and plx317-EGFP (0.5 μg) plasmids, diluted in Opti-MEM®I 1X and incubated as previously described. Immunofluorescence assays were performed 48 hours after transfection.

Briefly, after three washes in Dulbecco's Phosphate-Buffered Saline 1X (DPBS 1X, Gibco) supplemented with 1% FBS, cells were fixed for 5 minutes at 4°C with Fixation/Permeabilization Concentrate reagent (Cat#00512343, Invitrogen) diluted 1:4 in Bioscience™ Fixation Diluent (Cat#00522356, Invitrogen). Cells were then washed three times with DPBS 1X supplemented with 1% FBS and permeabilized for 30 minutes at 4°C in TF Perm/Wash Buffer 5x (Cat#519008102, BD Pharmingen) diluted to 1x in H_2O. Primary antibodies targeting NOTCH1, HES1, and HEY1 were diluted in the Permeabilization solution and added to each well for 30 minutes at 4 °C. Secondary antibodies were also diluted in the Permeabilization solution and subsequently incubated for 30 minutes at 4 °C. The employed antibodies are listed in **Table 5**. Cell nuclei were stained with DAPI 1X diluted in DPBS 1X (Gibco) for 2 minutes at RT. Coverslips were mounted with ProLongTM Gold Antifade Reagent (ThermoFisher) on SuperfrostTM Plus Adhesion Microscope Slides (Epredia) and observed through the Leica Stellaris 5 confocal microscope with a magnification of 63x. Fluorescent signals, expressed as Row Integrated Density (RID) relative to the cell nuclear area, were measured using ImageJ Fiji and normalized to the Mean Background Intensity to calculate the Corrected Total Cell Fluorescence (CTCF) value.

ANTIBODIES	SOURCE	IDENTIFIER
Mouse monoclonal anti-NOTCH1 (1:50)	Santa Cruz Biotechnology	Cat#sc373891
Rabbit monoclonal anti-HES1 (1:100)	Abcam	Cat#ab108937
Rabbit polyclonal anti-HEY1 (1:100)	Abcam	Cat#ab154077
Goat Anti-rabbit IgG H+L AlexaFluor 568 (1:400)	ThermoFisher	Cat#A11036
Goat Anti-mouse IgG H+L AlexaFluor 488 (1:400)	ThermoFisher	Cat#A11029

Table 5. Immunofluorescence antibodies

4.5 Skin biopsy processing

4.5.1 Primary cell cultures establishment

Primary keratinocytes and fibroblasts were isolated from DD patients' 3 mm skin punch biopsies and control samples derived from peripheral healthy areas of nevus excision. The skin samples were collected and washed three times in Hank's Balanced Solution without calcium and magnesium (HBSS; Gibco) at RT in sterile conditions. After being separated using sterile scalpels, the epidermis and dermis were minced and separately digested by collagenase from *Clostridium histolyticum* (Cat#C6885, Merck) with a concentration of 1 mg/ml at 37°C for 30 minutes. During the incubation, samples were vortexed every 10 minutes. The digestion was stopped by adding 5-6 ml of DMEM Embryomax (Cat#SLM-120B, Merck) supplemented with 10% FBS and 1% P/S. After centrifugation at 760 *rcf* for 5 minutes, pellets were resuspended in 1.5 ml of Amniopan (Cat#P04-70100; Pan Biotech) cell culture medium, transferred in 6 cm Petri dish, and grown at 37 °C with 5% CO_2 in a humidified atmosphere. Following keratinocytes adhesion to the petri dish, the Amniopan was replaced with EpiLife™ Medium containing 60 µM calcium (Cat#MEPI500CA; Gibco) supplemented with 1% P/S. On the contrary, fibroblasts were maintained in Amniopan cell culture medium.

4.5.2 *Ex vivo* real-time PCR

Total RNA was extracted from primary keratinocytes established from both patients and controls biopsies, Hacat, and ALL-SIL cell lines using the RNeasy Micro kit (QIAgen) and

quantified through NanoDrop and QuantiFluor RNA System High Standard kit with the Quantus™ Fluorometer (Promega). ALL-SIL is a T-cell acute lymphoblastic leukemia cell line and was employed as a control due to its enhanced NOTCH signaling activity. RNA samples were reverse transcribed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's protocol. Real-time PCR was performed using a 10 µl final volume solution containing Luna® Universal Probe qPCR Master Mix (New England Biolabs), TaqMan® Gene Expression Assay 20x (*HES1* Hs00172878_m1, *c-MCY* Hs00153408_m1, ThermoFisher), and 3 µl of the diluted cDNA template (10 ng/µl) on the QuantStudio 7 Flex instrument (Applied Biosystems) with the following amplification settings: 1 minute at 95 °C, followed by 40 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Human housekeeping gene RPL13A (Hs04194366_g1, ThermoFisher) was used for normalization, and relative quantification of gene expression was calculated according to the $2^{-\Delta\Delta CT}$ method.

4.5.3 *Ex vivo* immunofluorescence

Cultured keratinocytes established from both patients' and controls' skin biopsies were stained by immunofluorescence to characterize them and evaluate NOTCH1 signaling activation and ER stress. Cells were washed three times with Phosphate-Buffered Saline 1X (PBS 1X, Gibco) and fixed at RT for 10 minutes with 4% paraformaldehyde in PBS 1X. Permeabilization was performed using 0.4% Triton-X-100 in PBS 1X at RT for 10 minutes, and after three washes in PBS 1X, cells were blocked for 1 hour at RT employing 5% Bovine Serum Albumin (BSA), 5% FBS and 0.1% Triton-X-100 in PBS1X. Primary antibodies for Cytokeratin, Vimentin, SERCA2, NOTCH1, HES1, and XBP1 were incubated for 1 hour at RT, followed by AlexaFluor secondary antibodies hybridization for 1 hour at RT. Cells were then counterstained with DAPI 1X for 2 minutes at RT. Slides were mounted with ProLongTM Gold Antifade Reagent (ThermoFisher) and observed through the EVOS™ M5000 fluorescence microscope. Raw Integrated fluorescent target signals relative to the cell nuclear area were measured using the ImageJ Fiji software and normalized to the Mean Background Intensity to calculate the Corrected Total Cell Fluorescence (CTCF) value. Antibodies are reported in **Table 6**.

ANTIBODIES	SOURCE	IDENTIFIER
Mouse monoclonal anti-Cytokeratin	Agilent Dako	Cat#GA053
Rabbit monoclonal anti-Vimentin (2 μg/ml)	Abcam	Cat#ab92547
Rabbit monoclonal anti-SERCA2 (1:500)	Cell Signaling Technology	Cat# 9580
Mouse monoclonal anti-NOTCH1 (1:50)	Santa Cruz	Cat#sc373891
Rabbit monoclonal anti-HES1 (1:100)	Abcam	Cat# ab108937
Rabbit polyclonal anti-XBP1 (1:250)	Abcam	Cat# ab37152
Goat Anti-rabbit IgG H+L AlexaFluor 568 (1:400)	ThermoFisher	Cat#A11036
Goat Anti-mouse IgG H+L AlexaFluor 488 (1:400)	ThermoFisher	Cat#A11029

Table 6. Ex vivo immunofluorescence antibodies

4.5.4 RNA-sequencing

Skin samples from 7 patients and 5 controls were also employed for RNA-sequencing analysis. Biopsies were transported and incubated in 500 μl of RNALater (Cat#R0901, Merck) on ice for 1 hour and processed to extract RNA with the Nucleospin RNA kit (Cat#740955, Macherey-Nagel), according to the manufacturer's protocol. Briefly, after three washes in PBS 1X, samples were minced with two scalpels by adding 600 μl of Lysis Buffer RA1 supplemented with β-Mercaptoethanol in an untreated 10 cm Petri dish. RNA was eluted in 40 μl of RNAse-free water and quantified using a NanoDrop spectrophotometer and the QuantiFluor® RNA System High Standard kit with Quantus™ Fluorometer (Cat#E3310, Promega). In the presence of gDNA contamination evaluated by PCR with intronic primers, RNA samples were subjected to the Nucleospin RNA kit Clean-up protocol (Macherey-Nagel). RNA integrity number (RIN) was assessed using a High Sensitivity RNA ScreenTape kit and Agilent 4200 TapeStation (Agilent Technologies). Purified RNA samples were stored at -80°C until use.

DNA-free RNA samples with a 260/280 ratio of 1.8-2.2, RIN>6 and a concentration≥ 50 ng/μl were sent to Azenta Life Sciences to generate an RNA-sequencing library using the NEBNext Ultra II RNA Library Prep Kit for Illumina & NEBNext Poly(A) mRNA Magnetic Isolation Module according to the manufacturer's instructions. Paired-end sequencing (2x150 bp) was performed using Illumina technologies on the NovaSeq platform.

FastQ files quality was assessed by employing the FastQC software and subjected to trimming and UMI (Unique Molecular Identifiers) deduplication to remove adapter sequences

introduced during the library preparation. Paired-end reads from patients' and controls' samples were then aligned to the human reference genome (GRCh38/hg38) using the annotation file gencode.v45.chr_patch_hapl_scaff.annotation.gtf (Gencode) by employing the RNA-STAR software available on the Galaxy Europe Bioinformatic website. The counting of reads mapping to exons of each annotated gene for individual samples was assessed using the featureCounts tool. Subsequently, a Differential Gene Expression (DGE) analysis between patients and controls was performed using the DESeq2 software, also available on the Galaxy website. Differentially expressed gene counts were used for principal component analysis (PCA) to cluster the two group samples. Normalized counts were also filtered for p-value < 0.05 (adjusted for multiple testing with the Benjamini-Hochberg procedure) and abs(log2(FC)>1), implying FC > 2 or FC < 0.5, to extract the most differentially expressed genes, which were then visualized using volcano plot and heatmaps.

Normalized counts obtained from DESeq2 were employed to conduct Gene Set Enrichment Analysis (GSEA) using C5 Gene Ontology gene sets for molecular functions, cellular components, and biological processes (v2023.2.Hs.symbols) on GSEA 4.3.3 software. The analysis was performed using the default parameters, with the minimum gene set size set to 15 and the maximum gene set size to 500 and 1000 permutations. Considering that the control group consisted of fewer than seven samples, the permutation type was set to "gene set" instead of "phenotype". An Enrichment Map was generated using the Cytoscape software from the GSEA analysis results, filtered for a p-value cutoff of 0.001, and an FDR cutoff of 0.01 (for upregulated gene sets) or 0.1 (for downregulated gene sets) and visualized as a gene association network. GSEA outcomes were also employed for leading-edge analysis, selecting gene sets belonging to mitochondrial, ribosomal, and fatty acid/lipid metabolism, which resulted to be downregulated in patients (negative NES and FDR q-value < 0.1). A Gene Ontology chord plot was generated to represent the most significant DD patients' downregulated genes belonging to categories related to mitochondrial signaling and oxidative phosphorylation, selected based on log2(FC) < 0 and adjusted p-value ≤ 0.05.

GSEA leading edge analyses were also performed using GOBP_Regulation_of_Notch_Signaling_Pathway_signal and 110 gene sets including a wide range of pathways related to both innate and adaptive immune responses. Gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to evaluate gene

function and biological pathways using the GOseq and Pathview software overlaying log2(FC) values on KEGG pathways. Additionally, we uploaded the DGE list with an adjusted p-value <0.05 on g:Profiler webtool to identify statistically significant biological processes, pathways, and molecular functions associated with these genes. The tool compared these genes to different databases, including Gene Ontology, KEGG, and Reactome, among others, to highlight overexpressed biological categories, applying the Benjamini-Hochberg FDR method for multiple testing correction and setting a threshold of 0.05.

The STRING database was employed to evaluate protein-protein interaction. The differential gene list was entered into the STRING database to construct the genes network (confidence 0.8 and FDR $\leq$0.01).

4.5.5 Immunohistochemistry

For immunohistochemistry assays, 4 μm formalin-fixed paraffin-embedded (FFPE) serial sections of 4 patients and 7 control subjects skin biopsies were employed. Sections were deparaffinized for 20 minutes at 60 °C and rehydrated in decreasing alcohol scale. Epitope retrieval was performed by water bath for 40 minutes at 95°C in Ultra Cell Conditioning Solution pH8.2 (Cat#950-224, Ventana) for HES1 staining and 1 hour for c-MYC. Subsequently, endogenous peroxidase was blocked with 3% H_2O_2 for 10 minutes at RT. Sections were incubated with rabbit anti-human c-MYC (37°C 40 minutes; Cat#395R-18, Cell Marque TM) and rabbit anti-human HES1 (1:150, 4°C, ON; Cat#ab108937, Abcam). For target reveal, IHC Detection Kit-Micropolymer (Cat#ab236466, Abcam) was employed according to the manufacturer's protocol. Hematoxylin was used to counterstain the sections, which were mounted with coverslips. Images acquired at 10x magnification were analyzed using the QuPath software by counting cells with high, medium, and low target signal intensity normalized on the total cell number of four fields of equal size.

4.5.6 RNAscope assay

FFPE skin sections from patients and controls were also employed for a Fluorescent In Situ Hybridization assay to validate gene expression and map cell populations.
HES1 and *HEY1* mRNA were visualized using the RNAscope® Multiplex Fluorescent Reagent Kit v.2 (Cat#323100, Advanced Cell Diagnostics) according to the manufacturer's protocol. Targets were visualized employing TSA Vivid 650 and 570 fluorophores, respectively, and Opal Polaris 780 for the C3-UBC control probe. Keratin 15 immunofluorescence assay was performed following RNA-FISH staining according to the manufacturer's instructions, by incubating the sections overnight at 4°C with the chicken anti-human KRT15 antibody (1:150; Cat#833901, BioLegend) in 0.05% PBS-Tween supplemented with 4% Goat serum gently provided by Prof. Kasper Lab of the Karolinska Institutet, Sweden. Secondary antibody staining was performed using AlexaFluor 488 diluted 1:400 in 0.05% PBS-Tween supplemented with 4% Goat serum. Sections were counterstained with DAPI solution provided in the RNAscope kit. Images were acquired on a Nikon CrEST X-Light V3 Spinning disk confocal microscope with a 63x magnification and the NIS Elements software. Subsequently, images from both patients' and controls' sections were processed (maximum intensity projection, brightness adjustment) using ImageJ Fiji and QuPath software. Nuclei were identified and expanded to approximate cytoplasm and targets dots were quantified within these cells, by distinguishing the basal layer from the others based on KRT15 staining.

4.6 Statistical analysis

Data and statistical analysis were performed using GraphPad Prism 9 and R Studio. Normal distribution of data was tested by applying the Kolmogorov-Smirnov test available on GraphPad Prism 9. Statistical evaluation was performed using an unpaired Student's t-test or the Mann-Whitney test. All numerical data are presented as standard error (SE). Differences were considered statistically significant when p-values fell below 5% ($P < 0.05$). For RNA sequencing analysis, multiple test corrections were performed with the Benjamini-Hochberg procedure with a p-value below 0.05 considered significant.

5. Results

5.1 Patients phenotype, *ATP2A2* variants identification and *in silico* characterization

Nineteen clinically diagnosed DD patients were recruited for this study and nine of them have a family history of the disease. Even though showing skin symptoms compatible with DD, in one of the patients no variant was identified in the ATP2A2 gene. However, genotype-negative patients have been previously reported in the literature (Sakuntabhai, Burge, et al., 1999; Bchetnia et al., 2009; Nellen et al., 2017). The absence of detected *ATP2A2* variants does not necessarily indicate that there is no mutation in the gene. Some variants might be located in unscreened regions of *ATP2A2*, such as promoter, intronic, or 3' untranslated regions, which could impact the expression or function of the SERCA2 protein. However, we excluded this patient data from assays results.

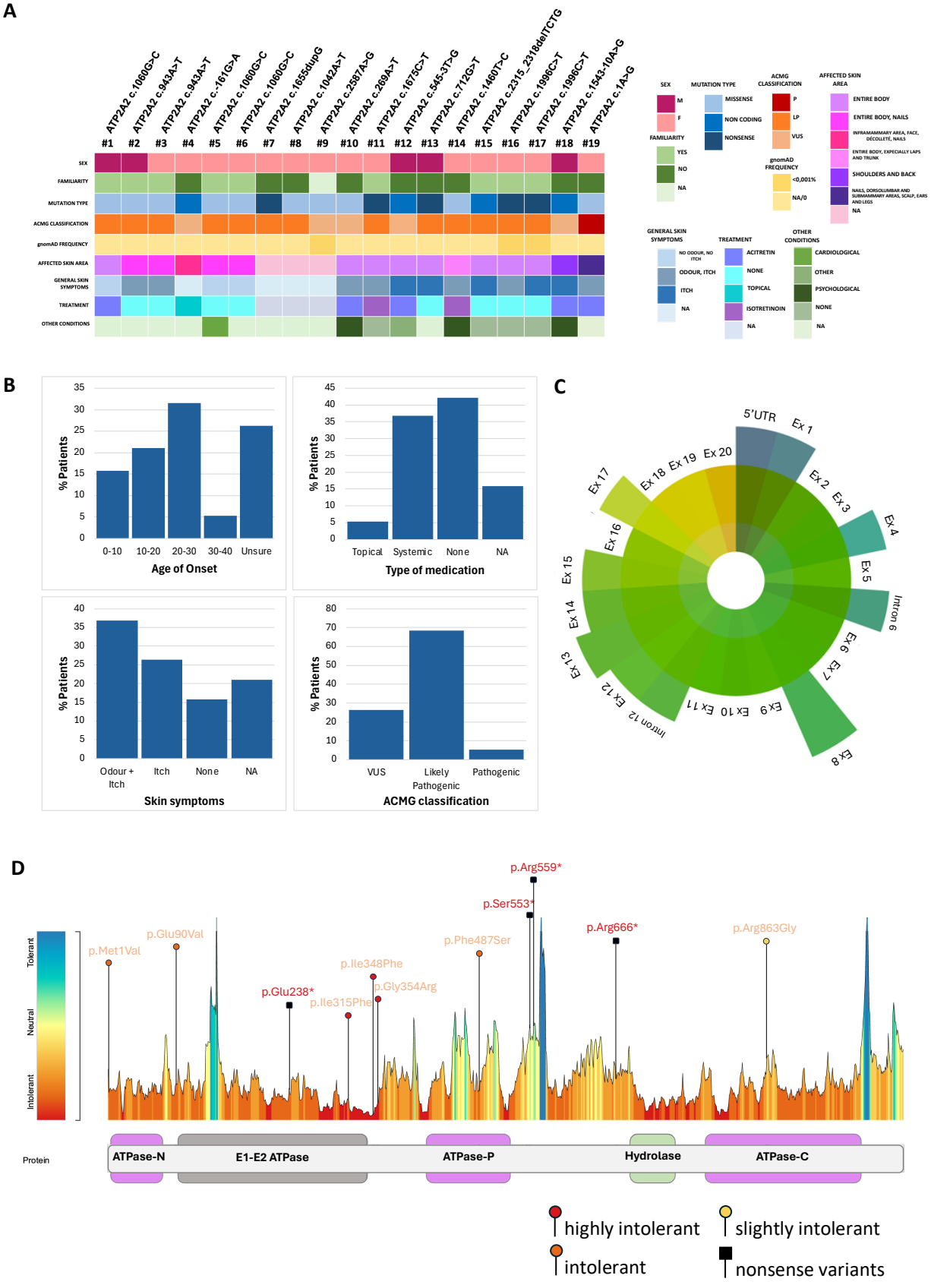
Fifteen different *ATPA2* variants were identified in this study, 10 (66.7%) of which not previously reported. The spectrum of variants included seven missense (46.7%), four nonsense (26.7%), a frameshift (6.7%), and three non coding (20%) mutations. All the variants, their predicted effect according to ACMG classification, and patients' phenotypes are summarized in **Table 7**, **Figure 27 A** and **27 B**. The majority of patients presented keratotic papules throughout the body, with six of them also showing nail abnormalities. Symptoms primarily associated with cutaneous lesions included itching and malodor. Some of the patients in this study are treated with oral retinoids (acitretin or isotretinoin) or topical treatments, while others do not receive any therapy. As reported in previous studies, DD patients often suffer from extracutaneous conditions, such as neurological disorders. In particular, among our patients cohort, three individuals were reported suffering from psychiatric, mood disorders, or cognitive impairments.

ID	Sex	Age of testing	Family history of disease	Variant	Affected skin areas	General skin symptoms	Other conditions
1	M	20	Yes	c.1060G>C (p.Gly354Arg)	Entire body	No odour, no itch	na
2	M	66	Yes	c.943A>T (p.Ile315Phe)	Entire body, nails	Odour, Itch	na
3	F	34	Yes	c.943A>T (p.Ile315Phe)	Entire body, nails (mild)	Odour, Itch	na
4	F	52	No	c.-161G>A	Inframammary area, face, décolleté, nails	na	na
5	F	55	Yes	c.1060G>C (p.Gly354Arg)	Entire body, nails	No odour, no itch	Heart valve disfunction
6	F	64	Yes	c.1060G>C (p.Gly354Arg)	Entire body, nails	No odour, no itch	na
7	F	43	No	c.1655dupG (p.Ser553*)	na	na	na
8	F	23	No	c.1042A>T (p.Ile348Phe)	na	na	na
9	F	na	na	c.2587A>G (p.Arg863Gly)	na	na	na
10	F	45	No	c.269A>T (p.Glu90Val)	Entire body	Odour, itch	Cognitive impairment
11	F	37	Yes	c.1675C>T (p.Arg559*)	Entire body	Odour, itch	None
12	M	27	No	c.545-3T>G	Entire body	Itch	Previous osteosarcoma
13	M	65	No	c.712G>T (p.Glu238*)	Entire body	Itch	na
14	F	37	No	c.1460T>C (p.Phe487Ser)	Entire body, expecially laps and trunk	Odour, Itch	Psychiatric disorders
15	F	69	Yes	c.2315_2318delTCTG	Entire body	Itch	None
16	F	63	Yes	c.1996C>T (p.Arg666*)	Entire body	Odour, Itch	None
17	F	28	Yes	c.1996C>T (p.Arg666*)	Entire body	Itch	None
18	M	52	No	c.1543-10A>G	Shoulders and back	Itch	Mood disorders
19	F	58	No	c.1A>G (p.Met1Val)	Nails, dorsolumbar and submammary areas, scalp, ears and legs	Odour, Itch	na

Table 7. Genetic and clinical features of recruited Darier Disease patients

Although functional studies specifically investigating these variants are lacking, previous research on different *ATP2A2* mutations, including missense, nonsense, and deletion ones, demonstrated that they all impact SERCA2 function by reducing protein expression, Ca^{2+}-ATPase activity, and Ca^{2+} transport, or by altering the protein's kinetic properties (Ahn et al., 2003; Dode et al., 2003; Sato et al., 2004; Leinonen et al., 2005; Miyauchi et al., 2006; Wang et al., 2011). Additionally, mutant SERCA2 protein is responsible for ER stress initiation which induces epidermal keratinocytes to round up, detach, and cause apoptosis (Y. Wang et al., 2011).

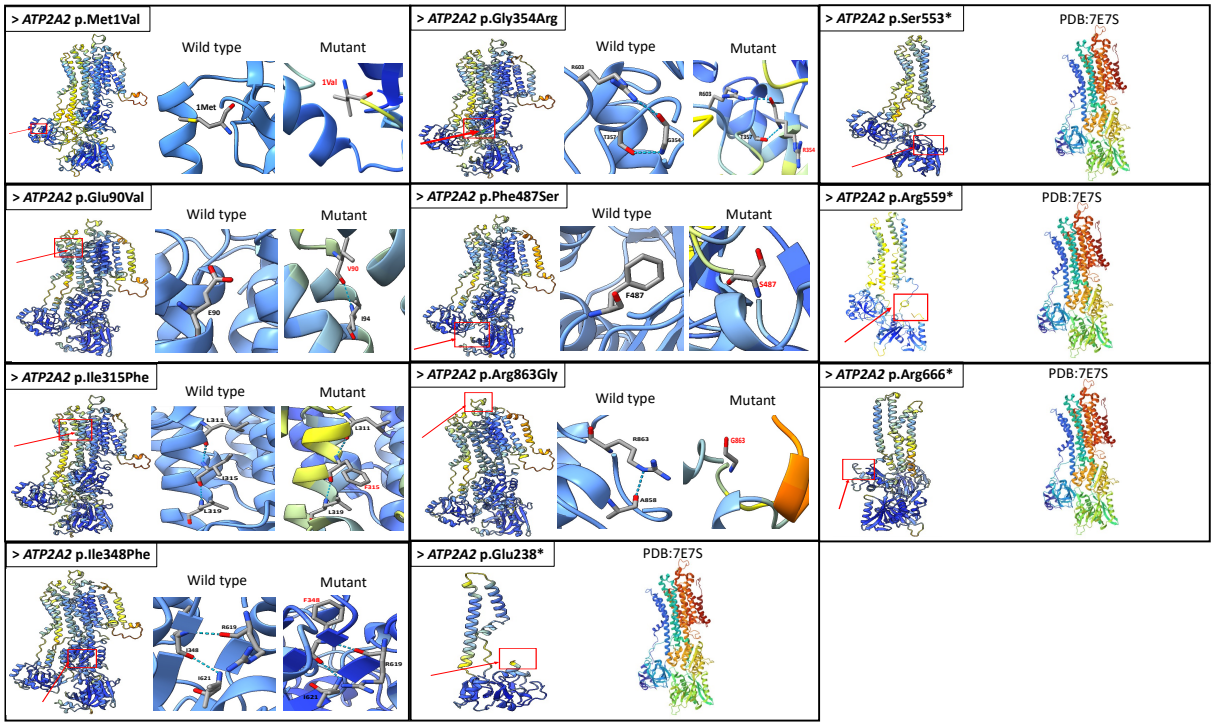
As previously reported, establishing a genotype-phenotype correlation of DD is difficult (Ruiz-Perez et al., 1999; Sakuntabhai, Burge, et al., 1999 Ringpfeil et al., 2001). The identified variants are distributed along the entire gene, without a distinctive hot spot (**Figure 27 C** and **27 D**). Artificial intelligence-based approaches, including Alphamissense, MetaDome and DDMut, were employed to evaluate missense variants' pathogenicity and their effects on protein stability. As reported in **Figure 27 E**, five out six variants (83.33%) were predicted to be likely pathogenic and located at positions that are intolerant or highly intolerant to variations. Altogether, these data support the pathogenicity of the mentioned variants. In order to evaluate the consequences of mutations based on a structural perspective, we generated SERCA2 protein models through ColabFold v.1.5.5, a platform for AlphaFold2-based protein structure predictions (**Figure 27 F**). Variant sites localization in the SERCA2 protein is shown in **Figure 27 G**. Among them, p.Glu90Val determines the formation of a hydrogen bond between V90 and the neighboring I94 on the β-strand, whereas p.Arg863Gly causes the loss of a hydrogen bond with A858 (**Figure 27 H**).


A
ATP2A2 c.1060G>C
ATP2A2 c.943A>T
ATP2A2 c.943A>T
ATP2A2 c.-161G>A
ATP2A2 c.1060G>C
ATP2A2 c.1060G>C
ATP2A2 c.1655dupG
ATP2A2 c.1042A>T
ATP2A2 c.2587A>G
ATP2A2 c.269A>T
ATP2A2 c.1675C>T
ATP2A2 c.545-3T>G
ATP2A2 c.712G>T
ATP2A2 c.1460T>C
ATP2A2 c.2315_2318delTCTG
ATP2A2 c.1996C>T
ATP2A2 c.1996C>T
ATP2A2 c.1543-10A>G
ATP2A2 c.1A>G
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18 #19
SEX
FAMILIARITY
MUTATION TYPE
ACMG CLASSIFICATION
gnomAD FREQUENCY
AFFECTED SKIN AREA
GENERAL SKIN SYMPTOMS
TREATMENT
OTHER CONDITIONS
M
F
YES
NO
NA
MISSENSE
NON CODING
NONSENSE
P
LP
VUS
<0,001%
NA/0
ENTIRE BODY
ENTIRE BODY, NAILS
INFRAMMAMMARY AREA, FACE, DECOLLETE, NAILS
ENTIRE BODY, EXPECIALLY LAPS AND TRUNK
SHOULDERS AND BACK
NAILS, DORSOLUMBAR AND SUBMAMMARY AREAS, SCALP, EARS AND LEGS
NO ODOUR, NO ITCH
ODOUR, ITCH
ITCH
ACITRETIN
NONE
TOPICAL
ISOTRETINOIN
CARDIOLOGICAL
OTHER
PSYCHOLOGICAL
B
% Patients
0-10
10-20
20-30
30-40
Unsure
Age of Onset
Topical
Systemic
None
Type of medication
Odour + Itch
Itch
None
NA
Skin symptoms
VUS
Likely Pathogenic
Pathogenic
ACMG classification
C
5'UTR
Ex 1
Ex 2
Ex 3
Ex 4
Ex 5
Intron 6
Ex 6
Ex 7
Ex 8
Ex 9
Ex 10
Ex 11
Intron 12
Ex 12
Ex 13
Ex 14
Ex 15
Ex 16
Ex 17
Ex 18
Ex 19
Ex 20
D
p.Arg559*
p.Ser553*
p.Arg666*
p.Arg863Gly
p.Glu90Val
p.Met1Val
p.Phe487Ser
p.Ile348Phe
p.Gly354Arg
p.Glu238*
p.Ile315Phe
Tolerant
Neutral
Intolerant
Protein
ATPase-N
E1-E2 ATPase
ATPase-P
Hydrolase
ATPase-C
highly intolerant
slightly intolerant
intolerant
nonsense variants

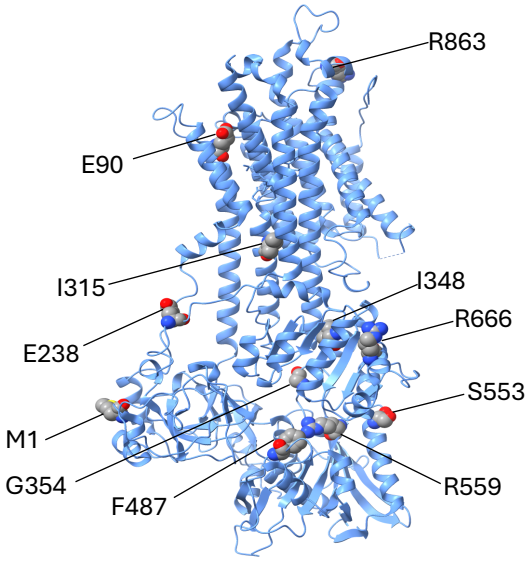
E

Position	Alphamissense score and effect	MetaDome tolerance score and effect	Predicted Stability Change DDMut and effect
ATP2A2:c.269A>T p.Glu90Val	0,9979 (LP)	0,35 (intolerant)	1.09 kcal/mol (stabilising)
ATP2A2:c.943A>T p.Ile315Phe	0,9932 (LP)	0,2 (intolerant)	-1.03 kcal/mol (destabilising)
ATP2A2:c.1042A>T p.Ile348Phe	0,992 (LP)	0,06 (highly intolerant)	-1.43 kcal/mol (destabilising)
ATP2A2:c.1060G>C p.Gly354Arg	0,9997 (LP)	0,17 (highly intolerant)	-0.32 kcal/mol (destabilising)
ATP2A2:c.1460T>C p.Phe487Ser	0,9999 (LP)	0,28 (intolerant)	-0.1 kcal/mol (destabilising)
ATP2A2:c.2587A>G p.Arg863Gly	0,1183 (LB)	0,6 (slightly intolerant)	-0.65 kcal/mol (destabilising)

F



G



H

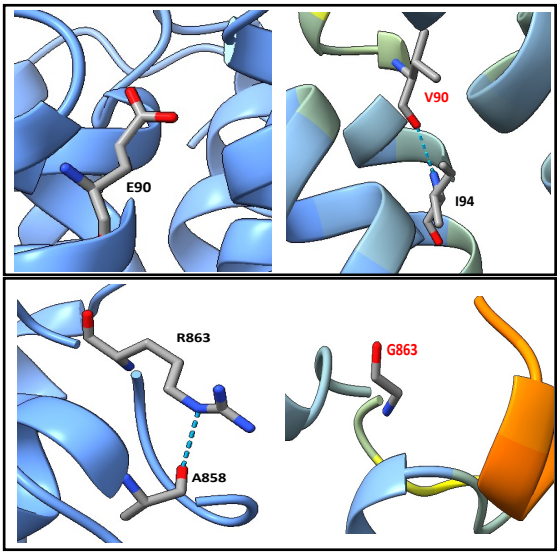


Figure 27. *ATP2A2* variants and SERCA2 protein structure
A) Table reporting relevant genetic and clinical features of the patient samples collected for this study. **B**) Distribution of key clinical and genetic characteristics among patients. The bar plots represent the percentage of patients across various categories, including age of disease onset, types of gene variants, ACMG (American College of Medical Genetics) classification, and types of treatments administered. **C**) Polar bar plot illustrating the frequency and distribution of genetic variants found in each exon of the ATP2A2 gene. **D**) Location of missense and nonsense variants in SERCA2 domains (RefSeq NM_170665, ENST00000539276.2). The tolerance landscape (MetaDome) is shown color-coded above the protein scheme. Legend: ATPase-N, cation transporter/ATPase, N-terminus; E1-E2 ATPase; ATPase-P, cation transport ATPase; Hydrolase, haloacid dehalogenase-like hydrolase; ATPase-C, cation transporter/ATPase, C-terminus. **E**) Alphamissense, MetaDome and DDMut-based evaluation of missense variants' pathogenicity and effects on protein stability. **F**) Evaluation of variants structural effects on SERCA2 protein by ColabFold v.1.5.5-AlphaFold2 tool. **G**) Structure of SERCA2 (PDB: 7E7S) indicating the identified genetic variants' sites. **H**) Enlargement of the SERCA2 structure showing the effect of p.Glu90Val variant in determining the formation of a hydrogen bond to I94 and of p.Arg863Gly in causing the loss of the hydrogen bond to A858.

5.2 *ATP2A2* variants overexpression *in vitro*

To gain further insight into the pathogenic mechanisms of DD, we transiently overexpressed the different *ATP2A2* variants of interest in HEK293T and HaCat cell lines. In particular, being HEK293T easily transfectable compared to HaCat cells, we employed them for western blotting assays, whereas HaCat were transfected for qPCR, gene reporter, and immunofluorescence assays. *ATP2A2* variants were introduced by site-directed mutagenesis in a plasmid containing the gene coding sequence fused with CopGFP and their presence was checked by Sanger sequencing method (**Figure 28**).

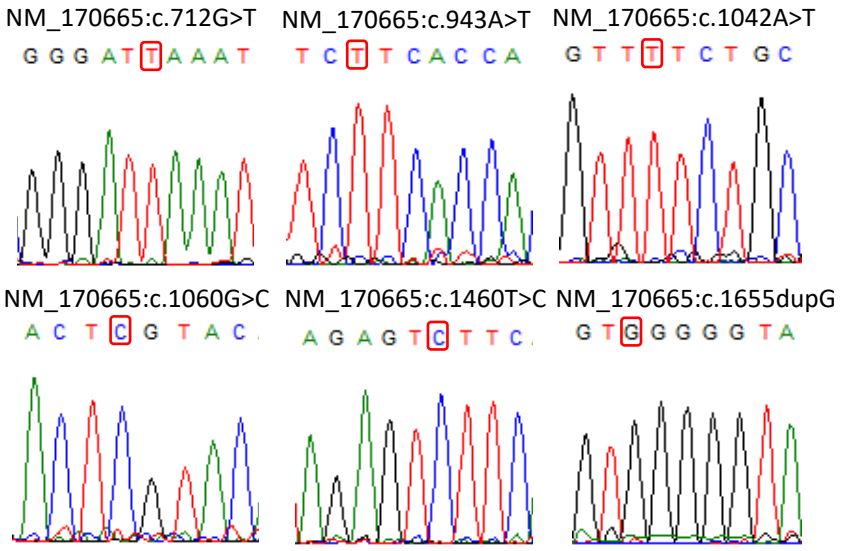


Figure 28. Site-directed mutagenesis results relative to some of the *ATP2A2* variants.

5.3 Real-time PCR (*ex vivo* and *in vitro*)

Primary keratinocytes together with HaCat, and T-cell acute lymphoblastic leukemia (ALL-SIL) cell lines-deriving cDNA was employed to perform real-time PCR in order to evaluate the NOTCH1 signaling. It is known that ALL-SIL cells have an active NOTCH1 pathway and for this reason were employed as positive controls. Results showed a significant reduction of *NOTCH1* and *HES1* expression levels in DD keratinocytes compared to control ones, and indicated HaCats as a valid cellular model, in particular for *HES1* analysis (**Figure 29 A**).

In vitro studies were performed by transfecting HaCat cells with *ATP2A2*-WT and mutant plasmids deriving from site-directed mutagenesis. Interestingly, *HES1* expression levels were reduced, but not in a statistically significant fashion, in the presence of *ATP2A2* variants compared to the WT form at 24 hours after transfection (**Figure 29 B**). In comparison, after 48 hours, they even showed an increase (**Figure 29 C**). Noteworthy, in a study conducted by Lee and colleagues, an increase in *HES1* mRNA levels was observed during ER stress as a consequence of PERK activation. They suggested that the initial decrease in HES1 protein levels during acute ER stress was due to translational inhibition by PERK, combined with the instability of HES1 (Hirata et al., 2002). This reduction relieves the transcriptional repression of its own promoter, resulting in an increase in mRNA levels. Therefore, although HES1 protein levels generally do not exceed baseline during ER stress, this feedback mechanism likely contributes to the cell's ability to maintain the appropriate overall levels of HES1 (J. E. Lee et al., 2018). Considering that ER stress is one of the main features observed in DD and confirmed also in our study, it could be responsible for *HES1* mRNA increase in our patients' cohort.

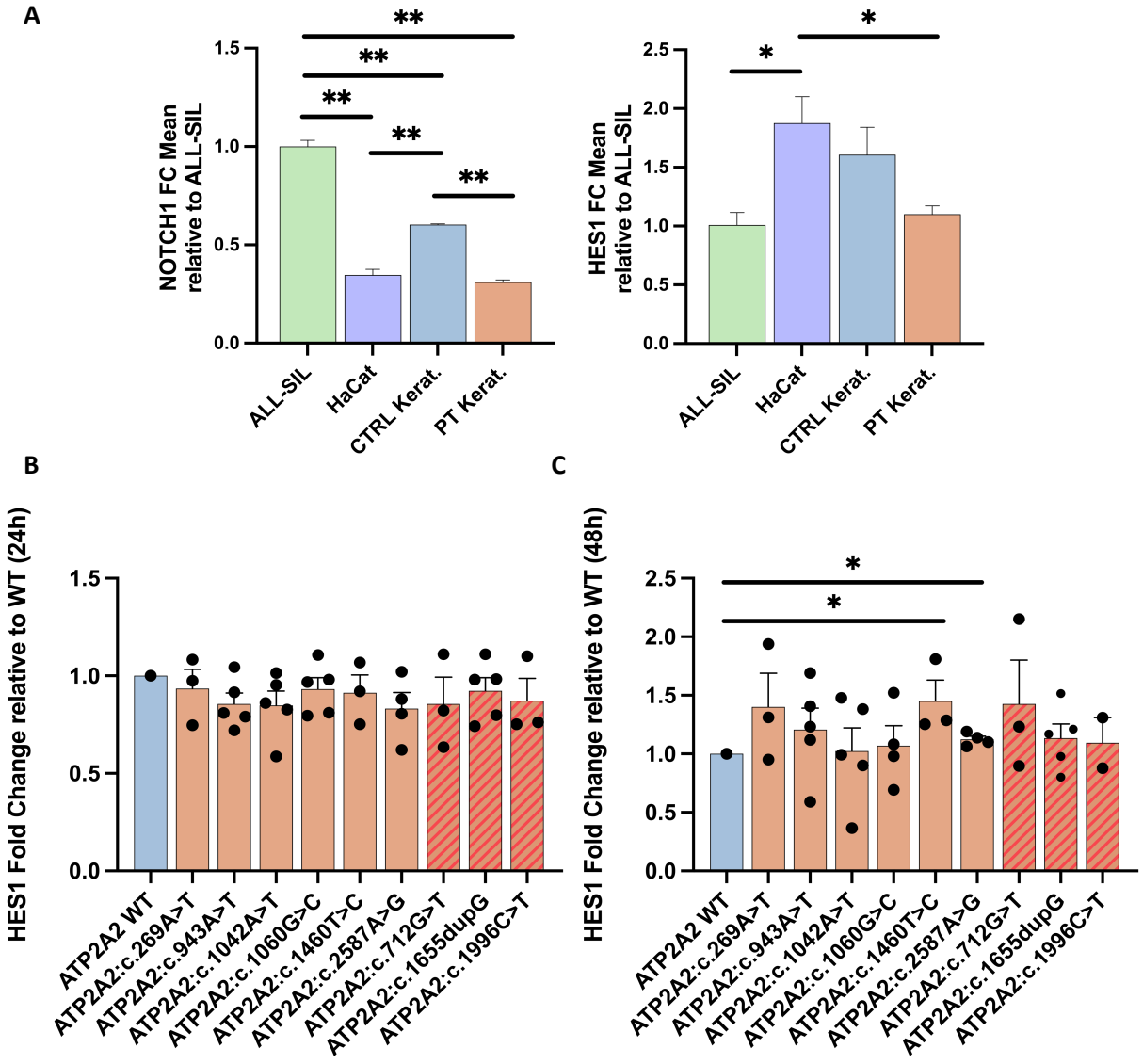


Figure 29. Real-time PCR.

A) Evaluation of *NOTCH1* and *HES1* expression levels in patients' and controls' primary keratinocytes, HaCat, and ALL-SIL cell lines. Results show a reduction of *NOTCH1* and *HES1* levels in patients' keratinocytes compared to controls' ones and confirmed HaCat cell line as a cellular model for NOTCH1 target evaluation. **B**) Evaluation of *HES1* expression levels in HaCat cells with *ATP2A2*-WT and mutant plasmids 24 and **C**) 48 hours after transfection. Mann-Whitney test on GraphPad Prism 9 software. P-value: *P<0.05; **P<0.005; not significant P>0.05.

5.4 Western blot

Western blot analysis was conducted to evaluate the NOTCH1 signaling pathway in the presence of SERCA2-WT and mutated forms. After 24 hours of HEK293T cells transfection, a reduction of the activated intracellular NOTCH1 domain (NOTCH1-ICD), c-MYC, and HES1 protein level in the presence of patients' variants compared to SERCA2-WT were observed (**Figure 30 A**). At 48 hours post-transfection, the target protein levels remained reduced except for c-MYC, although this difference was not statistically significant (**Figure 30 B**).

Western blot assays were also performed to analyze whether the mutant SERCA2 was expressed at normal levels or whether it might be subjected to degradation by UPR. At both 24 and 48 hours post-transfection, we observed a significant reduction in cells transfected with both missense and nonsense variants, with the latter leading to a greater decrease in protein levels.

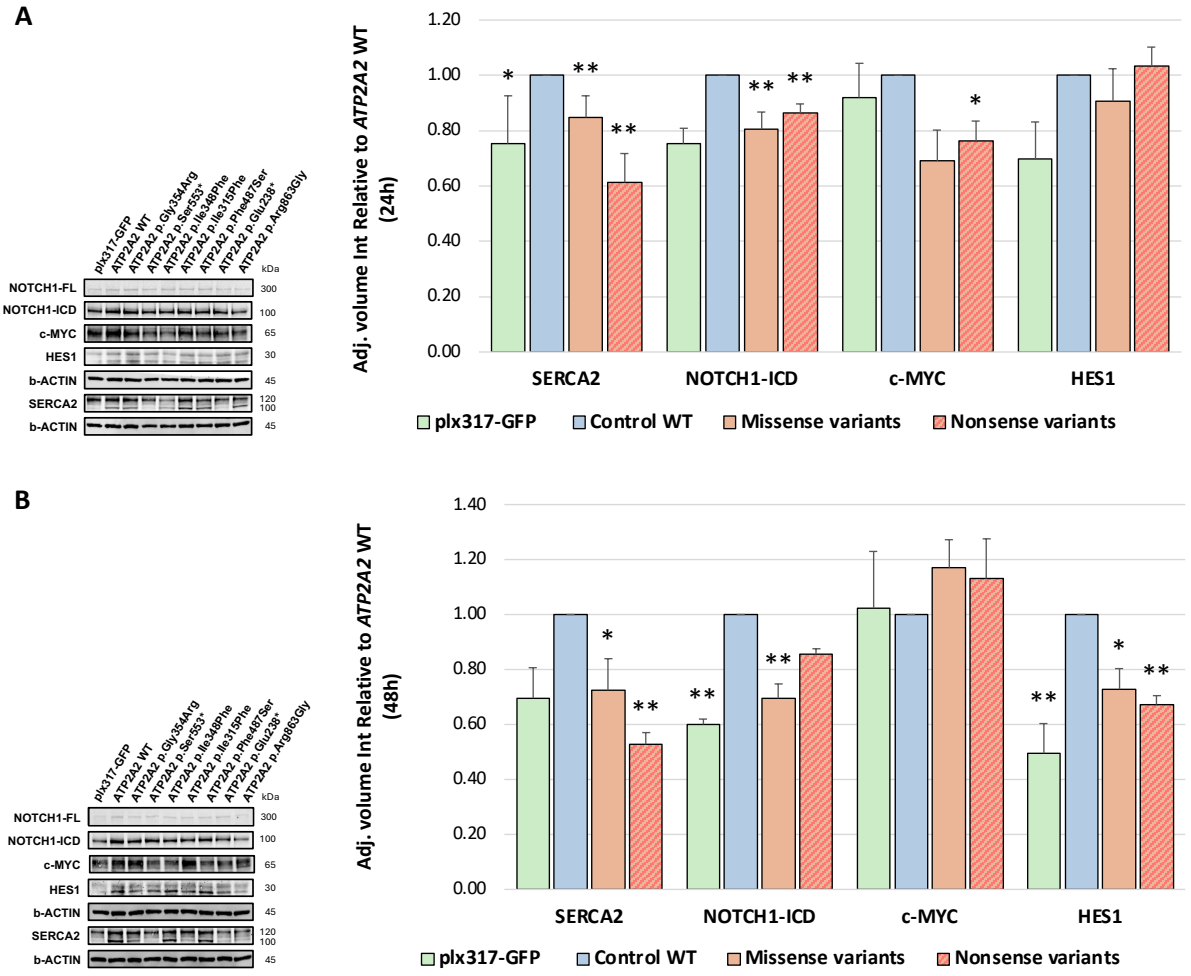


Figure 30. Western Blot.
Densitometric evaluation of SERCA2, NOTCH1-ICD, c-MYC, and HES1 protein levels by western blot in the presence of *ATP2A2* variants at 24h (**A**) and 48h (**B**) after the transfection of the HEK293T cell line. Results show statistically significant reduced levels of NOTCH1-ICD in the presence of missense and nonsense variants; HES1 and c-MYC levels were reduced at 24h compared to the overexpressed *ATP2A2* WT, while at 48h c-MYC resulted increased, even if the difference with the WT form was not significant. T-test p-value: *P<0.05; **P<0.005; not significant P>0.05.

5.5 Luciferase gene reporter assay

To examine *ATP2A2* variants' effect on the NOTCH1 signaling pathway, HaCat cells were employed to perform a gene reporter assay using a plasmid containing the Firefly Luciferase gene driven by an inducible promoter comprising four binding sites for CSL, thus under the control of NOTCH1 signaling activation. Firefly luciferase signal was normalized on the Renilla luciferase employed as control. Except for p.Ile348Phe, all variants of interest led to significantly decreased luciferase signal compared to the wild-type condition. In the presence of SERCA2 truncating variants, the luciferase signal was more reduced compared to missense ones (**Figure 31**).

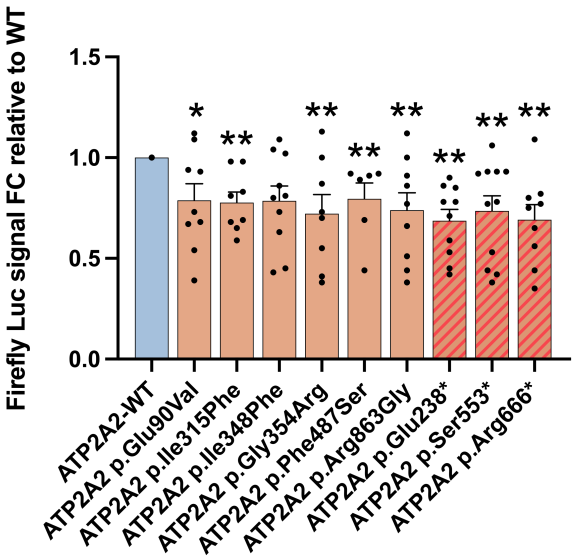


Figure 31. Evaluation of *ATP2A2* variants effect on the NOTCH1 signaling by gene reporter assay.
The graph illustrates the Firefly luciferase activity in HaCat cells transfected with the 4xCSL plasmid, a reporter construct responsive to NOTCH signaling activation. Firefly luciferase activity was normalized to the Renilla luciferase signal, expressed by a co-transfected plasmid. Results show a decreased Luciferase signal in the presence of *ATP2A2* missense and nonsense variants compared to the wild-type gene. Mann-Whitney test on GraphPad Prism 9 software. P-value: *P<0.05; **P<0.005; not significant P>0.05.

5.6 Immunofluorescence

5.6.1 Primary cell lines characterization

In order to molecularly characterize primary cell lines established from patients' and controls' skin biopsies (**Figure 32 A**), we performed immunofluorescence assays employing specific cell markers: vimentin and cytokeratin. Vimentin is a mesenchymal marker and was used to stain primary fibroblasts, while cytokeratin, which constitutes the epithelial cytoskeleton, was employed to stain keratinocytes (**Figure 32 B**).

5.6.2 ER stress analysis

As before mentioned (see 2.4.1), altered SERCA2 in epidermal cells is insoluble or partially soluble and forms aggregates resulting in calcium homeostasis impairment and ER stress with the consequent unfolded protein response (UPR) induction. UPR is characterized by the enhancement of different markers, including XBP1s. Following this evidence, we performed immunofluorescence assays on primary keratinocytes deriving from skin biopsies of controls and patients carrying missense variants to evaluate the ER stress. Acquired images showed a significant increase of XBP1 protein level in patient keratinocytes compared to control cells, as emerged by the more intense nuclear signal (**Figure 32 C** and **32 D**). These results align with the impact of SERCA2 alterations on ER stress exacerbation.

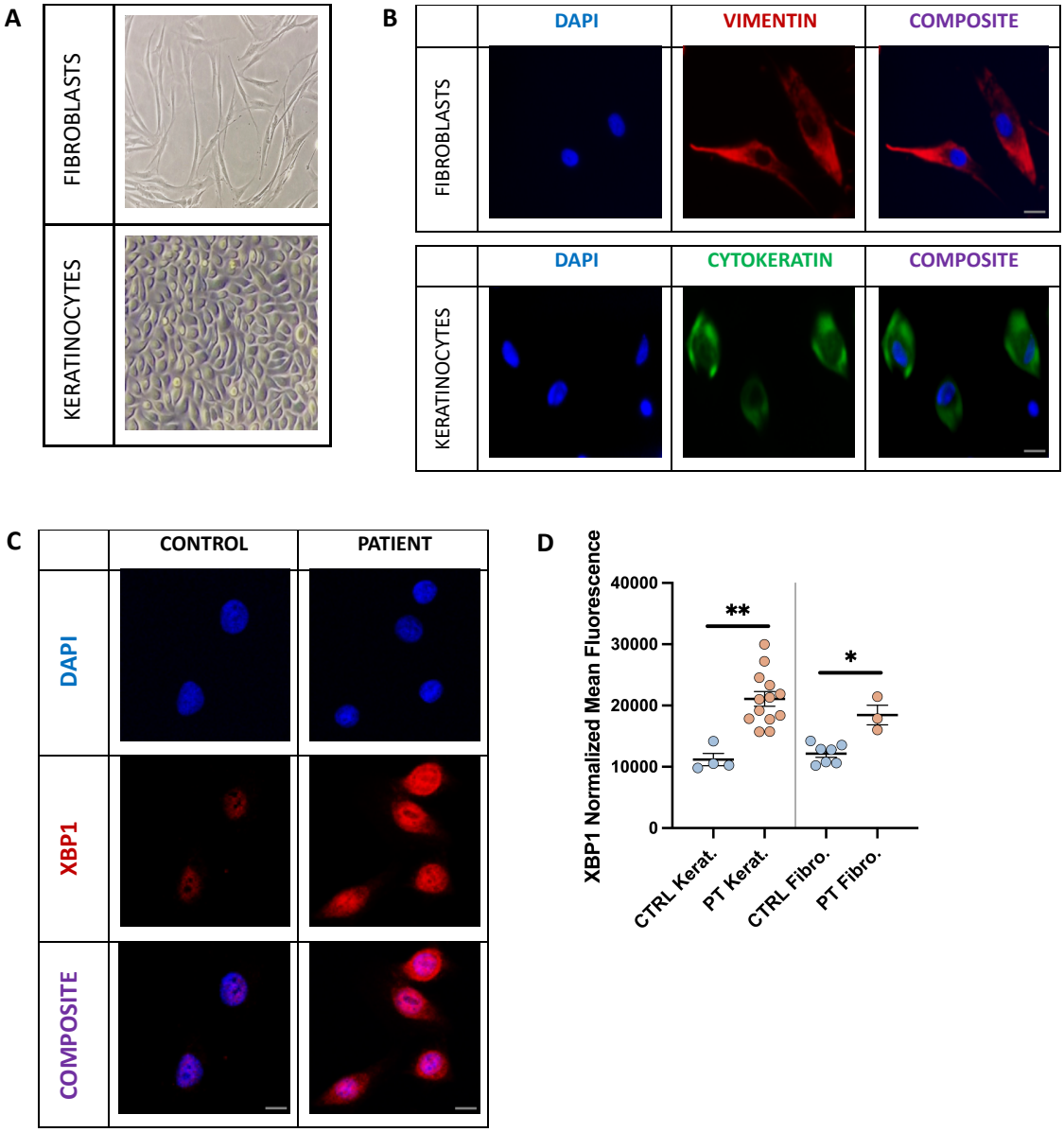


Figure 32. Primary cells characterization and ER stress evaluation.
A) Brightfield microscopy images of primary fibroblasts and keratinocytes. Fibroblasts exhibit a spindle-shaped morphology characteristic of mesenchymal cells, while keratinocytes display a cobblestone-like appearance typical of epithelial cells. **B**) Primary cell line characterization by immunofluorescence staining with vimentin (red) marking fibroblasts, and cytokeratin (green) highlighting keratinocytes. **C**) Immunofluorescence evaluation of XBP1 protein level in patient and control subjects' primary keratinocytes. **D**) XBP1 normalized mean fluorescence intensity was quantified by employing the ImageJ-Fiji software and resulted to be higher in DD patients compared to control primary keratinocytes and fibroblasts. Statistical comparison was performed through the Mann-Whitney test on GraphPad Prism 9 software. P-value: *P<0.05; **P<0.005; not significant P>0.05.

5.6.3 Evaluation of NOTCH1 activity in DD keratinocytes by immunofluorescence (*ex vivo*)

According to previous studies, SERCA2 plays an active role in the NOTCH pathway regulation, and its alterations would impede the correct NOTCH1 maturation and translocation towards the plasma membrane. Being stacked in the ER-Golgi compartment, NOTCH1 interaction with its ligands would be prevented, and the active form (NICD) could not translocate into the nucleus to activate the target transcription. In order to evaluate the effect of *ATP2A2* variants on SERCA2 protein and consequently on NOTCH1 signaling, patients' (2315_2318delTCTG, c.1543-10A>G) and controls' primary keratinocytes together with HaCat cells were employed to perform immunostaining assays for SERCA2, NOTCH1 (**Figure 33 A**) and HES1 (**Figure 33 B**). Considering the difficulties in obtaining primary cells, we also employed HaCats to assess their protein levels and use them when control keratinocytes were not available. Corrected total cell fluorescence (CTCF) was evaluated by measuring integrated density, cell area, and background mean fluorescence obtained through ImageJ software (CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings)). CTCF value resulted to be reduced for both SERCA2 and NOTCH1 as well as HES1 in patients' cells compared to control and HaCats (**Figure 33 C**).

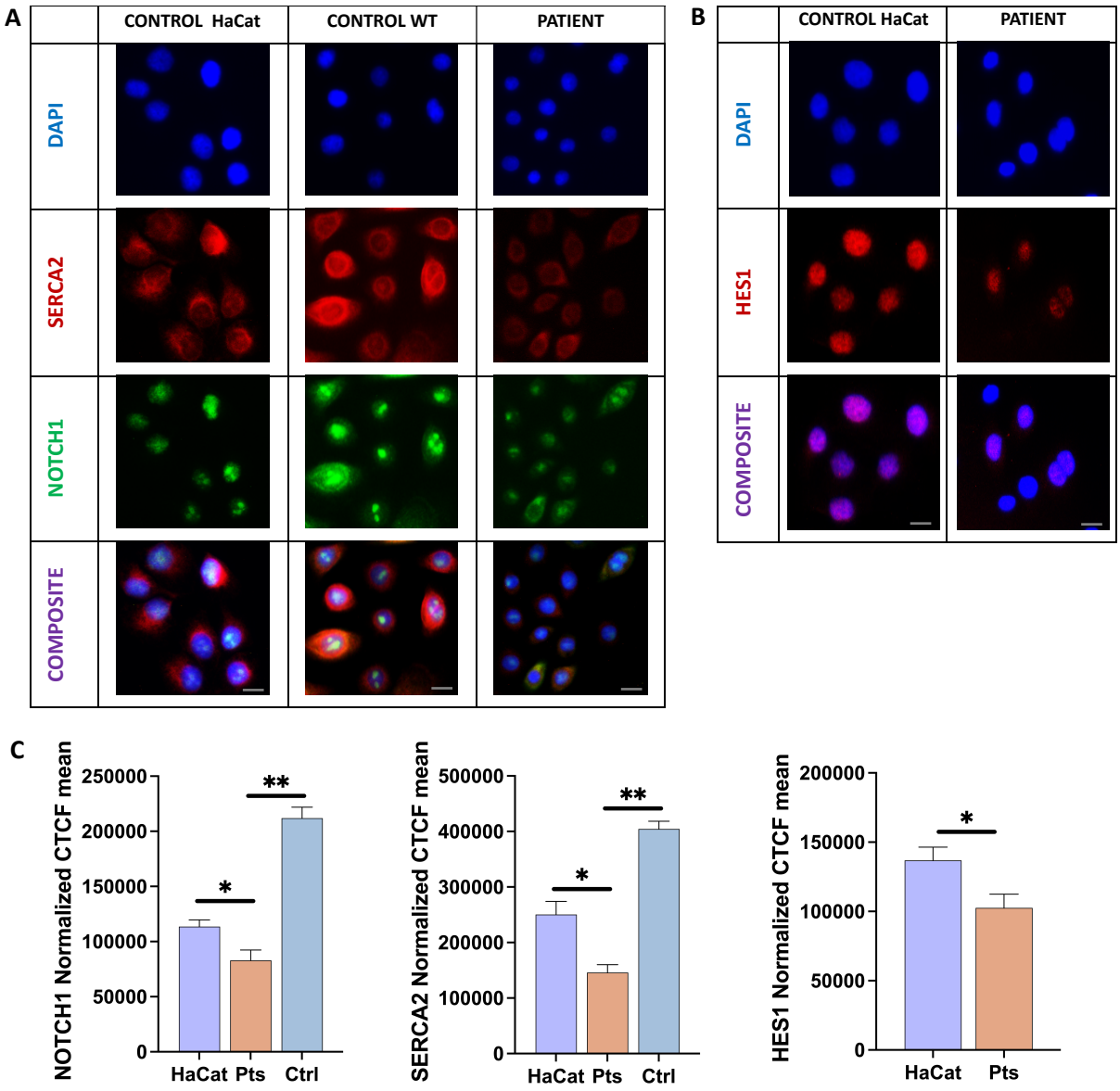
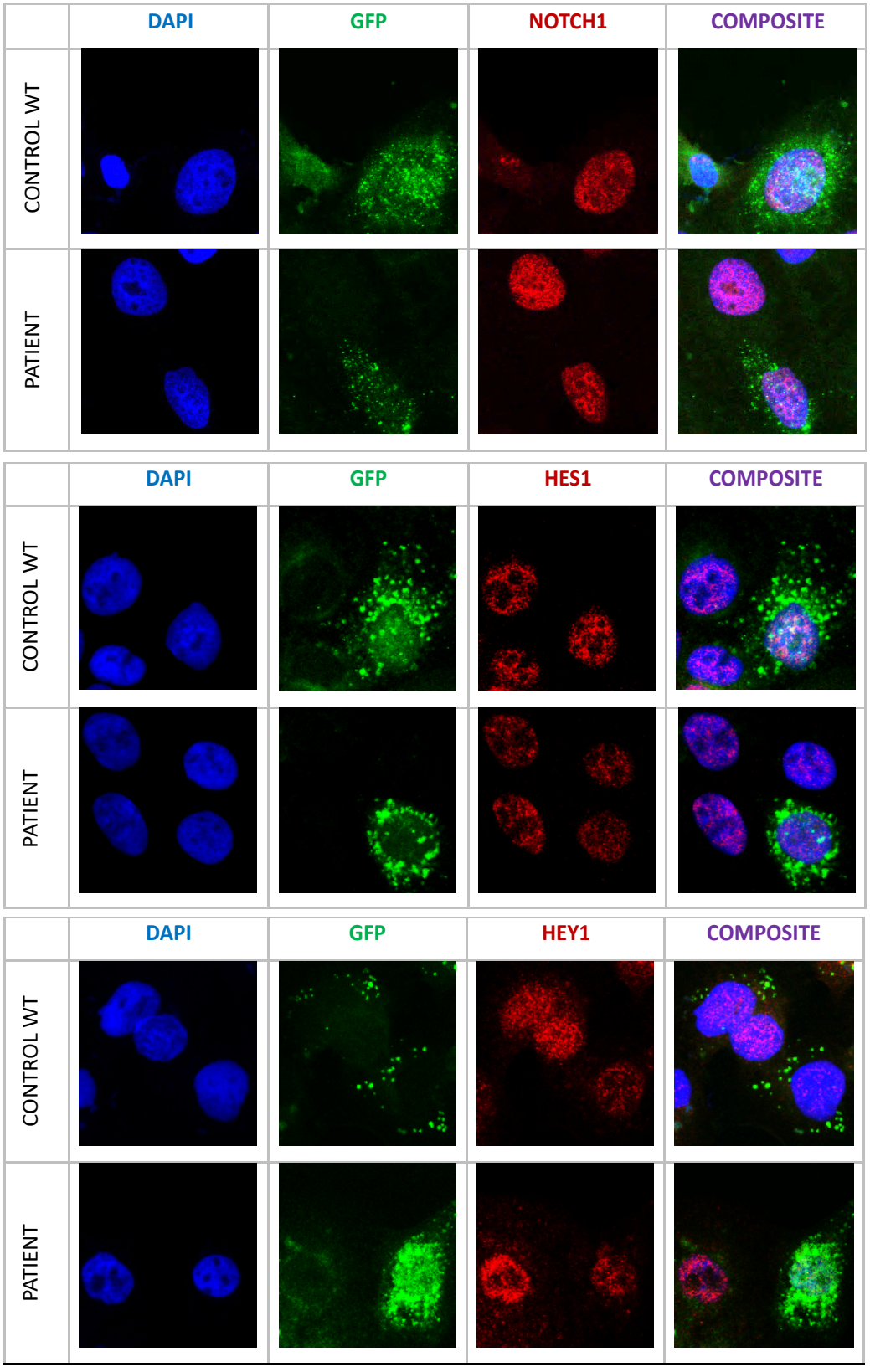


Figure 33. *Ex vivo* immunofluorescence assays.
Immunofluorescence evaluation of SERCA2, NOTCH1 (**A**) and HES1 (**B**) protein levels in patient and control subjects' primary keratinocytes and HaCat cells. CTCF (corrected total cell fluorescence) was quantified by employing the ImageJ-Fiji software and the statistical comparison performed using an unpaired two-tailed T-test showed a reduced expression of SERCA2, NOTCH1, and HES1 in DD patients compared to control primary keratinocytes and HaCat (**C**). Unpaired T-test was calculated through GraphPad Prism 9. P-value: *P<0.05; **P<0.005; not significant P>0.05.

5.6.4 Evaluation of NOTCH1 activity in the HaCat cell line by immunofluorescence (*in vitro*)

HES1, HEY1, and NOTCH1 levels were evaluated after the overexpression of *ATP2A2* WT and mutant in HaCat cells. Signal assessment was performed by Leica Stellaris 5 confocal microscope with 63x magnification, and images were analyzed by ImageJ-Fiji software to determine CTCF values differences between cells with high and low and/or negative CopGFP signal. Results showed a reduction of targets signal in cells with high CopGFP (transfected with variants) compared to low/negative CopGFP ones, thus indicating a negative effect of the SERCA2 variant on the NOTCH1 pathway (**Figure 34 A**). In the presence of overexpressed *ATP2A2*-WT, we observed an increase of targets signal compared to the not transfected cells. In particular, HES1 signal resulted statistically significant decreased in the presence of all variants, except for p.Ile348Phe and p.Arg863Gly, while from HEY1 analysis a not significant signal reduction was observed in the presence of overexpressed *ATP2A*- WT, p.Gly354Arg and p.Glu238* (**Figure 34 B**).

A


DAPI
GFP
NOTCH1
COMPOSITE
CONTROL WT
PATIENT
DAPI
GFP
HES1
COMPOSITE
CONTROL WT
PATIENT
DAPI
GFP
HEY1
COMPOSITE
CONTROL WT
PATIENT

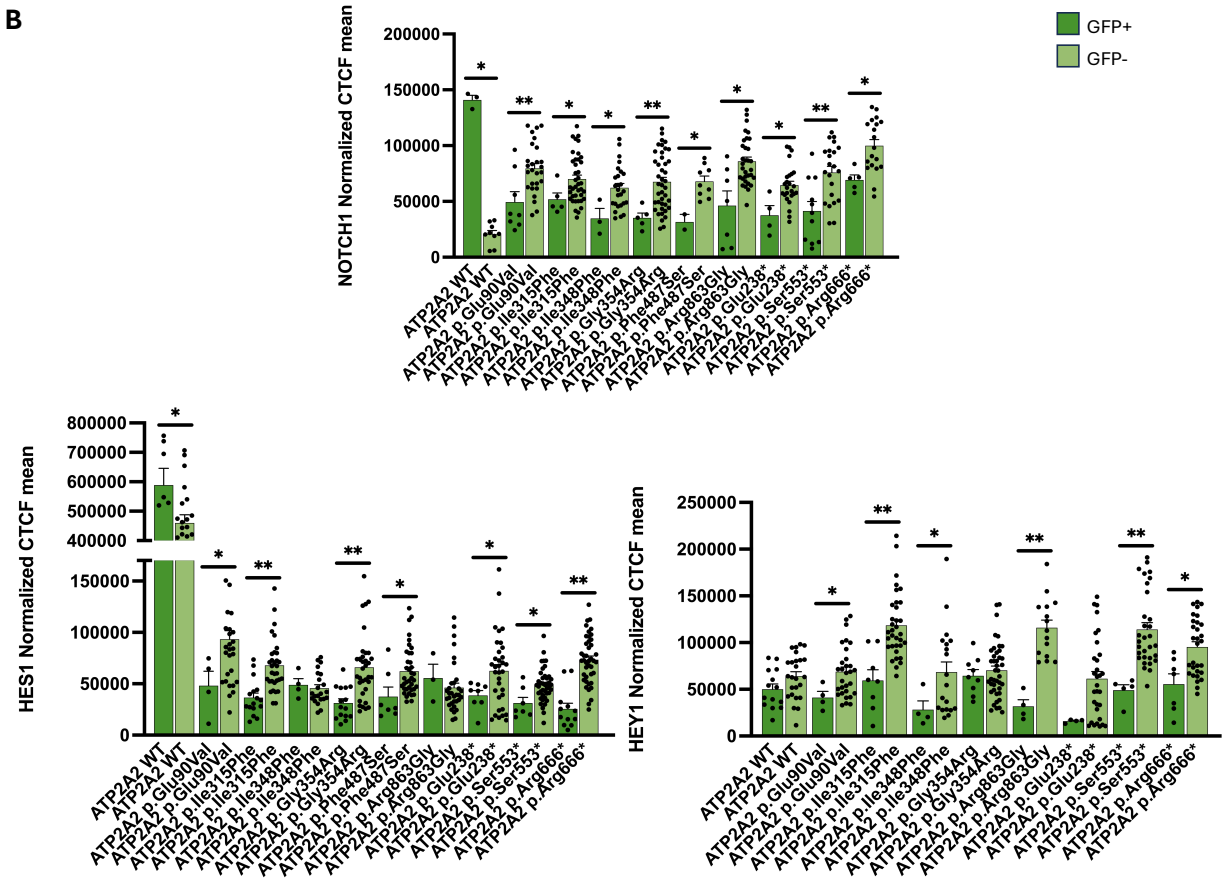


Figure 34. *In vitro* immunofluorescence assays.
A) Representative confocal images of HaCat cells transfected for 48 hours with plasmids encoding SERCA2 in the wild-type or mutant forms fused to Cop Green Fluorescent Protein (CopGFP). Signals for NOTCH1, HES1, and HEY1 are visualized in red. **B**) Corrected Total Cell Fluorescence (CTCF) values for the target proteins were calculated using ImageJ-Fiji software measurements. Comparative analysis reveals a reduction in target signals in cells with high GFP intensity compared to those lacking GFP signal, indicating decreased protein levels in the presence of the mutant *ATP2A2* variants. Statistical comparison was performed through the Mann-Whitney test on GraphPad Prism 9 software. P-value: *P<0.05; **P<0.005; not significant P>0.05.

5.7 Skin biopsies-derived RNA analysis

Total RNA purified from patient #18 (c.1543-10A>G) skin biopsy was employed to assess the effect of the *ATP2A2* genomic variant on the coding sequence. The obtained cDNA was used as the template for PCR reaction using a primer set overlapping the ATP2A2 gene coding sequence and the product was sequenced by Sanger method (see Material and Methods). The variant was predicted to determine the gain of a splicing acceptor site by SpliceAI (score 0.94) and results showed the retention of a 9-nucleotide intronic sequence (**Figure 35 A**).

Additionally, we analyzed the *.bam* file deriving from RNA-sequencing analysis (see paragraph 5.7.1) of the patient carrying the c.545-3T>G variant, leading to acceptor loss according to SpliceAI (score 0.95). We observed the deletion of 23 nucleotides at the 5' extremity of *ATP2A2* exon 7 in 11 reads (**Figure 35 B**).

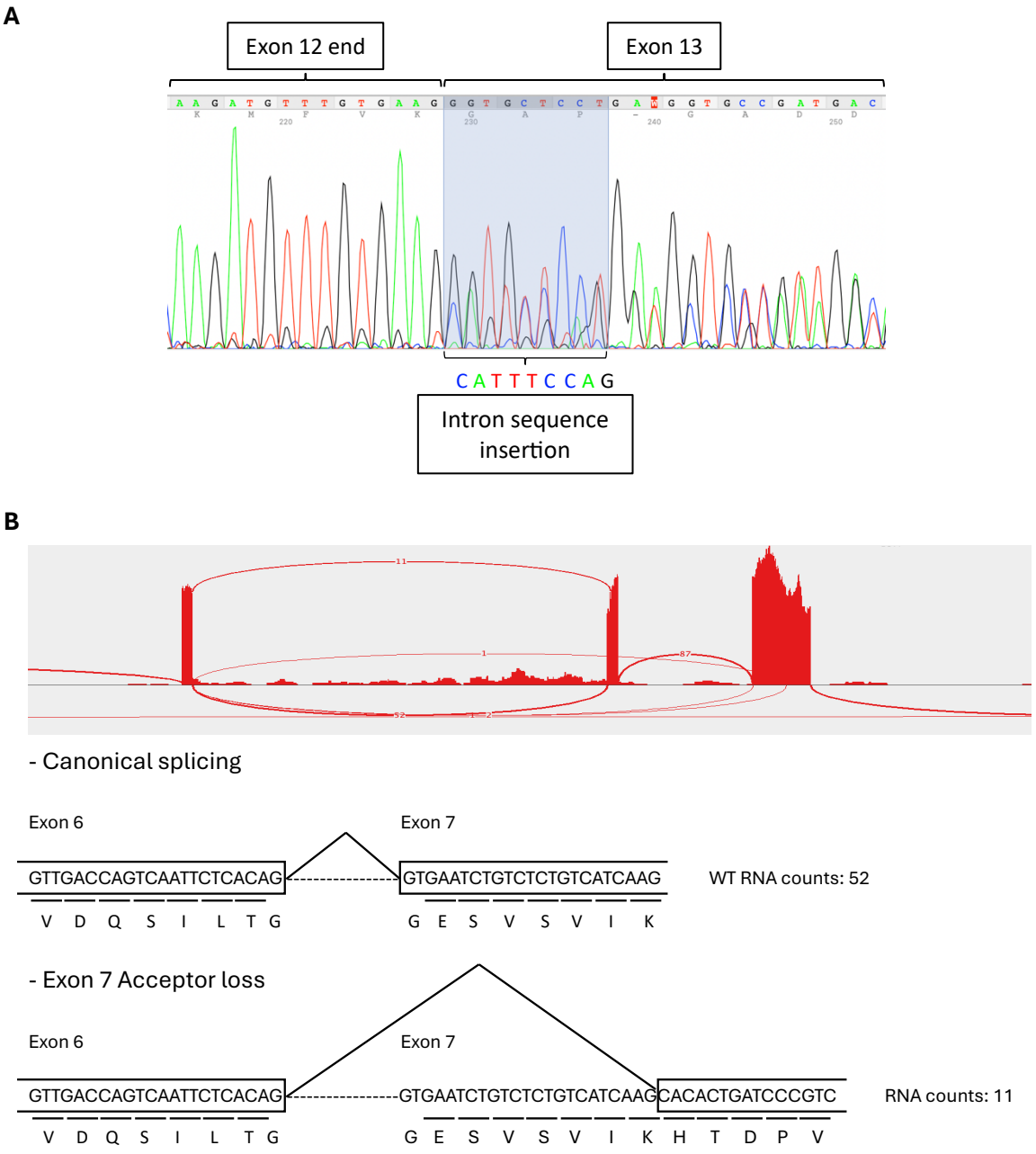


Figure 35. Evaluation of genomic variants effect on *ATP2A2* coding sequence.
A) Retention of 9-nucleotide intronic sequence in the presence of the c.1543-10A>G variant.
B) Evaluation of c.545-3T>G effect on the ATP2A2 gene coding sequence observed on IGV software from RNAseq-deriving *.bam* file. In 11 reads, the variant caused the loss of the canonical splicing site and 23 nucleotides at the 5' region of the exon 7.

5.7.1 RNA-Sequencing

High-throughput RNA-sequencing (RNA-seq) has been demonstrated to be an essential tool for transcriptome-wide analysis. RNA-seq can capture various types of RNAs, including messenger RNAs (mRNAs), microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) (Xiang et al., 2018).

To understand the transcriptional impact of *ATP2A2* variants and to evaluate other molecular pathways that could be involved in the onset of DD, 3 mm skin punch biopsies from patients and control subjects were used for RNA-sequencing analysis. In total, 7 patients (c.1060G>C, c.943A>T, c.269A>T, c.1460T>C, c.1996C>T m, c.1996C>T d, c.545-3T>G) and 5 controls RNA samples extracted from whole (epidermis and dermis) skin biopsies were evaluated.

Following the assessment of *.fastq* files quality, sequencing reads were mapped to the human reference genome GRCh38 using the RNA STAR tool and results showed a mean of 89.3% of uniquely mapped reads in patients and 88% in controls.

5.7.1.1 Differential Gene Expression analysis

In order to determine the biological variance for each condition and estimate differential gene expression (DGE) between DD patients and the control group, DESeq2 software was employed using count files deriving from the featureCounts tool as input. Counts for each gene in samples were normalized for sequencing depth and library composition and a heatmap of the sample-to-sample distance matrix showing closer subjects in dark blue and more distant in white was generated (**Figure 36 A**). A principal component analysis (PCA) plot was created from subjects normalized counts and indicated that expression profiles were separated by whether the samples were from patients or controls cohort, revealing a distinct clustering of the two groups with 45.68% and 21.9% variance (**Figure 36 B**).

Annotated DESeq2 results were employed to generate a volcano plot highlighting significant genes with FDR < 0.01 and $\log_2$(FC) of 0.58 (corresponding to a Fold Change of 1.5). In the plot, colored genes are the ones that passed the FDR and $\log_2$(FC) thresholds and are distinguished

in upregulated (red) and downregulated (blue) with top 10 most significant labeled (**Figure 36 C**).

Fibrillin 2 (FBN2) gene resulted to be the most statistically significant differentiated. It encodes the fibrillin 2 protein, a component of extracellular matrix microfibrils, mainly expressed in developing fetal tissues (Quondamatteo et al., 2002). In adult normal skin, it is barely expressed at the dermal-epidermal junction, but increased in the dermis during wound healing and sclerotic skin diseases, and in the presence of TGF-β or IL-4 in fibroblast cell culture (Ashcroft et al., 1997; Brinckmann et al., 2010). Although there are no data regarding the involvement of *FBN2* in Darier disease, its high expression in the patients in this study may be due to tissue remodeling mechanisms in lesion-affected areas or in response to inflammatory processes, resulting to be upregulated in patients compared to controls, as revealed by GSEA analysis. Another gene resulted to be upregulated in DD patient was the regulator of calcineurin 1 (RCAN1), involved in the modulation of mitochondrial function. Recent studies suggested the role of overexpressed RCAN1 in the induction of mitochondrial dysfunction and apoptosis through the activation of the c-Jun N-terminal kinase/mitochondrial fission factor (JNK/Mff) pathway in acute kidney injury (Xiao et al., 2022). It has also been observed that RCAN1 plays a role in inflammatory signaling modulation by inhibiting NF- κB (E. H. Lee et al., 2017) and the nuclear factor of activated T cells (NFAT) transcription factors (Hesser et al., 2004). The RCAN1 increased expression in this study patients could represent a compensatory mechanism to contrast inflammation. Additionally, calcium homeostasis disruption leads to RCAN1 exon 4 overexpression, resulting in neural death in Alzheimer's disease, and calcium overloading could mediate its upregulation (Sun et al., 2014). To our knowledge, there are currently no direct studies linking RCAN1 to Darier disease. However, according to previous studies on other conditions, further research could clarify whether RCAN1 plays a role also in Darier disease exacerbation and comorbidities development, i.e mental disorders frequently observed in DD patients. GBP6 is one of the guanylate-binding proteins (GBP) belonging to the superfamily of INF-inducible guanosine triphosphate hydrolases (GTPases) (B.-H. Kim et al., 2012). Previous studies indicated a higher expression of *GBP6* and other *GBPs* in skin cancer compared to normal tissues (Q. Wang et al., 2018). GBPs are also involved in host defense due to their antiviral and antimicrobial activities (Vestal & Jeyaratnam, 2011) and could control infection and autoimmunity (Shenoy et al., 2012). The upregulation of *GBP6* in DD patients

may be related to an inflammatory or immune response associated with the predisposition to secondary infections, which is common in individuals affected by this cutaneous condition.

LCE3E codifies for a member of the late cornified envelope protein family (LEP17) and is low expressed or undetectable in normal skin (Jackson et al., 2005). However, studies on psoriatic skin samples demonstrated an increased expression of *LCE3E* in lesional skin compared to unaffected areas, suggesting that skin barrier disruption could lead to this gene induction (de Cid et al., 2009). *In vitro* studies on cultured normal human keratinocytes demonstrated an *LCE3E* upregulation in the presence of ultraviolet irradiation. In the skin, this response could represent a protective adaption mechanism to UV exposure (Jackson et al., 2005). The elevated expression of *LCE3E* in patients with Darier disease might reflect an attempt by the skin to compensate for the loss of structural integrity and the need for enhanced protective mechanisms against environmental insults.

LRRC20 (Leucine-Rich Repeat Containing 20) is a gene encoding a protein that contains leucine-rich repeats. Leucine-rich repeats (LRRs) are motifs involved in protein-ligand interactions, found in numerous proteins with varied structures, locations, and functions in different organisms, including the innate immune system and cell adhesion (Kobe & Kajava, 2001; Nürnberger et al., 2004). Even though the specific function of *LRRC20* is not yet well understood, considering the multiple roles of LRRs-containing proteins, its increased expression may indicate an effort by the skin cells to strengthen intercellular connections or participate in pathways that manage inflammation and tissue repair.

Previous studies showed that the overexpression of *TMPRSS11D* significantly enhanced the *NOX2* and intracellular ROS level in polymorphonucleoid myeloid-derived suppressor cells (PMN-MDSCs), and observed an increase of UPR proteins level, suggesting that TMPRSS11D induces ER stress in PMN-MDSCs. Moreover, it has been proposed that TMPRSS11D could be co-localized with ER or mitochondria (Feng et al., 2023). In Darier disease the upregulation of *TMPRSS11D* may exacerbate ER stress and ROS production, suggesting a further keratinocytes destabilization, inflammation promotion, cell damage, and contribution to the characteristic DD skin lesions.

SLC7A1 is a cationic amino acid transporter of the SLC7 family, which are essential metabolic regulators in immune cells. In a previous study, *SLC7A1* expression has been found increased in CD8+ T cells as well as in naïve and memory CD4+ T cells in order to supply adequate arginine

needed for T cell function (Werner et al., 2016). In the context of Darier disease, elevated *SLC7A1* expression could contribute to heightened immune cell activity in response to chronic skin inflammation or barrier dysfunction.

N-acetylgalactosaminyltransferase 5 (*GALNT5*) gene encodes a member of the UDP-N-α-D galactosamine-polypeptide N-acetylgalactosaminyltransferases family (ppGalNAc-Ts, GalNAc-Ts or GALNTs), responsible for the initial synthesis of α-GalNAc1,3-O-Ser/Thr, or Tn antigen, at the beginning of mucin type O-linked glycosylation (Brooks et al., 2002). Although there is no direct evidence linking *GALNT5* to Darier disease, abnormalities in glycosylation could affect the stability and function of surface proteins in skin cells, potentially making them more vulnerable to injury or stress.

Among top 10 most significant genes identified in this study, two resulted to be downregulated in patients compared to controls, including *UGT3A2.*

UGT3A2 is involved in epidermal homeostasis maintenance (Lefèvre-Utile et al., 2022) and its downregulation could be associated to the impaired skin barrier. Indeed, its reduced expression has also been reported in psoriasis and eczema (Quaranta et al., 2014).

In order to compare gene expression across samples, gene counts were employed to generate a Z-score heatmap with differentially expressed genes in rows and samples in columns. As shown in **Figure 36 D**, the Z-score ranges from -3 up to +3 standard deviations, and differential gene expression is evident with predominantly positive Z-scores in the controls group and negative for patient samples (upper section) and vice versa in the lower part of the graph. A negative Z-score for a gene in a sample indicates that this value falls below the mean of the values for this gene across all samples by a number of standard deviations corresponding to the magnitude of the Z-score. If all counts are the same value (typically 0), the standard deviation is 0, making it impossible to calculate a Z-score for these genes and there is no differential expression.

To identify the most differentially expressed genes, annotated normalized counts were filtered by a p-value (adjusted for multiple testing with the Benjamini-Hochberg procedure for the Wald statistic) below 0.05 between patients and controls. Results revealed 3797 genes (5.97%) with a significant gene expression difference between the two groups. Subsequently, the

resulting data were further filtered to select genes based on their fold change (FC>2 or FC<0.5), obtaining 2079 genes representing 54.75% of the significantly differentially expressed ones. Among them 1070 genes resulted upregulated (log_2FC>1 (FC>2) and adjusted p-value <0.05) and 1009 downregulated (log_2FC<-1 (FC<0.5) and adjusted p-value <0.05).

Differentially expressed genes included different factors involved in keratinocyte differentiation and cornification pathways (*SPRR2A*, *SPRR2B*, *SPRR2C*, *SPRR2F*, *SPRR3*, *LCE3A*, *LCE3C*, *LCE3D*, *LCE3E*, *SPINK6*, *MMP1*, *HRNR*, *KRT6A*, *KRT6C*, *PI3*, *KLK13*, *KRT13*), immune cytokines and related receptors (*IL36A*, *IL36G*, *IL19*, *IL24*, *IL17A*, *IL17F*), and chemokines (*CXCL8*, *CCL7*, *CXCL1*, *CXCL5*), and antimicrobial protection (*DEFB4A*, *DEFB4B*, *S100A7A*, *S100A12*, *S100A9*, *S100A7*, *S100A8*, *LTF*). We also identified genes associated with antiviral response (*APOBEC3A*, *OASL*) and involved in cell cycle regulation (*FOSL1*, *CCNB1*, *HMGA2*), among others (**Figure 36 E**). Metabolic-associated genes resulted to be downregulated in DD skin, including *FBP2*, *DDC*, *UGT3A2*, *HGD*, *UPB1* and *APOA1*, compared to the controls' group.

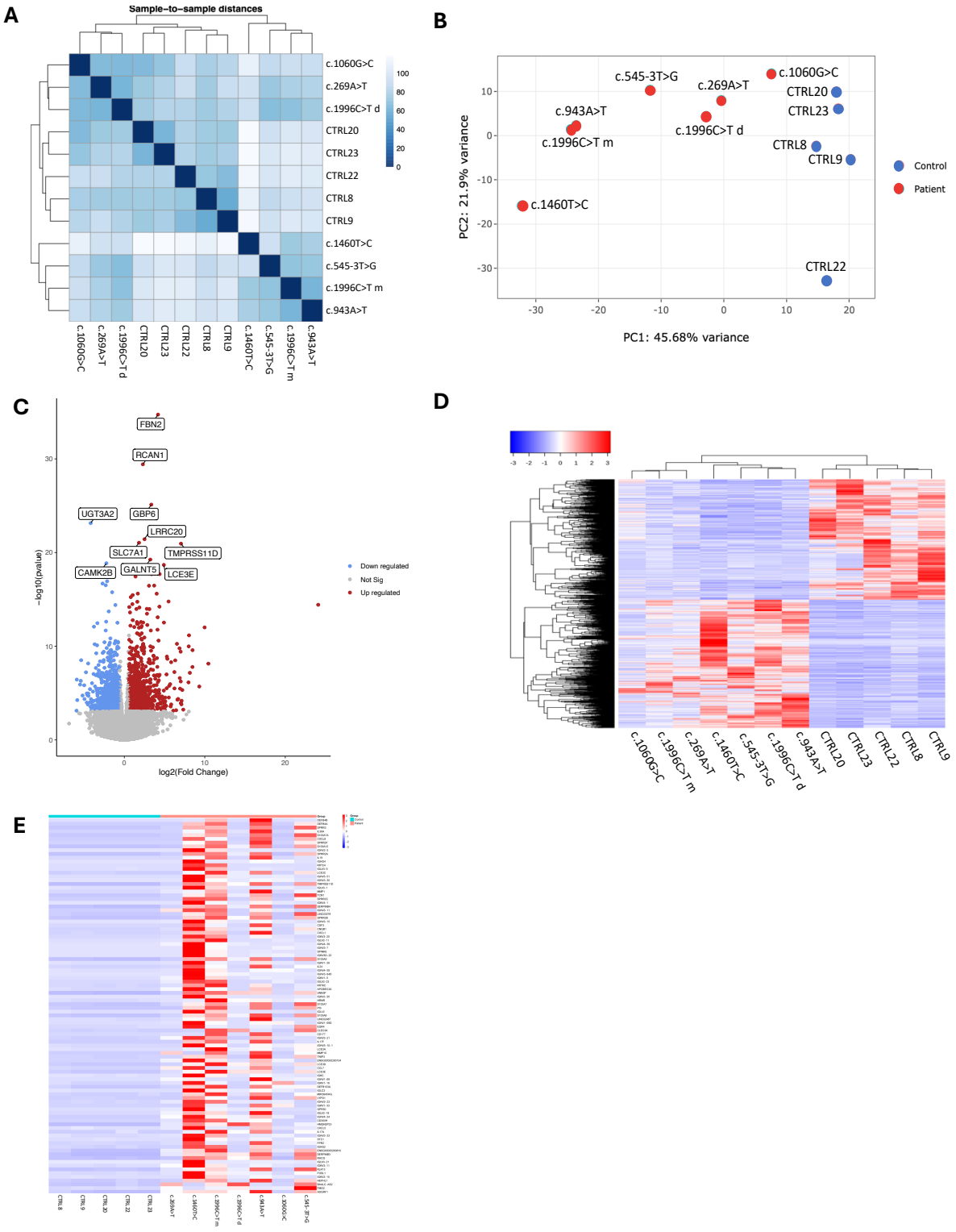


Figure 36. Differential Gene Expression analysis. A) Heatmap of the sample-to-sample distances. The color denotes the distance between the samples. Dark blue represents a shorter distance, meaning closer subjects, on the basis of the normalized counts. **B**) Principal component analysis displaying all 12 samples along PC1 and PC2, which describes 45.68% and 21.9% of the variability, respectively, within the expression data set. **C**) Volcano plot of differentially expressed genes. The log2 fold change of each gene is represented on the x-axis and the negative log10 of the p-value is on the y-axis. Only significant genes (FDR <0.01 and FC of 1.5) are colored. Red dots represent up-regulated genes and blue dots the

down-regulated ones. Top 10 most significant genes are labeled. **D**) Z-scores for the most differentially expressed genes in patients and controls. **E**) Z-scores heatmap of the top 100 up-regulated DEGs.

5.7.1.2 Gene Ontology analysis and KEGG pathways

Functional enrichment analyses were performed to determine whether the differentially expressed genes are enriched for transcripts belonging to broader or more specific categories and identify potentially impacted biological functions.

Gene Ontology analysis for cellular component, biological processes, and molecular function categories was performed using the GOseq software, which takes into account transcripts length bias. Differentially expressed genes with adjusted p-value < 0.05 were used and we obtained a category list ranked by Wallenius method.

Top 10 over-represented GO terms by p-value (adjusted for multiple testing with the Benjamini-Hochberg procedure) for each category were represented with bubble plots (**Figure 37 A**). Biological processes categories for most up- and down-regulated differentially expressed genes are shown in **Figure 37 B** and **37 C**, respectively.

The GOseq tool was also employed to identify KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways. The analysis resulted in a list of 229 KEGG terms, of whom 4 pathways (1.75%) were over-represented and only 1 (0.44%) was under-represented (Olfactory transduction pathway). Over-represented pathways included cell cycle, peroxisome proliferator-activated receptor (PPAR) signaling, and cytokines-cytokines receptor interaction. Differentially expressed genes in the pathway are indicated in colored boxes (green is for downregulated genes, with log2FC<0, and red for upregulated genes, with log2FC>0) (**Figure 37 D**).

Genes resulted to be up-regulated in the cell cycle pathway included cyclins and cyclin-dependent kinases (CKDs), a group of serine/threonine kinases fundamental for cell cycle regulation forming complexes for their stabilization and activation during the different phases (Malumbres, 2014). The activation of these complexes is induced by mitogenic signals and suppressed by cell cycle checkpoints in response to DNA damage (Otto & Sicinski, 2017). In a study on psoriasis, a condition characterized by keratinocyte hyperproliferation and inflammation, CDK1 levels were found to be elevated in lesions compared to the skin of healthy subjects and it was correlated with keratinocytes proliferation (Ni & Lai, 2020; Xue et al., 2022).

The mammalian polo-like kinase 1 (PLK1) is a serine/threonine kinase, which plays an essential role in mitosis and cytokinesis modulation, and its dysregulation leads to cell cycle aberrations that often stimulate cell proliferation and cell size increase (Mundt et al., 1997; Cholewa et al., 2013). Another upregulated gene was *CDC25A* which has been previously reported to promote cell proliferation by interacting with cyclins and cyclin-dependent kinases, facilitating progression through both the G1/S and G2/M phases of the cell cycle (T. Shen & Huang, 2012). This evidence could explain keratinocytes hyperproliferation in the Darier disease epidermis. Among downregulated genes, we found the stem cell factor (*SCF*) involved in wound repair through the production of growth factors and mast cells recruitment (Oriss et al., 2014; Z. Wang et al., 2020). Downregulated *SCF* expression can impair wound healing, leading to slower recovery and increased risk of infection, a condition usually observed in DD patients. Another downregulated gene encodes the growth arrest and DNA damage-inducible protein 45A (GADD45A), a member of the DNA damage-inducible 45 family, which plays a key role in cellular responses to DNA damage. It contributes to DNA repair, genomic stability maintenance, and can induce cell cycle arrest under physiological or environmental stress conditions (Salvador et al., 2013). Its reduced expression could impair cell cycle control by preventing cell cycle arrest under stress conditions, leading to accelerated proliferation and altered differentiation of keratinocytes in Darier disease.

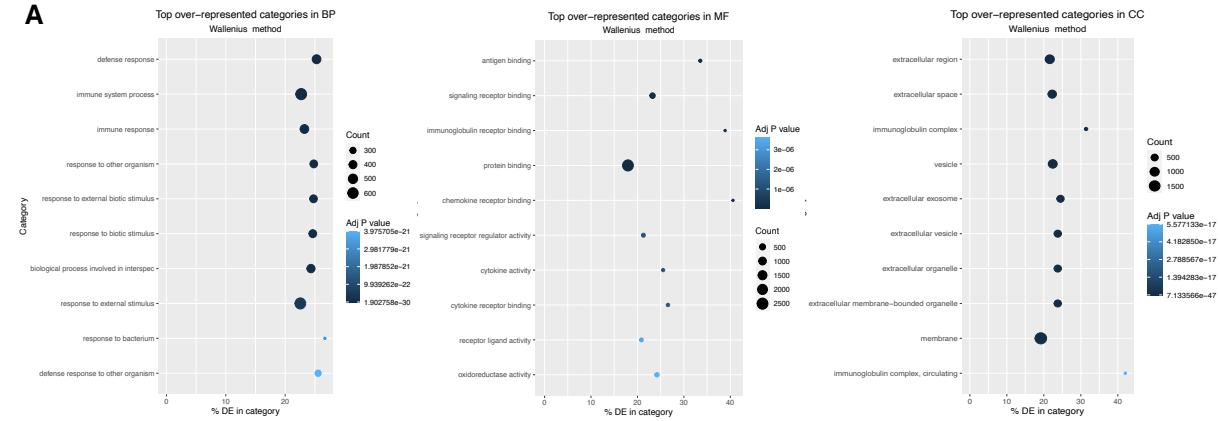
Another over-represented KEGG pathway was the peroxisome proliferator-activated receptor (PPAR) one, involved in critical biological processes, such as energy homeostasis and inflammation (Varga et al., 2011; Vázquez-Carrera & Wahli, 2022). The fatty acid-binding gene FABP was overexpressed in patients' samples; in the skin, it promotes epidermal proliferation by activating PPAR β/δ signaling (Kannan-Thulasiraman et al., 2010), which has been found overexpressed in this study. Additionally, also in other dermatologic conditions, including atopic dermatitis and psoriasis, characterized by inflammation, epidermal hyperplasia, altered permeability barrier function, and abnormal keratinocytes differentiation, an increase of *PPARß/δ* and a decreased *PPARα* and *PPARγ* expression were observed (Westergaard et al., 2003; Plager et al., 2007). FABP expression in keratinocytes contributes to skin water permeability barrier maintenance and differentiation by enhancing the expression of Keratin 1 mediated by fatty acid (Owada et al., 2002; Ogawa et al., 2011). Additionally, previous studies showed that epidermal FABP is also expressed in immune cells, where it plays a role in

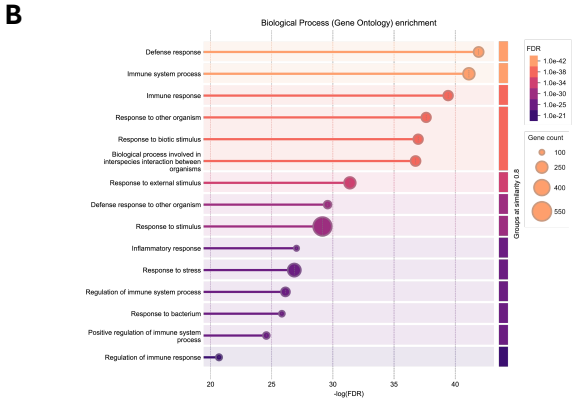
regulating T cell differentiation and macrophage pro-inflammatory functions (Moore et al., 2015; Y. Zhang et al., 2015). In DD patients FABP overexpression could affect lipid metabolism and skin barrier function and cause excessive inflammatory responses worsening the disease's clinical features. The fatty acid transporter gene (FATP) resulted downregulated in patients' skin. In a previous study, *FATP4*, a member of this transporters family, has been found mutated in Ichthyosis Prematurity Syndrome, a condition characterized by hyperkeratosis, acanthosis, and dermal inflammation (Klar et al., 2009), clinical features observed also in Darier disease. Although these genes mutations were not evaluated in this study, considering their function in the epidermis, their alterations could contribute to the DD symptoms manifestation.

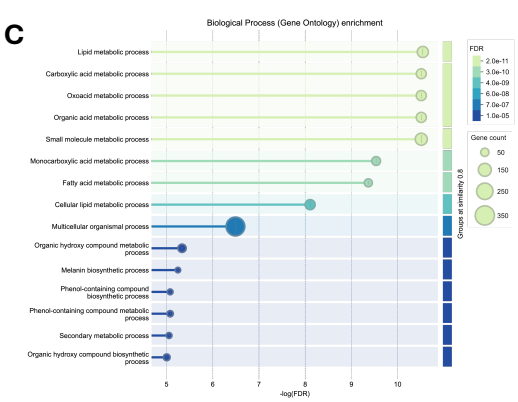
The cytokine-cytokine receptor interaction pathway was also over-represented in Darier disease patients. This is highlighted by the overexpression of genes encoding interleukins, which play a key role in immune responses and inflammation. The upregulation of these interleukins may contribute to the skin inflammation observed in Darier disease, highlighting a significant aspect of the disease's pathophysiology.

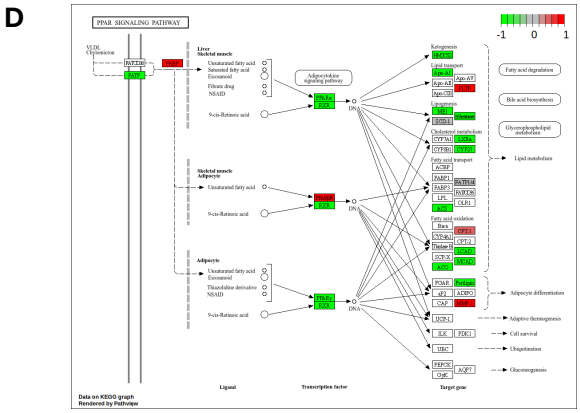
Our observations are consistent with the evidence that skin inflammation typically occurs when skin cells are activated by external or internal stimuli, such as chemicals, UV radiation, pathogen- and damage-associated molecular patterns. Depending on the nature of the stimulus, various signaling pathways, including PPAR, chemokine, and cytokine ones, are activated to trigger innate and adaptive immune responses, as outlined by Schwingen and colleagues (Schwingen et al., 2020). This aligns with the evidence that external stimuli are known to exacerbate Darier's disease.

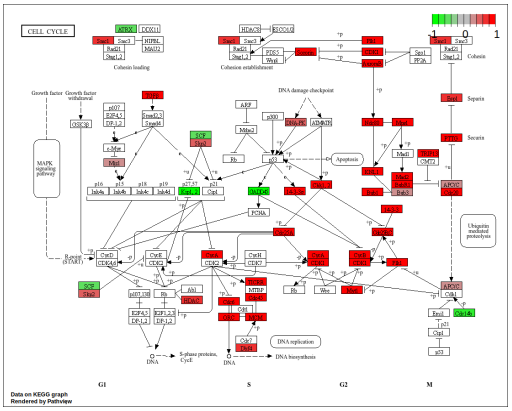
GO enrichment analysis for biological processes revealed that upregulated genes in patients' skin are involved in pathways including defense response, immunologic mechanisms, and response to external stimuli. A DEG network analysis was performed and visualized through the STRING tool (**Figure 37 E**) and a Manhattan plot generated using the list of statistically significant differentially expressed genes (adjusted p-value <0.05) through g:Profiler. We highlighted 15 categories associated to biological processes category, 8 KEGG terms and 2 HP (human phenotype). In addition to categories observed in GOseq results and reported in the list below the Manhattan plot with relative p-value, abnormalities related to skin morphology and physiology were detected (**Figure 37 F**).

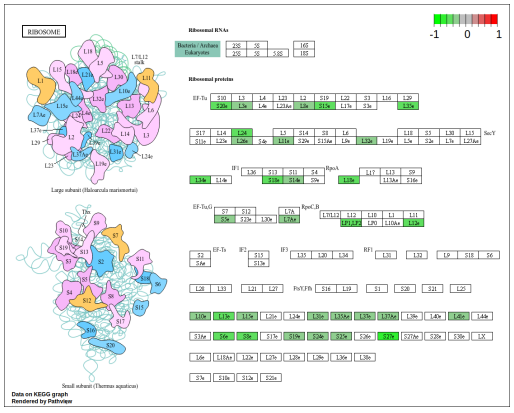

A
Top over−represented categories in BP
Wallenius method
defense response
immune system process
immune response
response to other organism
response to external biotic stimulus
response to biotic stimulus
biological process involved in interspec
response to external stimulus
response to bacterium
defense response to other organism
Category
% DE in category
Count
300
400
500
600
Adj P value
3.975705e−21
2.981779e−21
1.987852e−21
9.939262e−22
1.902758e−30
Top over−represented categories in MF
Wallenius method
antigen binding
signaling receptor binding
immunoglobulin receptor binding
protein binding
chemokine receptor binding
signaling receptor regulator activity
cytokine activity
cytokine receptor binding
receptor ligand activity
oxidoreductase activity
% DE in category
Adj P value
3e−06
2e−06
1e−06
Count
500
1000
1500
2000
2500
Top over−represented categories in CC
Wallenius method
extracellular region
extracellular space
immunoglobulin complex
vesicle
extracellular exosome
extracellular vesicle
extracellular organelle
extracellular membrane−bounded organelle
membrane
immunoglobulin complex, circulating
% DE in category
Count
500
1000
1500
Adj P value
5.577133e−17
4.182850e−17
2.788567e−17
1.394283e−17
7.133566e−47

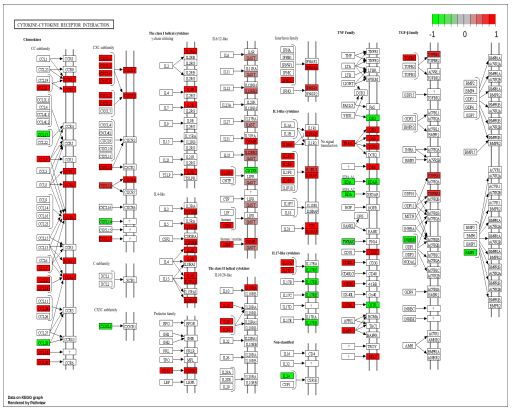

B
Biological Process (Gene Ontology) enrichment
Defense response
Immune system process
Immune response
Response to other organism
Response to biotic stimulus
Biological process involved in interspecies interaction between organisms
Response to external stimulus
Defense response to other organism
Response to stimulus
Inflammatory response
Response to stress
Regulation of immune system process
Response to bacterium
Positive regulation of immune system process
Regulation of immune response
-log(FDR)
FDR
1.0e-42
1.0e-38
1.0e-34
1.0e-30
1.0e-25
1.0e-21
Gene count
100
250
400
550
Groups at similarity 0.8


C
Biological Process (Gene Ontology) enrichment
Lipid metabolic process
Carboxylic acid metabolic process
Oxoacid metabolic process
Organic acid metabolic process
Small molecule metabolic process
Monocarboxylic acid metabolic process
Fatty acid metabolic process
Cellular lipid metabolic process
Multicellular organismal process
Organic hydroxy compound metabolic process
Melanin biosynthetic process
Phenol-containing compound biosynthetic process
Phenol-containing compound metabolic process
Secondary metabolic process
Organic hydroxy compound biosynthetic process
-log(FDR)
FDR
2.0e-11
3.0e-10
4.0e-09
6.0e-08
7.0e-07
1.0e-05
Gene count
50
150
250
350
Groups at similarity 0.6

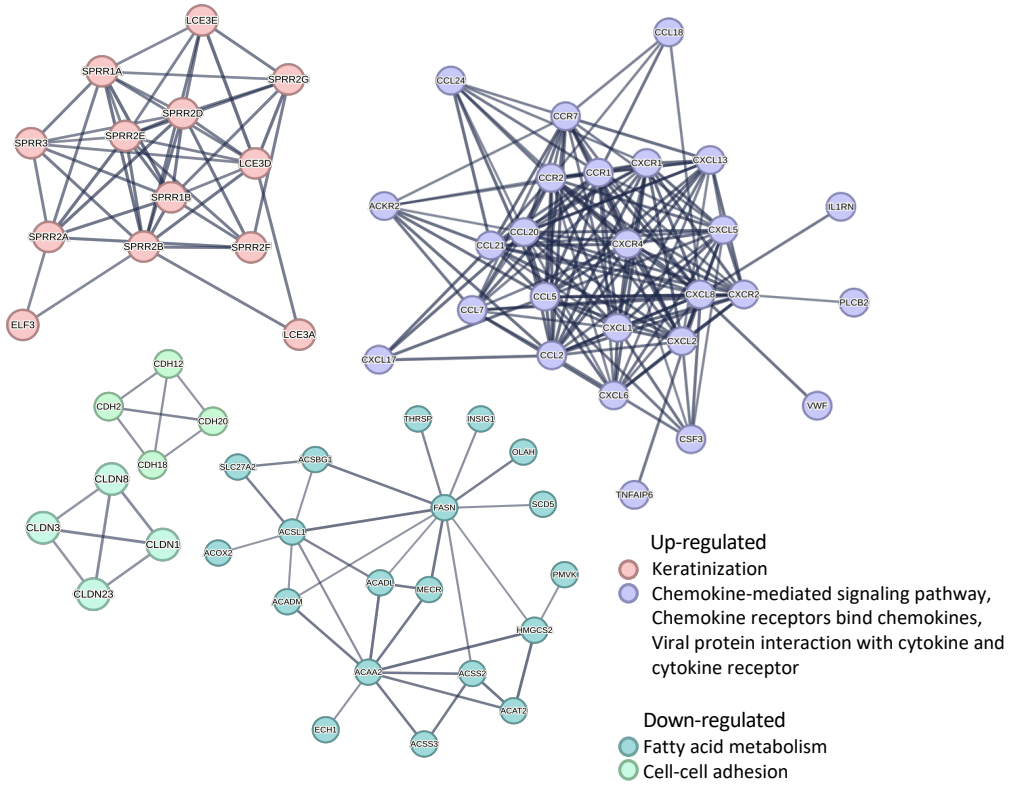

D
PPAR SIGNALING PATHWAY
Data on KEGG graph
Rendered by Pathview


CELL CYCLE
Data on KEGG graph
Rendered by Pathview

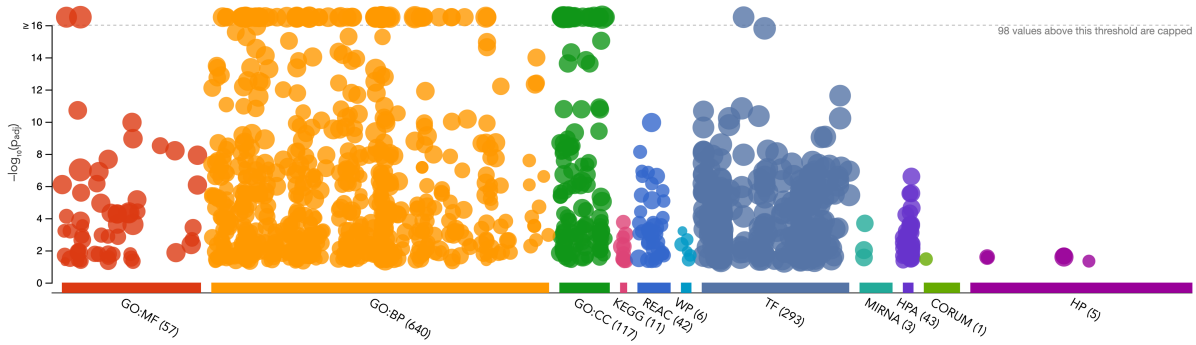

RIBOSOME
Data on KEGG graph
Rendered by Pathview


CYTOKINE-CYTOKINE RECEPTOR INTERACTION

E



F



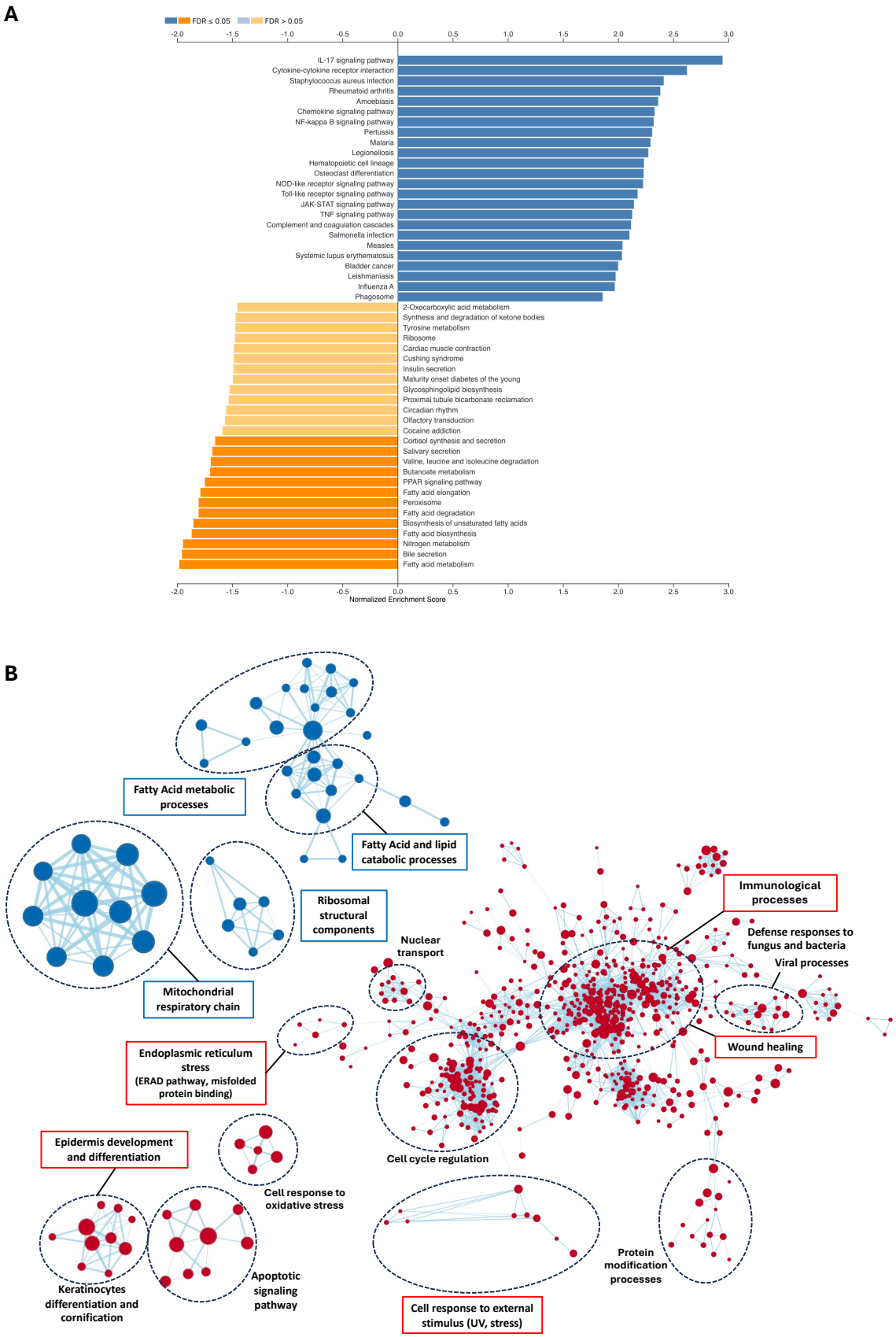
ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:BP	GO:0050896	response to stimulus	6.982×10^{-65}
2	GO:BP	GO:0010033	response to organic substance	2.469×10^{-48}
3	GO:BP	GO:0032501	multicellular organismal process	4.967×10^{-47}
4	GO:BP	GO:0048518	positive regulation of biological process	8.554×10^{-47}
5	GO:BP	GO:0032502	developmental process	5.041×10^{-44}
6	GO:BP	GO:0048856	anatomical structure development	2.380×10^{-43}
7	GO:BP	GO:0051716	cellular response to stimulus	4.195×10^{-40}
8	GO:BP	GO:0070887	cellular response to chemical stimulus	2.656×10^{-39}
9	GO:BP	GO:0048522	positive regulation of cellular process	2.074×10^{-38}
10	GO:BP	GO:0006950	response to stress	6.879×10^{-37}
11	GO:BP	GO:0002376	immune system process	2.108×10^{-36}
12	GO:BP	GO:0007275	multicellular organism development	1.020×10^{-34}
13	GO:BP	GO:0071310	cellular response to organic substance	1.162×10^{-34}
14	GO:BP	GO:0009605	response to external stimulus	2.136×10^{-34}
15	GO:BP	GO:1901564	organonitrogen compound metabolic process	2.665×10^{-34}
16	KEGG	KEGG:04110	Cell cycle	1.768×10^{-4}
17	KEGG	KEGG:03320	PPAR signaling pathway	1.471×10^{-3}
18	KEGG	KEGG:04061	Viral protein interaction with cytokine and cyto...	5.172×10^{-3}
19	KEGG	KEGG:01100	Metabolic pathways	5.692×10^{-3}
20	KEGG	KEGG:04141	Protein processing in endoplasmic reticulum	1.611×10^{-2}
21	KEGG	KEGG:04060	Cytokine-cytokine receptor interaction	3.362×10^{-2}
22	KEGG	KEGG:01212	Fatty acid metabolism	3.958×10^{-2}
23	KEGG	KEGG:04657	IL-17 signaling pathway	4.699×10^{-2}
24	HP	HP:0011122	Abnormality of skin physiology	2.198×10^{-2}
25	HP	HP:0011121	Abnormal skin morphology	2.567×10^{-2}
26	HP	HP:0025092	Epidermal acanthosis	4.622×10^{-2}

Figure 37. Gene Ontology, KEGG, and network analysis. A) Bubble plot of Gene Ontology (GO) function enrichment analysis. BP: biological processes; CC: cellular components; MF: molecular function. **B**) Gene ontology for the Biological process category of most up-regulated ($log_2FC>1$ (FC>2), adj p-value < 0.05) and **C**) downregulated ($log_2FC<-1$ (FC<0.5), adj p-value < 0.05) differentially expressed genes. **D**) KEGG overrepresented pathways highlighting upregulated genes (red) and downregulated ones (green) in DD skin compared to control samples. **E**) Network analysis of some DD representative gene clusters. The gene network of differentially expressed DEGs between patients and controls and gene clusters were identified with STRING (clustering method: MCL, FDR 0.01, confidence 0.8). **F**) Manhattan plot displaying functional enrichment analysis of differentially expressed genes (adj p-value < 0.05) using g:Profiler. Significant categories were identified for Gene Ontology Biological Process (GOBP), KEGG pathways, and Human Phenotype Ontology (HP) terms, among the others, with a Benjamini-Hochberg FDR threshold of 0.05. The table highlights selected enriched categories across these databases.

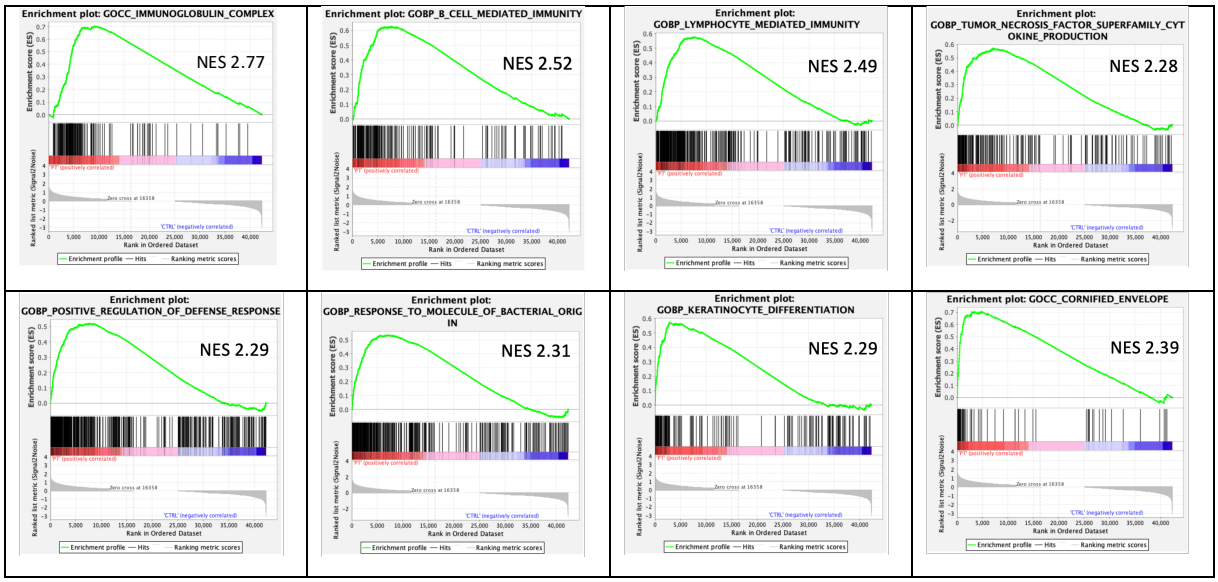
5.7.1.3 Gene Set Enrichment Analysis

A Gene Set Enrichment Analysis (GSEA) was performed to identify pathways that are enriched in our sample cohort compared to controls.

GSEA revealed consistent positive enrichment of immunological, defense response to external stimuli, including radiations, stress and bacteria, apoptosis, ER stress, cell adhesion regulation, epidermal development and differentiation pathways, while transcripts associated with mitochondrial (e.g. respiratory chain and oxidative phosphorylation), lipids and fatty acid metabolism, and ribosomal components showed a decrease in enrichment scores in patients compared to control subjects. Pathway enrichment analysis results were visualized and interpreted in Cytoscape and Webgestalt tools (**Figure 38 A-D**).


A
FDR ≤ 0.05
FDR > 0.05
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
IL-17 signaling pathway
Cytokine-cytokine receptor interaction
Staphylococcus aureus infection
Rheumatoid arthritis
Amoebiasis
Chemokine signaling pathway
NF-kappa B signaling pathway
Pertussis
Malaria
Legionellosis
Hematopoietic cell lineage
Osteoclast differentiation
NOD-like receptor signaling pathway
Toll-like receptor signaling pathway
JAK-STAT signaling pathway
TNF signaling pathway
Complement and coagulation cascades
Salmonella infection
Measles
Systemic lupus erythematosus
Bladder cancer
Leishmaniasis
Influenza A
Phagosome
2-Oxocarboxylic acid metabolism
Synthesis and degradation of ketone bodies
Tyrosine metabolism
Ribosome
Cardiac muscle contraction
Cushing syndrome
Insulin secretion
Maturity onset diabetes of the young
Glycosphingolipid biosynthesis
Proximal tubule bicarbonate reclamation
Circadian rhythm
Olfactory transduction
Cocaine addiction
Cortisol synthesis and secretion
Salivary secretion
Valine, leucine and isoleucine degradation
Butanoate metabolism
PPAR signaling pathway
Fatty acid elongation
Peroxisome
Fatty acid degradation
Biosynthesis of unsaturated fatty acids
Fatty acid biosynthesis
Nitrogen metabolism
Bile secretion
Fatty acid metabolism
Normalized Enrichment Score
B
Fatty Acid metabolic processes
Fatty Acid and lipid catabolic processes
Ribosomal structural components
Mitochondrial respiratory chain
Nuclear transport
Immunological processes
Defense responses to fungus and bacteria
Viral processes
Wound healing
Endoplasmic reticulum stress (ERAD pathway, misfolded protein binding)
Epidermis development and differentiation
Cell response to oxidative stress
Keratinocytes differentiation and cornification
Apoptotic signaling pathway
Cell cycle regulation
Protein modification processes
Cell response to external stimulus (UV, stress)

C



D

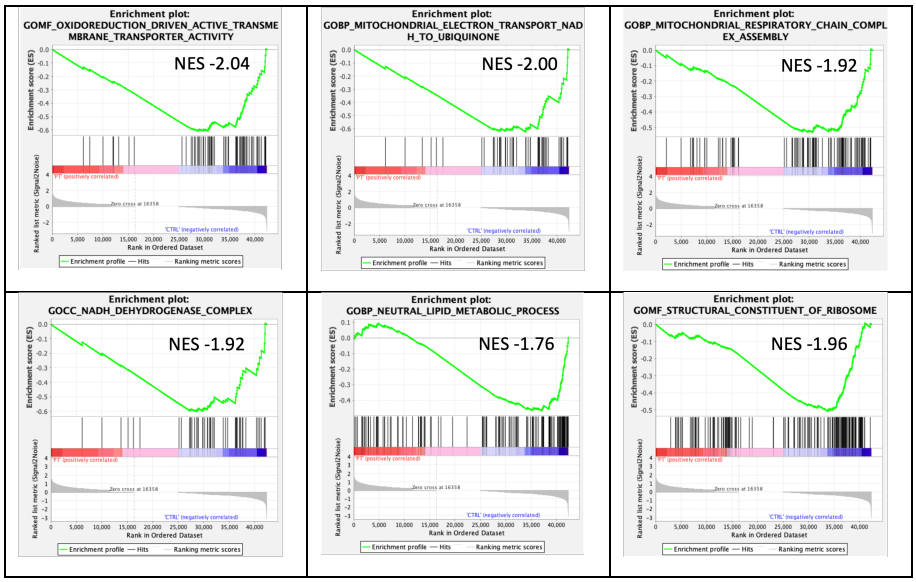


Figure 38. Gene Set Enrichment Analysis. A) Bar chart highlighting the most significantly enriched pathways from DEGs. Pathways are ranked by Normalized Enrichment Score (NES), while bar colors indicate the False Discovery Rate (FDR) values, with darker colors representing lower FDR values. **B**) Network analysis (Cytoscape enrichment map) of significant gene sets revealed by Gene Set Enrichment Analysis. Only significantly enriched terms were considered (FDR cutoff 0.01 for down-regulated or 0.1 for up-regulated gene sets). The node color is dependent on normalized enrichment score (NES) values: red nodes represents up-regulated gene sets, whereas blue nodes represents down-regulated ones. **C**) Gene set enrichment analysis (GSEA) plots displaying representative DD patients-enriched pathways related to immunological and defense responses and to epidermal differentiation. To the far left (red) the plot shows a correlation of the gene set with the patient phenotype and to the far right a correlation with the controls one. The vertical black lines indicate the position of each gene within the ranked gene list. The green curve represents the running sum of the enrichment score of

the GSEA. **D**) Gene set enrichment analysis (GSEA) plots displaying representative control-enriched pathways related to mitochondria, lipids and ribosomes.
ES (enrichment score), GOBP (Gene Ontology Biological Process), GOMF (Gene Ontology Molecular Function), GOCC (Gene Ontology Cellular Component).

In order to investigate specific genes that most strongly contribute to the detected enrichment signal of a pathway, a leading-edge analysis was performed. In particular, we focused our attention on the pathways that resulted enriched in downregulated genes (**Figure 39 A-E**). From the leading-edge analysis of mitochondria-related gene sets, including mitochondrial respiratory chain, membrane component, and oxidative phosphorylation, we identified 156 unique genes and selected the most downregulated ones with a log2(FC)<0 and p-value below 0.05 according to previous DEG analysis results. Among them, genes belonging to the mitochondrial encoded NADH dehydrogenase subunit family (*MT-NDs*), cytochrome c oxidase assembly factors (*COA5* and *COA8*), membrane proteins encoding genes (*TMEM223* and *IMMP2L*), and genes codifying for proteins involved in calcium homeostasis (*MICU3* and *SNCA*) were included (**Figure 39 E**).
Interestingly, to our knowledge the involvement of mitochondria in DD has been reported only in a recent study (Hunt et al., 2024) and could be relevant in the pathophysiology of this condition since the role of mitochondria has been reported also in other skin pathologies (Hunt et al., 2023) and the importance of contact sites between ER and mitochondria in Ca^{2+} signaling (Marchi et al., 2018).
Considering the multiple functions of fatty acids and lipids in the epidermis, including the generation of a proper barrier permeability (Uchida & Holleran, 2008) and the role of ER in lipid metabolism, the downregulation of their related pathways could contribute to skin barrier dysfunction and abnormal keratinization in DD and reflect the ER function and calcium homeostasis disruption.

Features observed in psoriasis, an autoimmune skin condition, including inflammation, abnormal keratinocytes differentiation, and epidermal hyperproliferation have been linked to T lymphocytes infiltration in the skin (Kuenzli & Saurat, 2003). Even though psoriasis and Darier are two different skin conditions, these characteristics are common. In our study, an enrichment in up-regulated genes belonging to immune-related pathways, including lymphocyte-induced ones, cytokine production, B- and T-cell mediated immunity, was

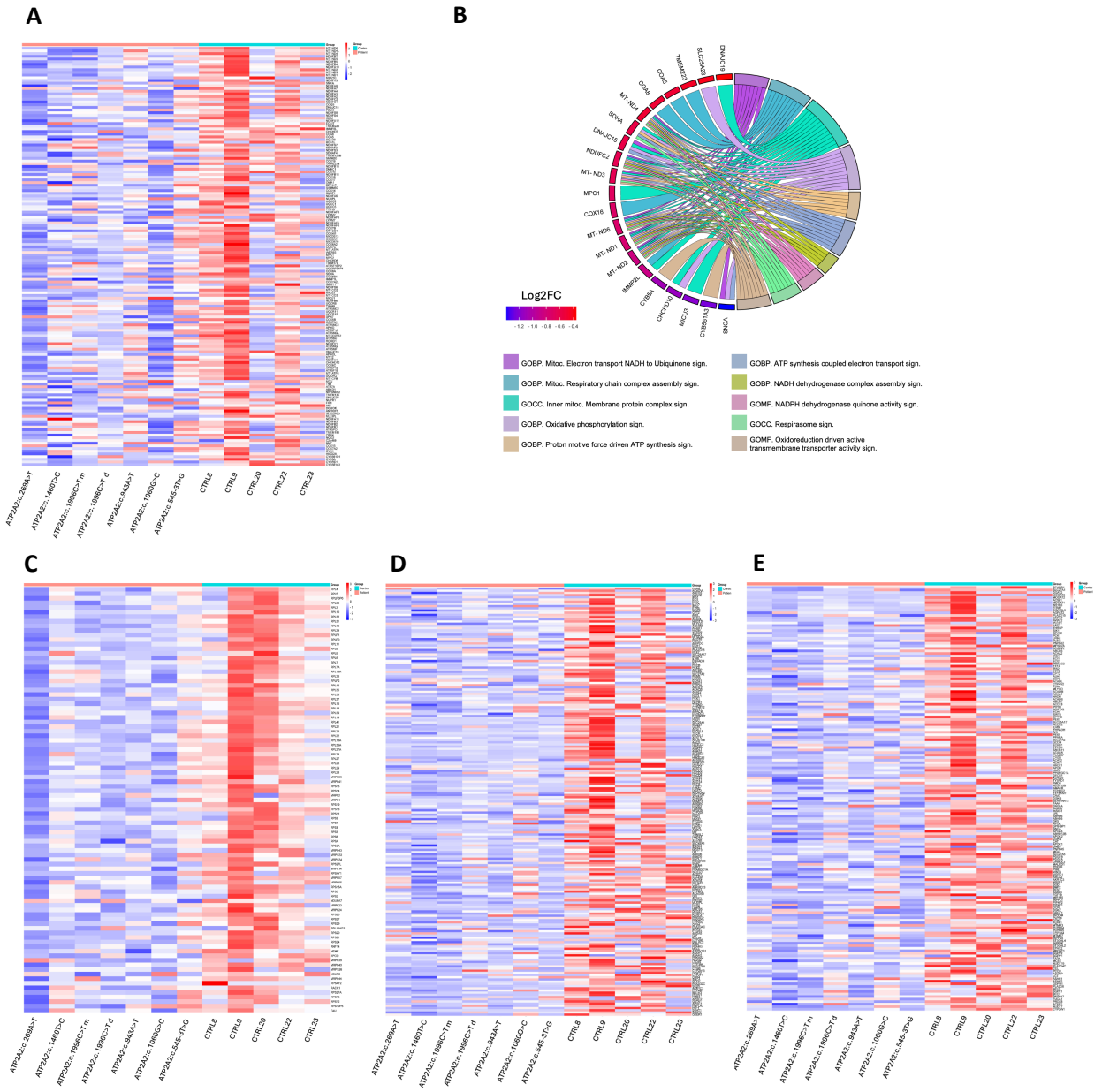
observed in the patients' group. In DD lesional skin, we found enhanced IL17 and T-helper 17 (Th17) pathways compared to control subjects. As mentioned before, the cytokine level increase was also confirmed by the overrepresented KEGG pathways where, among the others, *IL17A* and *F* were upregulated. These interleukins contribute to the immune activity against fungi and bacteria by promoting neutrophil recruitment, stimulating antimicrobial peptide production, and strengthening barrier function (Mills, 2023).

Additionally, pathways related to the reaction to extracellular stimuli, including defense response to fungus and bacteria, were upregulated in patients' skin.

GSEA analysis also showed enrichment in up-regulated pathways related to apoptosis and epidermal repair pathways with increased expression of genes associated with keratinocyte differentiation and cornification, among others (**Figure 38 C**).

A GSE leading edge analysis was also performed to identify genes overlapping between NOTCH and immunologically and inflammatory-related gene sets. In particular, from GSEA previous results, GOBP_Regulation_of_Notch_Signaling_Pathway_signal and 110 gene sets including a wide range of pathways related to both innate and adaptive immune responses, as well as various associated cellular mechanisms, such as lymphocyte proliferation, T cell activation, and cytokine production with an FDR q-value cutoff of 0.001 were selected. Shared upregulated genes included *PRKCI*, *TCIM*, *MFNG*, *STAT3*, *NOD2*, *ADAM10*, *IL6ST*, *SRC*, *MIR126*, and *GATA2*. ADAM10 is a disintegrin and metalloproteinase protein (Wolfsberg et al., 1995) which plays a pivotal role in the NOTCH signaling activation (Hartmann et al., 2002). It has been observed that different proinflammatory cytokines, including TGF-β, also found upregulated in our study DD patient, can activate ADAM10-mediated proteolysis of E-cadherin (Maretzky et al., 2008), affecting keratinocytes cell-cell adhesion and differentiation (Tinkle et al., 2004). The E-cadherins release from keratinocytes surface and the increase of their soluble level in serum was also observed in other inflammatory skin conditions, including eczema and psoriasis (Matsuyoshi et al., 1995). The ADAM10 modulation could represent a promising target for DD treatment. Another overexpressed gene belonging to the NOTCH gene set and shared with the majority of immune-inflammatory gene sets was *NOD2*. The receptor encoded by this gene detects a component of the bacterial wall peptidoglycan (Kawai & Akira, 2009) and mediates immune response by inducing proinflammatory pathways with the consequent increased levels of interleukins (IL6), chemokine ligands (CXC-chemokine), defensins and recruitment of

neutrophils and inflammatory monocytes, among others (Y.-G. Kim et al., 2011; Coulombe et al., 2012). SRC protein is a signal-transducing non-receptor protein kinase that plays different roles, including cell proliferation and migration (S. M. Thomas & Brugge, 1997; Irby & Yeatman, 2000). In normal human skin, *SRC* is expressed in the basal layer and diminishes during keratinocytes differentiation (Serrels et al., 2009). It was observed that SRC tyrosine kinases are activated in psoriasis with a hyperplasia grade correlated to their expression level (Ayli et al., 2008). Additionally, SRC family kinases (SFKs) physically interact with the intracellular NOTCH domain and phosphorylate its ankyrin region, by modulating NOTCH transcriptional activity. In particular, the increased expression of SFK reduces the interaction between NOTCH and MAML, impeding Notch target genes transcription (LaFoya et al., 2018). Considering the role of SCR in modulating the keratinocytes proliferation/differentiation balance, promoting proliferation and inhibiting differentiation, and the Notch signaling, it could contribute to DD skin phenotype (**Figure 39 F**).


A
B
C
D
E
Log2FC
GOBP. Mitoc. Electron transport NADH to Ubiquinone sign.
GOBP. Mitoc. Respiratory chain complex assembly sign.
GOCC. Inner mitoc. Membrane protein complex sign.
GOBP. Oxidative phosphorylation sign.
GOBP. Proton motive force driven ATP synthesis sign.
GOBP. ATP synthesis coupled electron transport sign.
GOBP. NADH dehydrogenase complex assembly sign.
GOMF. NADPH dehydrogenase quinone activity sign.
GOCC. Respirasome sign.
GOMF. Oxidoreduction driven active transmembrane transporter activity sign.

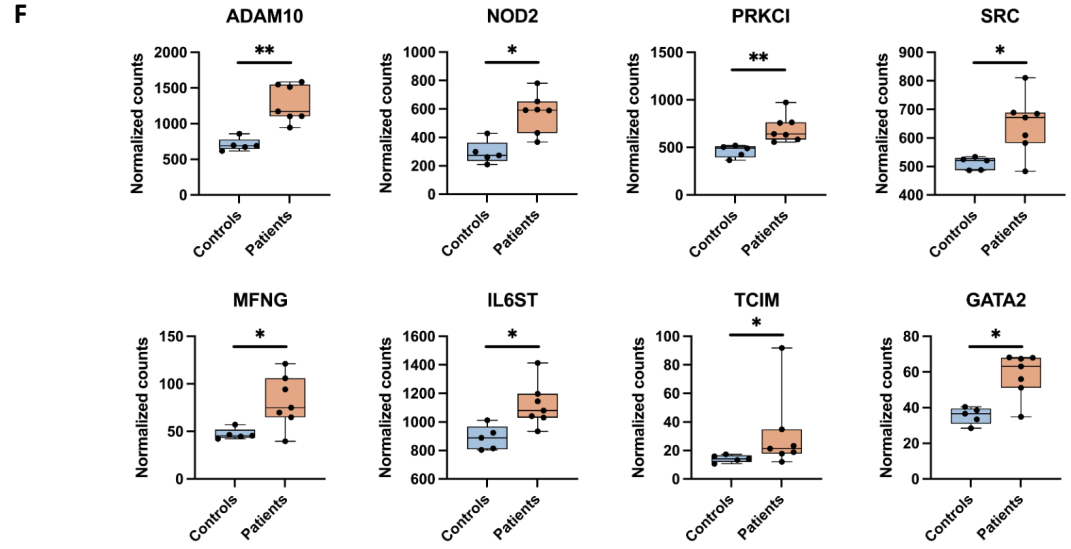

F
ADAM10
NOD2
PRKCI
SRC
MFNG
IL6ST
TCIM
GATA2
Normalized counts
Controls
Patients

Figure 39. GSEA Leading Edge analysis. **A**) Heatmap deriving from GSEA leading edge analysis relative to mitochondrial mechanisms gene sets. Data show an enrichment in downregulated genes in patients compared to control subjects. **B**) GO chord plot representing most significant DD patients' downregulated genes belonging to Gene Ontology categories related to mitochondrial signaling and oxidative phosphorylation, selected based on their Log2(FC) < 0 and adj p-value ≤ 0.05. **C**) GSEA leading edge-derived heatmaps showing the downregulation of genes related to ribosomal components, **D**) fatty acids, and **E**) lipid metabolism. **F**) Normalized counts of selected genes deriving from GSEA leading edge analysis between 110 immunological- and inflammatory-related gene sets and the GOBP regulation of Notch signaling pathway one.

5.8 Immunohistochemistry

In order to evaluate histological features and NOTCH1 target expression in DD patients deriving skin samples compared to control ones, we performed immunohistochemical analyses.

In patients' epidermis we observed the presence of typical DD histological signs, including "corps ronds" and "grains" (**Figure 40 A**).

HES1 and c-MYC protein levels were evaluated, and their reduction was observed in patients' epidermis by measuring their nuclear signal intensity using the QuPath software (**Figure 40 B-D**).

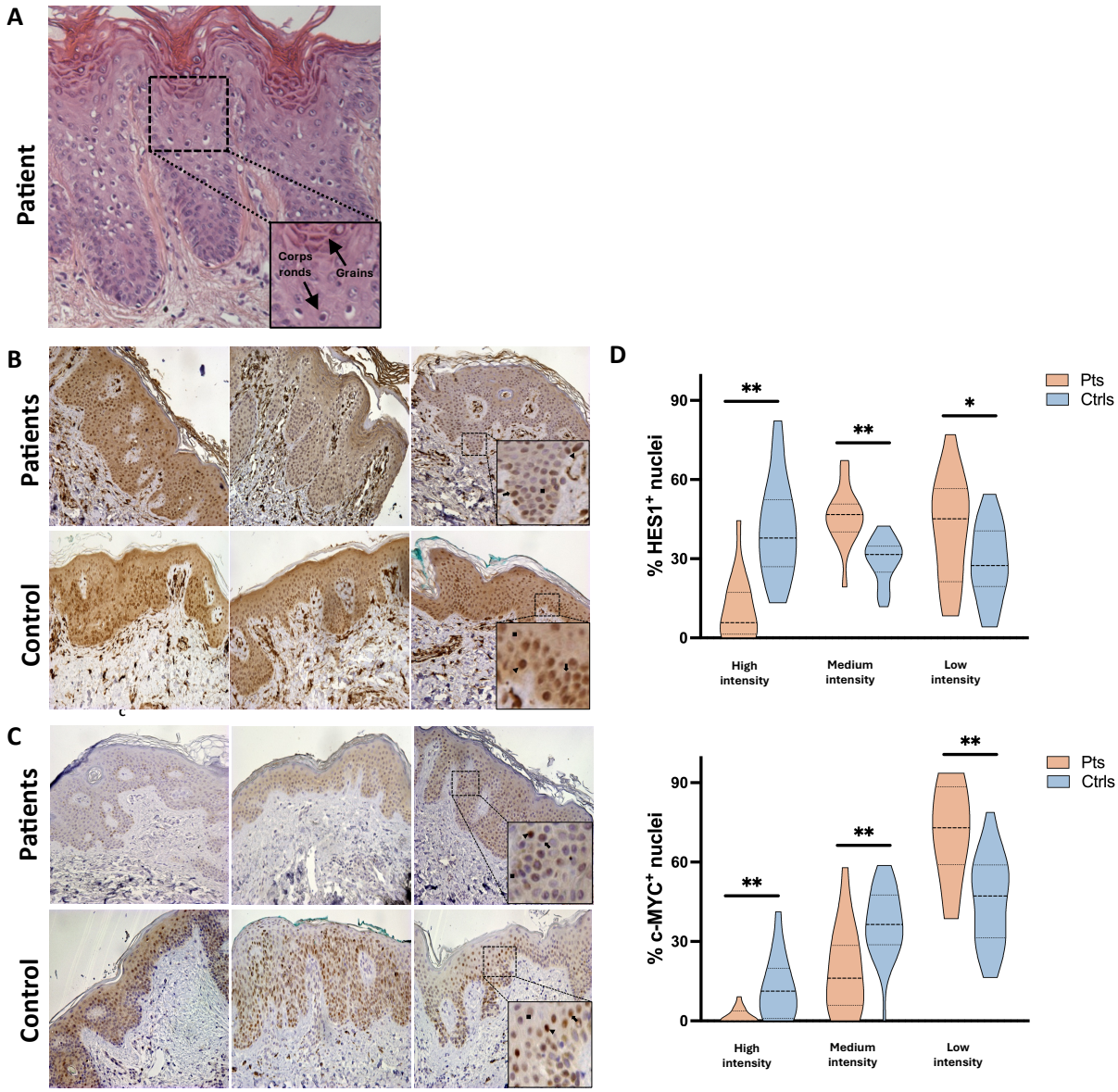
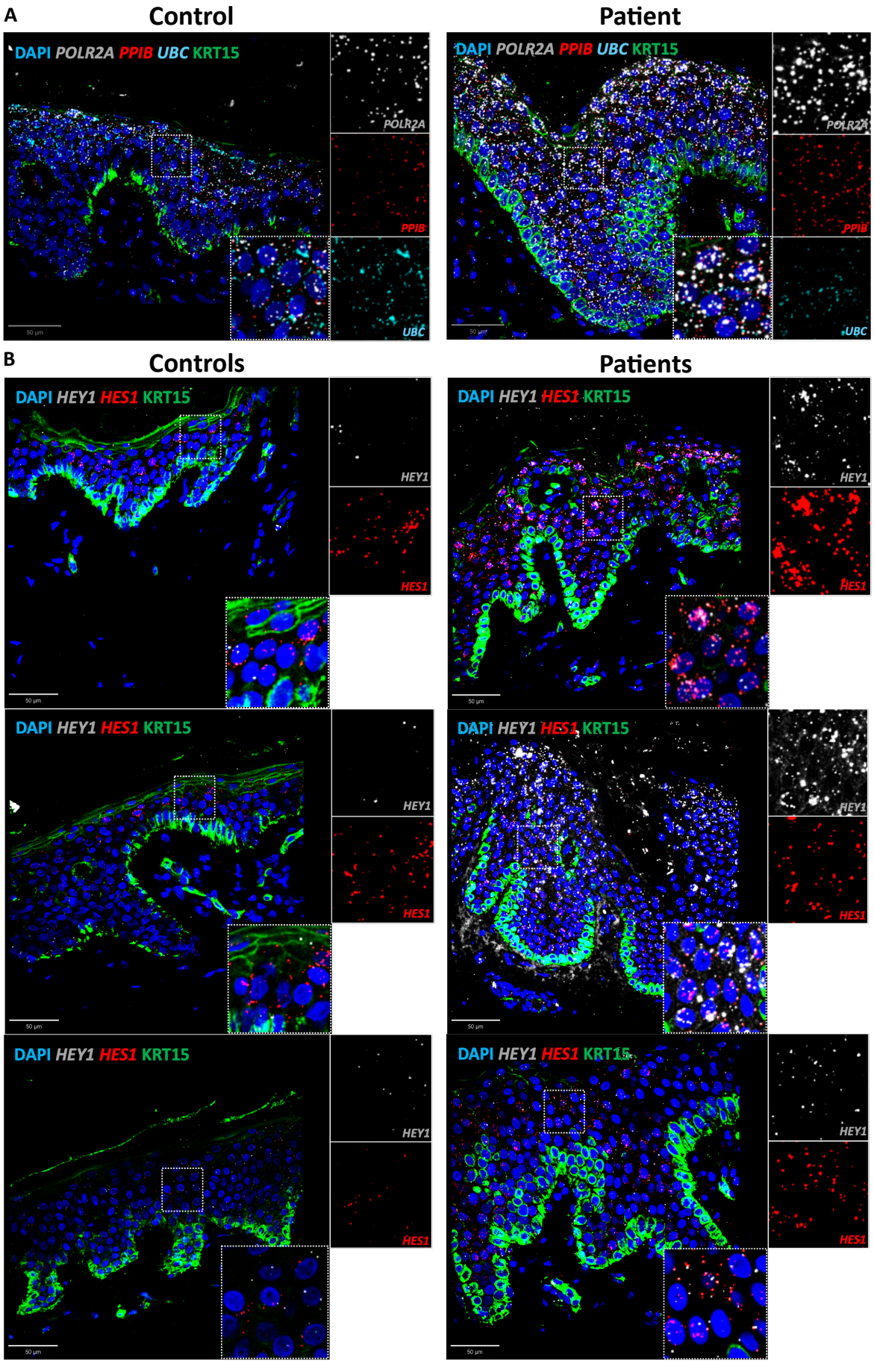


Figure 40. Histological analysis of skin biopsies. A) The typical DD hallmarks of grains and corps ronds identified in the H&E staining (arrows). Formalin-fixed, paraffin-embedded tissue sections were stained with anti-HES1 (**B**) and anti-cMYC (**C**) in 4 patients and 7 control cases. **D**) For nuclei counts, the QuPath software was employed considering 4 fields per image. The percentage of high-, medium- and low-intensity stained nuclei was calculated by relating them to the total cell number in each field. Mann-Whitney p value: *P<0.05; **P<0.005; not significant P>0.05. Microscope magnification was set at 10x. Figure legend: Triangle: high intensity; Arrow: medium intensity; Square: low intensity.

5.9 RNAscope

To evaluate *HES1* and *HEY1* expression in the presence of SERCA2 variants, 5 patients- and 3 controls-deriving skin sections were employed to perform RNAscope assay, a dual fluorescence in situ hybridization method (F. Wang et al., 2012). We used an anti-KRT15 antibody as a basal layer marker in order to distinguish it from the above suprabasal ones, where we expected to find our targets of interest. Experiments were performed in triplicate, and three images were acquired for each sample, with 60x magnification. After assessing the absence of signal in the negative control, images were analyzed using the QuPath software with a StarDist-based script for nuclei/cell segmentation and GraphPrism 9 for statistical analysis. Cells were distinguished in basal and suprabasal according to the KRT15 signal (green), and dots number for the two targets of interest were counted for all the cells. Interestingly, we observed a higher mRNA amount of housekeeping genes (positive control) (**Figure 41 A**) and target probes in patients compared to control subjects (**Figure 41 B**). For each target, cells were categorized based on the number of detected dots: High (≥5 dots), Dim (1–4 dots), and Negative (<1 dot). According to previous observations, *HES1* and *HEY1* were more expressed in the suprabasal layer, in both patients and controls. However, the percentage of HES1 High and Dim cells in patients was higher compared to controls both in the basal and suprabasal layer (**Figure 41 C**). The same tendency was also observed for HEY1. We also reported the mean dots number/cell for each target, confirming a statistically significant difference between controls and patients with a higher amount in these latter (**Figure 41 D**). Patients' group resulted to have a higher percentage of positive cells compared to the controls' one (**Figure 41 E**).


A
Control
Patient
DAPI POLR2A PPIB UBC KRT15
POLR2A
PPIB
UBC
B
Controls
Patients
DAPI HEY1 HES1 KRT15
HEY1
HES1

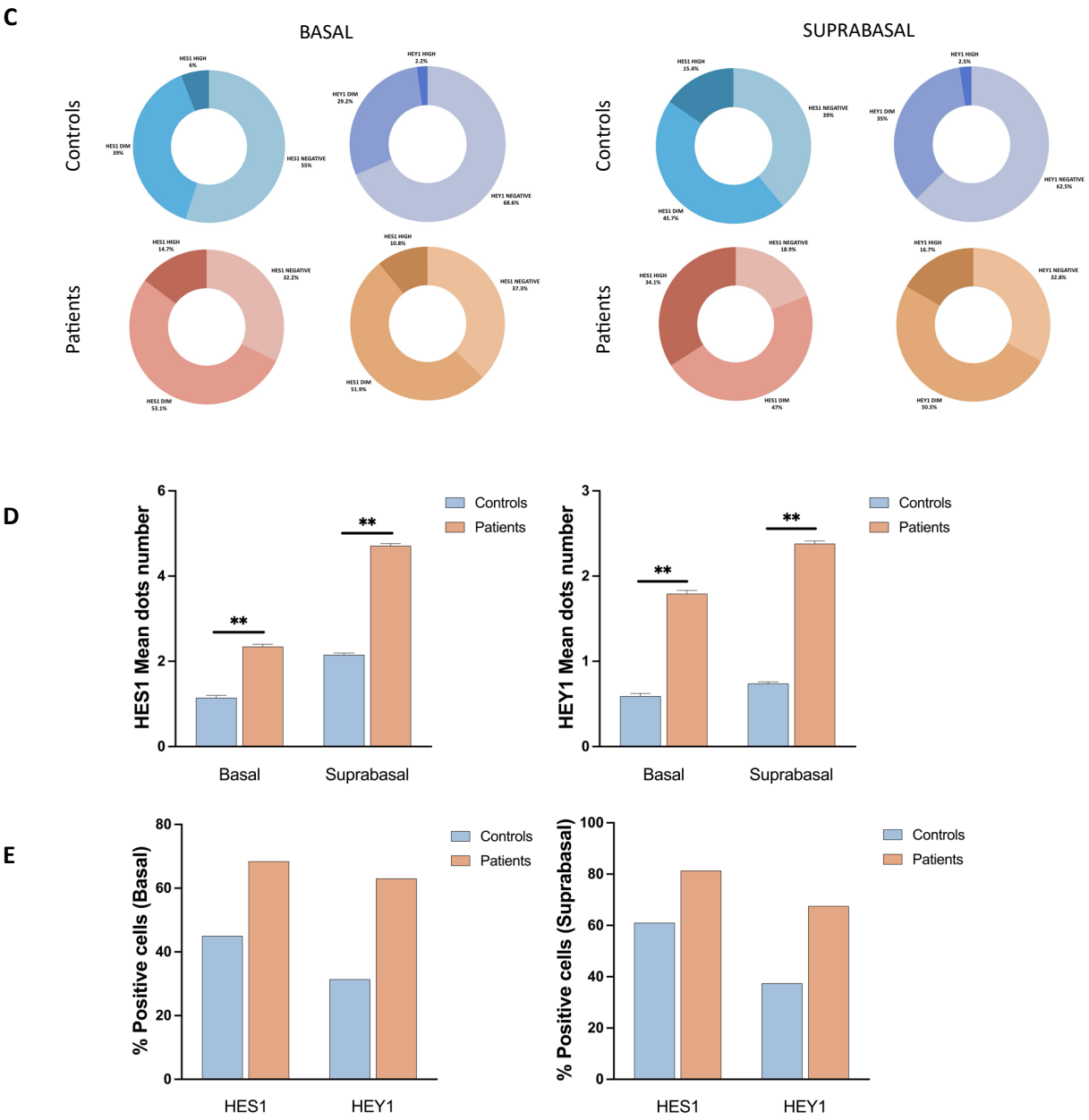


Figure 41. RNAscope assay. **A**) RNAscope staining using positive control probes for housekeeping genes (*POLR2A*, *PPIB*, *UBC*) shows an increased number of dots in the patient sample compared to controls. **B**) RNAscope results using probes for the target genes of interest (*HES1* and *HEY1*) revealed a higher number of dots in patient samples compared to controls. **C**) Percentage of *HES1* and *HEY1* categories on the basis of their dots number: High (≥5 dots), Dim (1–4 dots), and Negative (<1 dot). **D**) Bar graphs showing the average number of dots per target in the basal and suprabasal layers. Statistical analysis was performed using the Mann-Whitney test. *P<0.05; **P<0.005; not significant P>0.05. **E**) Bar graphs indicating a higher percentage of positive cells for both targets in the basal and suprabasal layers of the patients' group compared to controls.

6. Discussion and Conclusions

Darier Disease is a rare genodermatosis, autosomal dominantly inherited, determined by ATP2A2 gene alterations.

Mutations in *ATP2A2* result in impaired keratinocytes calcium homeostasis, decreased cell-cell adhesion in the epidermis, and apoptosis (Bachar-Wikström & Wikström, 2021). These mechanisms are thought to lead to the characteristic skin phenotype of Darier Disease with hyperkeratotic papules and plaques. However, the molecular mechanism by which SERCA2 mutations cause disease abnormalities is not well clarified.

Our findings provide important insights into the molecular mechanisms underlying Darier disease, particularly focusing on the NOTCH1 pathway. Through a combination of *in vitro* and *in vivo* assays, we observed a reduction in NOTCH1 signaling activity. Indeed, the NOTCH1 targets protein level was decreased in both cell line models and primary patient-derived cells. To perform *in vitro* studies, we employed HaCat and HEK293T cell lines transfected with plasmids carrying *ATP2A2* variants identified in affected patients. These models allowed us to dissect the direct impact of patient-specific mutations on the NOTCH1 signaling. The observed reduction in this pathway highlights and is in line with the previously reported interplay between *ATP2A2* mutations and impaired NOTCH1 function in leukemia, which may also contribute to the pathogenesis of the Darier disease.

Furthermore, these findings were also validated using primary cells directly deriving from patients and healthy controls biopsies. The reduction in NOTCH1 signaling across both primary and model cell lines suggests that this pathway may represent a critical node in disease onset and progression.

These results align with previous evidence indicating the pivotal role of NOTCH signaling in skin homeostasis and its potential disruption in keratinization disorders. Our data contribute to the growing body of knowledge suggesting that *ATP2A2* mutations can impair cellular processes not only through calcium signaling dysregulation, but also through perturbations in the maturation and function of proteins critical for skin integrity, such as NOTCH1. Indeed, as previously discussed, SERCA2 function alterations would impair the proper NOTCH1 maturation and trafficking toward the plasma membrane.

Interestingly, our RNA-based analysis presented a more nuanced picture. Despite the observed decrease in NOTCH1 signaling activity at the protein level, with reduced HES1, HEY1, and c-MYC, our real-time PCR results did not consistently show a reduction in the expression of these NOTCH target genes. Actually, in some cases, we even observed an increase in their expression level.

Furthermore, in order to evaluate the expression of NOTCH1 target genes directly on skin sections deriving from DD patients and controls, we employed the RNAscope technology, a highly sensitive and specific in situ hybridization technique, which allows mRNA visualization and quantification.

Interestingly, our analysis revealed an increased mRNA amount in DD samples compared to control subjects, not only for the probes of interest (*HES1* and *HEY1*) but also for the housekeeping genes used as positive controls. These findings suggest a global increase in mRNA levels in the affected skin.

The observed discrepancies between mRNA and protein levels raise important questions about the complexity of the pathway regulation and suggest that post-transcriptional or post-translational mechanisms may be involved in modulating NOTCH1 signaling activity in Darier disease.

The increase in NOTCH1 target gene expression, despite reduced signaling activity, could indicate a compensatory response, where the cell attempts to upregulate the pathway to counteract the effects of impaired signaling. This may reflect a form of feedback regulation, where alterations in one aspect of the signaling cascade leads to changes in gene expression as the cell tries to restore balance. Another possibility is that the observed increase in target gene expression could be related to the dysregulation of other upstream or downstream factors within the signaling network, potentially complicating the interpretation of NOTCH1 pathway activity based on RNA levels alone.

These RNA expression findings suggest that the regulation of NOTCH signaling in Darier disease is likely more intricate than initially anticipated and may involve additional layers of control, such as changes in the stability or translation of mRNA, as well as alterations in protein maturation or function. Further investigations, including proteomic approaches or functional

assays to assess the activity of NOTCH-related proteins, will be necessary to clarify the relationship between mRNA expression and functional changes in the pathway.

In our case, several factors could be considered to explain the observed discrepancy between gene expression and protein translation. One key aspect is the role of ER stress, which could lead to an upregulation of mRNA levels. As described in the Introduction, ER stress is known to activate different signaling, such as the unfolded protein response (UPR), to restore cellular homeostasis. These pathways can enhance transcription of certain genes as part of a compensatory mechanism. However, this increase in mRNA does not necessarily translate into higher protein levels, as translational efficiency can be impaired. Noteworthy, sustained ER stress is involved in the Darier Disease pathogenesis and we also confirmed it in our study by evaluating XBP1 protein level in both controls and patients-derived keratinocytes. Previous studies reported increased mRNA levels in mouse and human cells during ER stress. In particular, it has been shown that this upregulation depends on PERK-dependent translation attenuation, which as mentioned is a UPR marker (Szabat et al., 2011; J. E. Lee et al., 2018). In addition, HES1 regulates itself by repressing its own expression (Takebayashi et al., 1994) and both protein and mRNA are unstable (Hirata et al., 2002). Moreover, it has been suggested that the loss of HES1 protein could lead to the *HES1* mRNA upregulation. During ER stress, the reduced HES1 translation mediated by PERK determines the increase of its mRNA due to the loss of negative self-regulation. Noteworthy, in the presence of ER stressors, including Thapsigargin, a well-known SERCA2 inhibitor, HES1-depleted cells viability was compromised (J. E. Lee et al., 2018), which would explain one of the typical features of DD, apoptosis. Additionally, RNA sequencing results revealed a downregulation of genes associated with ribosomal components. A reduction in ribosomal gene expression could impair overall translational capacity, contributing to lower protein synthesis despite elevated mRNA levels. This evidence, combined with the endoplasmic reticulum (ER) stress observed in Darier disease, suggests a complex interplay between transcriptional upregulation, translational efficiency, and protein degradation, all of which may influence the disease phenotype.

Another characteristic feature of DD is chronic skin inflammation, which is prevalent and harmful in most patients. However, skin inflammation in DD is not mainly considered a primary *ATP2A2* variants consequence but is often attributed to epidermal barrier function disruption

that leads to an enhanced risk of infections and malodor and increased Th17-related genes expression. In particular, Amar et al identified a specific dysbiosis of Darier disease microbiome characterized by the loss of microbial variety and commensals that could be beneficial to counteract infections. Thus, recurrent infections in DD could be responsible for chronic inflammation at the skin lesion level (Amar et al., 2023).
However, other studies identified the Th-17 pathway as a direct effect of the ATP2A2 gene mutations in immune cells resulting in Th17 biasing in T cells (Ettinger et al., 2023).

Actually, among the differentially expressed genes we identified ones involved in the inflammatory response and in the wound healing process. In particular, the mRNA levels of proinflammatory cytokines, including Interleukin 6 (*IL-6*), *IL-1* family, and transforming growth factor (*TGF-β*) were up-regulated.
In skin wounds, these factors are crucial in regulating the re-epithelialization process and keratinocytes migration (Gallucci et al., 2004; Pastar et al., 2014). TGFβ1, which resulted to be up-regulated in the patient cohort, promotes keratinocyte migration by stimulating MMPs. For proper wound healing, keratinocytes should be able to detach from the underlying basal lamina and to migrate through the fibrin and newly synthesized extracellular matrix (ECM) of the wound, a process facilitated by MMPs (Krampert et al., 2004). Actually, among up-regulated DEGs we identified *MMP1*, *MMP2*, *MMP3* and *MMP10*. Different keratins, including KRT6 and KRT16, whose expression is regulated by growth factors present within the wound environment, were up-regulated in patients samples and are known to be involved in keratinocytes migration (Freedberg et al., 2001).
Consequently, considering the crucial role of cytokines, chemokines, and growth factors in the wound healing process, alterations in their expression could explain, at least in part, why DD skin lesions do not heal properly and show recurrent infections. A better understanding of the influence of deregulated cytokines on *ATP2A2*-defective keratinocytes proliferation, differentiation, and wound repair mechanisms may help to design treatment strategies to improve wound healing and microbial infection regression in affected patients.
In addition to its role in skin homeostasis, recent studies highlighted the involvement of NOTCH signaling in the regulation of inflammation. In mice, the disruption of cutaneous Notch signaling, through the deletion of RBP-J or the combined loss of Notch1 and Notch2, leads to

the overexpression of proinflammatory cytokines, resulting in a chronic inflammatory condition that resembles atopic dermatitis (AD) (Demehri et al., 2008; Dumortier et al., 2010). Similarly, in human patients with AD, reduced expression of NOTCH receptors has been observed in the affected epidermis, further supporting the critical role of NOTCH signaling in the regulation of skin inflammation (Dumortier et al., 2010). Chronic inflammation triggers the expression and release of various factors that can activate key pathways involved in cell proliferation and differentiation.

These observations are particularly relevant to our study of Darier disease, where chronic inflammation contributes to disease exacerbation. Our findings align with these reports in that the dysregulation of NOTCH signaling in Darier disease may not only impact skin homeostasis but also exacerbate the inflammatory condition within the skin. Chronic inflammation, as seen in AD and other inflammatory skin diseases, can activate key pathways involved in cell proliferation and differentiation, which are tightly regulated by NOTCH signaling. The altered NOTCH pathway in Darier disease might, therefore, contribute to the perpetuation of inflammatory responses, further complicating the disease's clinical presentation.

In conclusion, throughout this study, we investigated the genetic, histopathological, and transcriptomic features of Darier Disease, emphasizing the role of *ATP2A2* variants in altering SERCA2 function and resulting in NOTCH1 pathway dysregulation. However, further investigations are necessary to elucidate how the observed altered NOTCH1 signaling modulation interacts with other pathways implicated in Darier disease.

Additionally, while several studies contributed to gain insight into the understanding of this genodermatosis pathogenesis, further research is needed to identify targeted, effective, and less prone to side effects therapies.

7. References

Abe, M., Inoue, C., Yokoyama, Y., & Ishikawa, O. (2011). Successful treatment of Darier's disease with adapalene gel. *Pediatric Dermatology*, *28*(2), 197–198. https://doi.org/10.1111/j.1525-1470.2009.01077.x

Aboufares El Alaoui, A., Buhl, E., Galizia, S., Hodge, J. J. L., de Vivo, L., & Bellesi, M. (2023). Increased interaction between endoplasmic reticulum and mitochondria following sleep deprivation. *BMC Biology*, *21*(1), 1. https://doi.org/10.1186/s12915-022-01498-7

Adams, M. P., Mallet, D. G., & Pettet, G. J. (2012). Active regulation of the epidermal calcium profile. *Journal of Theoretical Biology*, *301*, 112–121. https://doi.org/10.1016/j.jtbi.2012.02.017

Agrawal, N., Frederick, M. J., Pickering, C. R., Bettegowda, C., Chang, K., Li, R. J., Fakhry, C., Xie, T.-X., Zhang, J., Wang, J., Zhang, N., El-Naggar, A. K., Jasser, S. A., Weinstein, J. N., Treviño, L., Drummond, J. A., Muzny, D. M., Wu, Y., Wood, L. D., … Myers, J. N. (2011). Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science (New York, N.Y.)*, *333*(6046), 1154–1157. https://doi.org/10.1126/science.1206923

Ahn, W., Lee, M. G., Kim, K. H., & Muallem, S. (2003). Multiple effects of SERCA2b mutations associated with Darier's disease. *The Journal of Biological Chemistry*, *278*(23), 20795–20801. https://doi.org/10.1074/jbc.M301638200

Akazawa, C., Sasai, Y., Nakanishi, S., & Kageyama, R. (1992). Molecular characterization of a rat negative regulator with a basic helix-loop-helix structure predominantly expressed in the developing nervous system. *The Journal of Biological Chemistry*, *267*(30), 21879–21885.

Akinduro, O., Sully, K., Patel, A., Robinson, D. J., Chikh, A., McPhail, G., Braun, K. M., Philpott, M. P., Harwood, C. A., Byrne, C., O'Shaughnessy, R. F. L., & Bergamaschi, D. (2016). Constitutive Autophagy and Nucleophagy during Epidermal Differentiation. *The Journal of Investigative Dermatology*, *136*(7), 1460–1470. https://doi.org/10.1016/j.jid.2016.03.016

Alalaiwe, A., Chen, C.-Y., Chang, Z.-Y., Sung, J.-T., Chuang, S.-Y., & Fang, J.-Y. (2021). Psoriasiform Inflammation Is Associated with Mitochondrial Fission/GDAP1L1 Signaling in Macrophages. *International Journal of Molecular Sciences*, *22*(19), 10410. https://doi.org/10.3390/ijms221910410

Albers, R. W. (1967). Biochemical aspects of active transport. *Annual Review of Biochemistry*, *36*, 727–756. https://doi.org/10.1146/annurev.bi.36.070167.003455

Allaoui, A., Botteaux, A., Dumont, J. E., Hoste, C., & De Deken, X. (2009). Dual oxidases and hydrogen peroxide in a complex dialogue between host mucosae and bacteria. *Trends in Molecular Medicine*, *15*(12), 571–579. https://doi.org/10.1016/j.molmed.2009.10.003

Almanza, A., Carlesso, A., Chintha, C., Creedican, S., Doultsinos, D., Leuzzi, B., Luís, A., McCarthy, N., Montibeller, L., More, S., Papaioannou, A., Püschel, F., Sassano, M. L., Skoko, J., Agostinis, P., de Belleroche, J., Eriksson, L. A., Fulda, S., Gorman, A. M., … Samali, A. (2019). Endoplasmic reticulum stress signalling—From basic mechanisms to clinical applications. *The FEBS Journal*, *286*(2), 241–278. https://doi.org/10.1111/febs.14608

Almeida, A., Lobo, M. de L., Moura, C., & Rivera, I. (2019). Darier disease: First molecular study of a Portuguese family. *Heliyon*, *5*(9), e02520. https://doi.org/10.1016/j.heliyon.2019.e02520

Amar, Y., Rogner, D., Silva, R. L., Foesel, B. U., Ud-Dean, M., Lagkouvardos, I., Steimle-Grauer, S. A., Niedermeier, S., Kublik, S., Jargosch, M., Heinig, M., Thomas, J., Eyerich, S., Wikström, J. D., Schloter, M., Eyerich, K., Biedermann, T., & Köberle, M. (2023). Darier's disease exhibits a unique cutaneous microbial dysbiosis associated with inflammation and body malodour. *Microbiome*, *11*(1), 162. https://doi.org/10.1186/s40168-023-01587-x

Anderson, J. M., & Van Itallie, C. M. (2009). Physiology and function of the tight junction. *Cold Spring Harbor Perspectives in Biology*, *1*(2), a002584. https://doi.org/10.1101/cshperspect.a002584

Anuset, D., Goutorbe, C., Bernard, P., & Reguiai, Z. (2014). Efficacy of oral alitretinoin for the treatment of Darier disease: A case report. *Journal of the American Academy of Dermatology*, *71*(2), e46-48. https://doi.org/10.1016/j.jaad.2014.01.876

Archer, C. B., Elias, P. M., Lowe, N. J., & Griffiths, W. A. (1989). Extensive spinal hyperostosis in a patient receiving isotretinoin—Progression after 4 years of etretinate therapy. *Clinical and Experimental Dermatology*, *14*(4), 319–321. https://doi.org/10.1111/j.1365-2230.1989.tb01993.x

Artavanis-Tsakonas, S., Rand, M. D., & Lake, R. J. (1999). Notch signaling: Cell fate control and signal integration in development. *Science (New York, N.Y.)*, *284*(5415), 770–776. https://doi.org/10.1126/science.284.5415.770

Ashcroft, G. S., Kielty, C. M., Horan, M. A., & Ferguson, M. W. (1997). Age-related changes in the temporal and spatial distributions of fibrillin and elastin mRNAs and proteins in acute cutaneous wounds of healthy humans. *The Journal of Pathology*, *183*(1), 80–89. https://doi.org/10.1002/(SICI)1096-9896(199709)183:1<80::AID-PATH1104>3.0.CO;2-N

Aster, J. C., Simms, W. B., Zavala-Ruiz, Z., Patriub, V., North, C. L., & Blacklow, S. C. (1999). The folding and structural integrity of the first LIN-12 module of human Notch1 are calcium-dependent. *Biochemistry*, *38*(15), 4736–4742. https://doi.org/10.1021/bi982713o

Ayli, E. E., Li, W., Brown, T. T., Witkiewicz, A., Elenitsas, R., & Seykora, J. T. (2008). Activation of Src-family tyrosine kinases in hyperproliferative epidermal disorders. *Journal of Cutaneous Pathology*, *35*(3), 273–277. https://doi.org/10.1111/j.1600-0560.2007.00807.x

Aymard, E., Barruche, V., Naves, T., Bordes, S., Closs, B., Verdier, M., & Ratinaud, M.-H. (2011). Autophagy in human keratinocytes: An early step of the differentiation? *Experimental Dermatology*, *20*(3), 263–268. https://doi.org/10.1111/j.1600-0625.2010.01157.x

Baba-Aissa, F., Raeymaekers, L., Wuytack, F., De Greef, C., Missiaen, L., & Casteels, R. (1996). Distribution of the organellar Ca2+ transport ATPase SERCA2 isoforms in the cat brain. *Brain Research*, *743*(1–2), 141–153. https://doi.org/10.1016/s0006-8993(96)01037-2

Bachar-Wikström, E., & Wikström, J. D. (2021). Darier Disease—A Multi-organ Condition? *Acta Dermato-Venereologica*, *101*(4), adv00430. https://doi.org/10.2340/00015555-3770

Bahar, E., Kim, H., & Yoon, H. (2016). ER Stress-Mediated Signaling: Action Potential and Ca(2+) as Key Players. *International Journal of Molecular Sciences*, *17*(9), 1558. https://doi.org/10.3390/ijms17091558

Balaban, R. S., Nemoto, S., & Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell*, *120*(4), 483–495. https://doi.org/10.1016/j.cell.2005.02.001

Barbieri, J. S., Wanat, K., & Seykora, J. (2014). Skin: Basic Structure and Function. In L. M. McManus & R. N. Mitchell (A c. Di), *Pathobiology of Human Disease* (pp. 1134–1144). Academic Press. https://doi.org/10.1016/B978-0-12-386456-7.03501-2

Baumgartner, H. K., Gerasimenko, J. V., Thorne, C., Ferdek, P., Pozzan, T., Tepikin, A. V., Petersen, O. H., Sutton, R., Watson, A. J. M., & Gerasimenko, O. V. (2009). Calcium elevation in mitochondria is the main Ca2+ requirement for mitochondrial permeability transition pore (mPTP) opening. *The Journal of Biological Chemistry*, *284*(31), 20796–20803. https://doi.org/10.1074/jbc.M109.025353

Bchetnia, M., Benmously, R., Ben Brick, A. S., Charfeddine, C., Ben Ameur, Y., Fajraoui, M., Debbiche, A., Ben Ayed, M., Mokni, M., Fenniche, S., Mokhtar, I., & Abdelhak, S. (2009). New mutations of Darier disease in Tunisian patients. *Archives of Dermatological Research*, *301*(8), 615–619. https://doi.org/10.1007/s00403-009-0963-5

Berg, D., & Bassett, A. S. (1993). Darier's disease: Current understanding of pathogenesis and future role of genetic studies. *International Journal of Dermatology*, *32*(6), 397–400. https://doi.org/10.1111/j.1365-4362.1993.tb02806.x

Berridge, M. J., Bootman, M. D., & Roderick, H. L. (2003). Calcium signalling: Dynamics, homeostasis and remodelling. *Nature Reviews. Molecular Cell Biology*, *4*(7), 517–529. https://doi.org/10.1038/nrm1155

Bhat, R. M., Ullal, K. R., Pinto, A. C., & Sukumar, D. (2010). Darier-White disease in siblings responding to isotretinoin. *Indian Dermatology Online Journal*, *1*(1), 18–20. https://doi.org/10.4103/2229-5178.73252

Bikle, D. D., Xie, Z., & Tu, C.-L. (2012). Calcium regulation of keratinocyte differentiation. *Expert Review of Endocrinology & Metabolism*, *7*(4), 461–472. https://doi.org/10.1586/eem.12.34

Birch-Machin, M. A., Tindall, M., Turner, R., Haldane, F., & Rees, J. L. (1998). Mitochondrial DNA deletions in human skin reflect photo- rather than chronologic aging. *The Journal of Investigative Dermatology*, *110*(2), 149–152. https://doi.org/10.1046/j.1523-1747.1998.00099.x

Blanchet-Bardon, C., Nazzaro, V., Rognin, C., Geiger, J. M., & Puissant, A. (1991). Acitretin in the treatment of severe disorders of keratinization. Results of an open study. *Journal of the American Academy of Dermatology*, *24*(6 Pt 1), 982–986. https://doi.org/10.1016/0190-9622(91)70158-x

Blanpain, C., & Fuchs, E. (2009). Epidermal homeostasis: A balancing act of stem cells in the skin. *Nature Reviews. Molecular Cell Biology*, *10*(3), 207–217. https://doi.org/10.1038/nrm2636

Blanpain, C., Lowry, W. E., Pasolli, H. A., & Fuchs, E. (2006). Canonical notch signaling functions as a commitment switch in the epidermal lineage. *Genes & Development*, *20*(21), 3022–3035. https://doi.org/10.1101/gad.1477606

Blaumueller, C. M., Qi, H., Zagouras, P., & Artavanis-Tsakonas, S. (1997). Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane. *Cell*, *90*(2), 281–291. https://doi.org/10.1016/s0092-8674(00)80336-0

Bobe, R., Bredoux, R., Corvazier, E., Andersen, J. P., Clausen, J. D., Dode, L., Kovács, T., & Enouf, J. (2004). Identification, expression, function, and localization of a novel (sixth) isoform of the human sarco/endoplasmic reticulum Ca2+ATPase 3 gene. *The Journal of Biological Chemistry*, *279*(23), 24297–24306. https://doi.org/10.1074/jbc.M314286200

Bobe, R., Bredoux, R., Corvazier, E., Lacabaratz-Porret, C., Martin, V., Kovács, T., & Enouf, J. (2005). How many Ca(2)+ATPase isoforms are expressed in a cell type? A growing family of membrane proteins illustrated by studies in platelets. *Platelets*, *16*(3–4), 133–150. https://doi.org/10.1080/09537100400016847

Bonora, M., Wieckowski, M. R., Chinopoulos, C., Kepp, O., Kroemer, G., Galluzzi, L., & Pinton, P. (2015). Molecular mechanisms of cell death: Central implication of ATP synthase in mitochondrial permeability transition. *Oncogene*, *34*(12), 1475–1486. https://doi.org/10.1038/onc.2014.96

Bouchard, C., Thieke, K., Maier, A., Saffrich, R., Hanley-Hyde, J., Ansorge, W., Reed, S., Sicinski, P., Bartek, J., & Eilers, M. (1999). Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. *The EMBO Journal*, *18*(19), 5321–5333. https://doi.org/10.1093/emboj/18.19.5321

Brandl, C. J., deLeon, S., Martin, D. R., & MacLennan, D. H. (1987). Adult forms of the Ca2+ATPase of sarcoplasmic reticulum. Expression in developing skeletal muscle. *The Journal of Biological Chemistry*, *262*(8), 3768–3774.

Bray, S. J. (2006). Notch signalling: A simple pathway becomes complex. *Nature Reviews. Molecular Cell Biology*, *7*(9), 678–689. https://doi.org/10.1038/nrm2009

Brenner, C., Deplus, R., Didelot, C., Loriot, A., Viré, E., De Smet, C., Gutierrez, A., Danovi, D., Bernard, D., Boon, T., Pelicci, P. G., Amati, B., Kouzarides, T., de Launoit, Y., Di Croce, L., & Fuks, F. (2005). Myc represses transcription through recruitment of DNA methyltransferase corepressor. *The EMBO Journal*, *24*(2), 336–346. https://doi.org/10.1038/sj.emboj.7600509

Bretones, G., Delgado, M. D., & León, J. (2015). Myc and cell cycle control. *Biochimica Et Biophysica Acta*, *1849*(5), 506–516. https://doi.org/10.1016/j.bbagrm.2014.03.013

Brinckmann, J., Hunzelmann, N., Kahle, B., Rohwedel, J., Kramer, J., Gibson, M. A., Hubmacher, D., & Reinhardt, D. P. (2010). Enhanced fibrillin-2 expression is a general feature of wound healing and sclerosis: Potential alteration of cell attachment and storage of TGF-beta. *Laboratory*

Investigation; a Journal of Technical Methods and Pathology, *90*(5), 739–752. https://doi.org/10.1038/labinvest.2010.49

Brini, M., & Carafoli, E. (2009). Calcium pumps in health and disease. *Physiological Reviews*, *89*(4), 1341–1378. https://doi.org/10.1152/physrev.00032.2008

Britzolaki, A., Saurine, J., Flaherty, E., Thelen, C., & Pitychoutis, P. M. (2018). The SERCA2: A Gatekeeper of Neuronal Calcium Homeostasis in the Brain. *Cellular and Molecular Neurobiology*, *38*(5), 981–994. https://doi.org/10.1007/s10571-018-0583-8

Britzolaki, A., Saurine, J., Klocke, B., & Pitychoutis, P. M. (2020). A Role for SERCA Pumps in the Neurobiology of Neuropsychiatric and Neurodegenerative Disorders. *Advances in Experimental Medicine and Biology*, *1131*, 131–161. https://doi.org/10.1007/978-3-030-12457-1_6

Brooks, S. A., Dwek, M. V., & Schumacher, U. (2002). O-Linked (mucin-type) glycoproteins. In *Functional and Molecular Glycobiology*. Garland Science.

Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cumano, A., Roux, P., Black, R. A., & Israël, A. (2000). A novel proteolytic cleavage involved in Notch signaling: The role of the disintegrin-metalloprotease TACE. *Molecular Cell*, *5*(2), 207–216. https://doi.org/10.1016/s1097-2765(00)80417-7

Bublitz, M., Poulsen, H., Morth, J. P., & Nissen, P. (2010). In and out of the cation pumps: P-type ATPase structure revisited. *Current Opinion in Structural Biology*, *20*(4), 431–439. https://doi.org/10.1016/j.sbi.2010.06.007

Burge, S. (1994). Darier's disease—The clinical features and pathogenesis. *Clinical and Experimental Dermatology*, *19*(3), 193–205. https://doi.org/10.1111/j.1365-2230.1994.tb01165.x

Burge, S. M., & Buxton, P. K. (1995). Topical isotretinoin in Darier's disease. *The British Journal of Dermatology*, *133*(6), 924–928. https://doi.org/10.1111/j.1365-2133.1995.tb06927.x

Burge, S. M., & Garrod, D. R. (1991). An immunohistological study of desmosomes in Darier's disease and Hailey-Hailey disease. *The British Journal of Dermatology*, *124*(3), 242–251. https://doi.org/10.1111/j.1365-2133.1991.tb00568.x

Burge, S. M., & Wilkinson, J. D. (1992). Darier-White disease: A review of the clinical features in 163 patients. *Journal of the American Academy of Dermatology*, *27*(1), 40–50. https://doi.org/10.1016/0190-9622(92)70154-8

Burgoon, C. F., Graham, J. H., Urbach, F., & Musgnug, R. (1963). Effect of vitamin A on epithelial cells of skin. The use of vitamin A in the treatment of diseases characterized by abnormal keratinization. *Archives of Dermatology*, *87*, 63–80. https://doi.org/10.1001/archderm.1963.01590130069012

Burk, S. E., Lytton, J., MacLennan, D. H., & Shull, G. E. (1989). cDNA cloning, functional expression, and mRNA tissue distribution of a third organellar Ca2+ pump. *The Journal of Biological Chemistry*, *264*(31), 18561–18568.

Candi, E., Schmidt, R., & Melino, G. (2005). The cornified envelope: A model of cell death in the skin. *Nature Reviews. Molecular Cell Biology*, *6*(4), 328–340. https://doi.org/10.1038/nrm1619

Carreras-Sureda, A., Pihán, P., & Hetz, C. (2018). Calcium signaling at the endoplasmic reticulum: Fine-tuning stress responses. *Cell Calcium*, *70*, 24–31. https://doi.org/10.1016/j.ceca.2017.08.004

Castella, P., Sawai, S., Nakao, K., Wagner, J. A., & Caudy, M. (2000). HES-1 repression of differentiation and proliferation in PC12 cells: Role for the helix 3-helix 4 domain in transcription repression. *Molecular and Cellular Biology*, *20*(16), 6170–6183. https://doi.org/10.1128/MCB.20.16.6170-6183.2000

Celli, A., Crumrine, D., Meyer, J. M., & Mauro, T. M. (2016). Endoplasmic Reticulum Calcium Regulates Epidermal Barrier Response and Desmosomal Structure. *The Journal of Investigative Dermatology*, *136*(9), 1840–1847. https://doi.org/10.1016/j.jid.2016.05.100

Celli, A., Mackenzie, D. S., Crumrine, D. S., Tu, C. L., Hupe, M., Bikle, D. D., Elias, P. M., & Mauro, T. M. (2011). Endoplasmic reticulum Ca2+ depletion activates XBP1 and controls terminal differentiation in keratinocytes and epidermis. *The British Journal of Dermatology*, *164*(1), 16–25. https://doi.org/10.1111/j.1365-2133.2010.10046.x

Celli, A., Mackenzie, D. S., Zhai, Y., Tu, C.-L., Bikle, D. D., Holleran, W. M., Uchida, Y., & Mauro, T. M. (2012). SERCA2-controlled Ca^2+-dependent keratinocyte adhesion and differentiation is mediated via the sphingolipid pathway: A therapeutic target for Darier's disease. *The Journal of Investigative Dermatology*, *132*(4), 1188–1195. https://doi.org/10.1038/jid.2011.447

Celli, A., Sanchez, S., Behne, M., Hazlett, T., Gratton, E., & Mauro, T. (2010). The epidermal Ca(2+) gradient: Measurement using the phasor representation of fluorescent lifetime imaging. *Biophysical Journal*, *98*(5), 911–921. https://doi.org/10.1016/j.bpj.2009.10.055

Chemaly, E. R., Troncone, L., & Lebeche, D. (2018). SERCA control of cell death and survival. *Cell Calcium*, *69*, 46–61. https://doi.org/10.1016/j.ceca.2017.07.001

Chin, L., Liégeois, N., DePinho, R. A., & Schreiber-Agus, N. (1996). Functional interactions among members of the Myc superfamily and potential relevance to cutaneous growth and development. *The Journal of Investigative Dermatology. Symposium Proceedings*, *1*(2), 128–135.

Cholewa, B. D., Liu, X., & Ahmad, N. (2013). The role of polo-like kinase 1 in carcinogenesis: Cause or consequence? *Cancer Research*, *73*(23), 6848–6855. https://doi.org/10.1158/0008-5472.CAN-13-2197

Cichorek, M., Wachulska, M., Stasiewicz, A., & Tymińska, A. (2013). Skin melanocytes: Biology and development. *Postepy Dermatologii I Alergologii*, *30*(1), 30–41. https://doi.org/10.5114/pdia.2013.33376

Clayton, E., Doupé, D. P., Klein, A. M., Winton, D. J., Simons, B. D., & Jones, P. H. (2007). A single type of progenitor cell maintains normal epidermis. *Nature*, *446*(7132), 185–189. https://doi.org/10.1038/nature05574

Coe, H., & Michalak, M. (2009). Calcium binding chaperones of the endoplasmic reticulum. *General Physiology and Biophysics*, *28 Spec No Focus*, F96–F103.

Coller, H. A., Grandori, C., Tamayo, P., Colbert, T., Lander, E. S., Eisenman, R. N., & Golub, T. R. (2000). Expression analysis with oligonucleotide microarrays reveals that MYC regulates genes involved in growth, cell cycle, signaling, and adhesion. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(7), 3260–3265. https://doi.org/10.1073/pnas.97.7.3260

Cooper, S. M., & Burge, S. M. (2003). Darier's disease: Epidemiology, pathophysiology, and management. *American Journal of Clinical Dermatology*, *4*(2), 97–105. https://doi.org/10.2165/00128071-200304020-00003

Cordle, J., Redfieldz, C., Stacey, M., van der Merwe, P. A., Willis, A. C., Champion, B. R., Hambleton, S., & Handford, P. A. (2008). Localization of the delta-like-1-binding site in human Notch-1 and its modulation by calcium affinity. *The Journal of Biological Chemistry*, *283*(17), 11785–11793. https://doi.org/10.1074/jbc.M708424200

Cornelissen, L. H., Oomens, C. W. J., Huyghe, J. M., & Baaijens, F. P. T. (2007). Mechanisms that play a role in the maintenance of the calcium gradient in the epidermis. *Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)*, *13*(4), 369–376. https://doi.org/10.1111/j.1600-0846.2007.00239.x

Coulombe, F., Fiola, S., Akira, S., Cormier, Y., & Gosselin, J. (2012). Muramyl dipeptide induces NOD2-dependent Ly6C(high) monocyte recruitment to the lungs and protects against influenza virus infection. *PloS One*, *7*(5), e36734. https://doi.org/10.1371/journal.pone.0036734

Covino, R., Hummer, G., & Ernst, R. (2018). Integrated Functions of Membrane Property Sensors and a Hidden Side of the Unfolded Protein Response. *Molecular Cell*, *71*(3), 458–467. https://doi.org/10.1016/j.molcel.2018.07.019

Dai, X., & Segre, J. A. (2004). Transcriptional control of epidermal specification and differentiation. *Current Opinion in Genetics & Development*, *14*(5), 485–491. https://doi.org/10.1016/j.gde.2004.07.002

Dally, S., Bredoux, R., Corvazier, E., Andersen, J. P., Clausen, J. D., Dode, L., Fanchaouy, M., Gelebart, P., Monceau, V., Del Monte, F., Gwathmey, J. K., Hajjar, R., Chaabane, C., Bobe, R., Raies, A., &

Enouf, J. (2006). Ca2+-ATPases in non-failing and failing heart: Evidence for a novel cardiac sarco/endoplasmic reticulum Ca2+-ATPase 2 isoform (SERCA2c). *The Biochemical Journal*, *395*(2), 249–258. https://doi.org/10.1042/BJ20051427

Dally, S., Corvazier, E., Bredoux, R., Bobe, R., & Enouf, J. (2010). Multiple and diverse coexpression, location, and regulation of additional SERCA2 and SERCA3 isoforms in nonfailing and failing human heart. *Journal of Molecular and Cellular Cardiology*, *48*(4), 633–644. https://doi.org/10.1016/j.yjmcc.2009.11.012

Danese, A., Patergnani, S., Bonora, M., Wieckowski, M. R., Previati, M., Giorgi, C., & Pinton, P. (2017). Calcium regulates cell death in cancer: Roles of the mitochondria and mitochondria-associated membranes (MAMs). *Biochimica Et Biophysica Acta. Bioenergetics*, *1858*(8), 615–627. https://doi.org/10.1016/j.bbabio.2017.01.003

Dantonio, P. M., Klein, M. O., Freire, M. R. V. B., Araujo, C. N., Chiacetti, A. C., & Correa, R. G. (2018). Exploring major signaling cascades in melanomagenesis: A rationale route for targetted skin cancer therapy. *Bioscience Reports*, *38*(5), BSR20180511. https://doi.org/10.1042/BSR20180511

Dawson, S. R., Turner, D. L., Weintraub, H., & Parkhurst, S. M. (1995). Specificity for the hairy/enhancer of split basic helix-loop-helix (bHLH) proteins maps outside the bHLH domain and suggests two separable modes of transcriptional repression. *Molecular and Cellular Biology*, *15*(12), 6923–6931. https://doi.org/10.1128/MCB.15.12.6923

de Cid, R., Riveira-Munoz, E., Zeeuwen, P. L. J. M., Robarge, J., Liao, W., Dannhauser, E. N., Giardina, E., Stuart, P. E., Nair, R., Helms, C., Escaramís, G., Ballana, E., Martín-Ezquerra, G., den Heijer, M., Kamsteeg, M., Joosten, I., Eichler, E. E., Lázaro, C., Pujol, R. M., … Estivill, X. (2009). Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. *Nature Genetics*, *41*(2), 211–215. https://doi.org/10.1038/ng.313

Deepika, K. (2020). Darier's Disease. *Pondicherry Journal of Nursing*, *13*(2), 43–45. https://doi.org/10.5005/jp-journals-10084-12138

Dellon, A. L., Chretien, P. B., & Peck, G. L. (1977). Successful treatment of Darier's disease by partial-thickness removal of skin. *Plastic and Reconstructive Surgery*, *59*(6), 823–830. https://doi.org/10.1097/00006534-197706000-00006

Demehri, S., Liu, Z., Lee, J., Lin, M.-H., Crosby, S. D., Roberts, C. J., Grigsby, P. W., Miner, J. H., Farr, A. G., & Kopan, R. (2008). Notch-deficient skin induces a lethal systemic B-lymphoproliferative disorder by secreting TSLP, a sentinel for epidermal integrity. *PLoS Biology*, *6*(5), e123. https://doi.org/10.1371/journal.pbio.0060123

Denning, M. F. (2004). Epidermal keratinocytes: Regulation of multiple cell phenotypes by multiple protein kinase C isoforms. *The International Journal of Biochemistry & Cell Biology*, *36*(7), 1141–1146. https://doi.org/10.1016/j.biocel.2003.12.004

Desruisseaux, M. S., Nagajyothi, null, Trujillo, M. E., Tanowitz, H. B., & Scherer, P. E. (2007). Adipocyte, adipose tissue, and infectious disease. *Infection and Immunity*, *75*(3), 1066–1078. https://doi.org/10.1128/IAI.01455-06

Dhitavat, J., Cobbold, C., Leslie, N., Burge, S., & Hovnanian, A. (2003). Impaired trafficking of the desmoplakins in cultured Darier's disease keratinocytes. *The Journal of Investigative Dermatology*, *121*(6), 1349–1355. https://doi.org/10.1046/j.1523-1747.2003.12557.x

Di Meglio, P., Perera, G. K., & Nestle, F. O. (2011). The multitasking organ: Recent insights into skin immune function. *Immunity*, *35*(6), 857–869. https://doi.org/10.1016/j.immuni.2011.12.003

Dicken, C. H., Bauer, E. A., Hazen, P. G., Krueger, G. G., Marks, J. G., McGuire, J. S., & Schachner, L. A. (1982). Isotretinoin treatment of Darier's disease. *Journal of the American Academy of Dermatology*, *6*(4 Pt 2 Suppl), 721–726. https://doi.org/10.1016/s0190-9622(82)80052-2

Dode, L., Andersen, J. P., Leslie, N., Dhitavat, J., Vilsen, B., & Hovnanian, A. (2003). Dissection of the functional differences between sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA) 1 and 2 isoforms and characterization of Darier disease (SERCA2) mutants by steady-state and transient

kinetic analyses. *The Journal of Biological Chemistry*, *278*(48), 47877–47889. https://doi.org/10.1074/jbc.M306784200

Dodiuk-Gad, R., Cohen-Barak, E., Ziv, M., Shani-Adir, A., Amichai, B., Zlotogorski, A., Shalev, S., & Rozenman, D. (2013). Health-related quality of life among Darier's disease patients. *Journal of the European Academy of Dermatology and Venereology: JEADV*, *27*(1), 51–56. https://doi.org/10.1111/j.1468-3083.2011.04355.x

Dogan, S., Karaduman, A., Erkin, G., & Gokoz, O. (2011). Effective treatment of linear Darier's disease with topical retinoids: Case report and review of the literature. *Acta Dermatovenerologica Croatica: ADC*, *19*(3), 206–209.

Donati, V., Peres, C., Nardin, C., Scavizzi, F., Raspa, M., Ciubotaru, C. D., Bortolozzi, M., Pedersen, M. G., & Mammano, F. (2022). Calcium Signaling in the Photodamaged Skin: In Vivo Experiments and Mathematical Modeling. *Function (Oxford, England)*, *3*(1), zqab064. https://doi.org/10.1093/function/zqab064

Dotto, G. P. (1999). Signal transduction pathways controlling the switch between keratinocyte growth and differentiation. *Critical Reviews in Oral Biology and Medicine: An Official Publication of the American Association of Oral Biologists*, *10*(4), 442–457. https://doi.org/10.1177/10454411990100040201

Driskell, R. R., Jahoda, C. A. B., Chuong, C.-M., Watt, F. M., & Horsley, V. (2014). Defining dermal adipose tissue. *Experimental Dermatology*, *23*(9), 629–631. https://doi.org/10.1111/exd.12450

Dumortier, A., Durham, A.-D., Di Piazza, M., Vauclair, S., Koch, U., Ferrand, G., Ferrero, I., Demehri, S., Song, L. L., Farr, A. G., Leonard, W. J., Kopan, R., Miele, L., Hohl, D., Finke, D., & Radtke, F. (2010). Atopic dermatitis-like disease and associated lethal myeloproliferative disorder arise from loss of Notch signaling in the murine skin. *PloS One*, *5*(2), e9258. https://doi.org/10.1371/journal.pone.0009258

Dyring-Andersen, B., Løvendorf, M. B., Coscia, F., Santos, A., Møller, L. B. P., Colaço, A. R., Niu, L., Bzorek, M., Doll, S., Andersen, J. L., Clark, R. A., Skov, L., Teunissen, M. B. M., & Mann, M. (2020). Spatially and cell-type resolved quantitative proteomic atlas of healthy human skin. *Nature Communications*, *11*(1), 5587. https://doi.org/10.1038/s41467-020-19383-8

Eckert, R. L., Crish, J. F., Banks, E. B., & Welter, J. F. (1997). The epidermis: Genes on - genes off. *The Journal of Investigative Dermatology*, *109*(4), 501–509. https://doi.org/10.1111/1523-1747.ep12336477

Elbaz, Y., & Schuldiner, M. (2011). Staying in touch: The molecular era of organelle contact sites. *Trends in Biochemical Sciences*, *36*(11), 616–623. https://doi.org/10.1016/j.tibs.2011.08.004

Elias, P., Ahn, S., Brown, B., Crumrine, D., & Feingold, K. R. (2002). Origin of the epidermal calcium gradient: Regulation by barrier status and role of active vs passive mechanisms. *The Journal of Investigative Dermatology*, *119*(6), 1269–1274. https://doi.org/10.1046/j.1523-1747.2002.19622.x

Elias, P. M. (2005). Stratum corneum defensive functions: An integrated view. *The Journal of Investigative Dermatology*, *125*(2), 183–200. https://doi.org/10.1111/j.0022-202X.2005.23668.x

Elias, P. M. (2012). Structure and function of the stratum corneum extracellular matrix. *The Journal of Investigative Dermatology*, *132*(9), 2131–2133. https://doi.org/10.1038/jid.2012.246

Elsholz, F., Harteneck, C., Muller, W., & Friedland, K. (2014). Calcium—A central regulator of keratinocyte differentiation in health and disease. *European Journal of Dermatology: EJD*, *24*(6), 650–661. https://doi.org/10.1684/ejd.2014.2452

Ettinger, M., Burner, T., Sharma, A., Chang, Y.-T., Lackner, A., Prompsy, P., Deli, I. M., Traxler, J., Wahl, G., Altrichter, S., Langer, R., Tsai, Y.-C., Varkhande, S. R., Schoeftner, L. C., Iselin, C., Gratz, I. K., Kimeswenger, S., Guenova, E., & Hoetzenecker, W. (2023). Th17-associated cytokines IL-17 and IL-23 in inflamed skin of Darier disease patients as potential therapeutic targets. *Nature Communications*, *14*(1), 7470. https://doi.org/10.1038/s41467-023-43210-5

Exadaktylou, D., Kurwa, H. A., Calonje, E., & Barlow, R. J. (2003). Treatment of Darier's disease with photodynamic therapy. *The British Journal of Dermatology*, *149*(3), 606–610. https://doi.org/10.1046/j.1365-2133.2003.05494.x

Farb, R. M., Lazarus, G. S., Chiaramonti, A., Goldsmith, L. A., Gilgor, R. S., & Balakrishnan, C. V. (1980). The effect of 13-cis retinoic acid on epidermal lysosomal hydrolase activity in Darier's disease and pityriasis rubra pilaris. *The Journal of Investigative Dermatology*, *75*(2), 133–135. https://doi.org/10.1111/1523-1747.ep12521639

Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A., & Artavanis-Tsakonas, S. (1990). Molecular interactions between the protein products of the neurogenic loci Notch and Delta, two EGF-homologous genes in Drosophila. *Cell*, *61*(3), 523–534. https://doi.org/10.1016/0092-8674(90)90534-l

Feng, S., Zhao, J., Yang, T., & Li, L. (2023). TMPRSS11D/ALR-mediated ER stress regulates the function of myeloid-derived suppressor cells in the cervical cancer microenvironment. *International Immunopharmacology*, *124*(Pt A), 110869. https://doi.org/10.1016/j.intimp.2023.110869

Fisher, A. L., Ohsako, S., & Caudy, M. (1996). The WRPW motif of the hairy-related basic helix-loop-helix repressor proteins acts as a 4-amino-acid transcription repression and protein-protein interaction domain. *Molecular and Cellular Biology*, *16*(6), 2670–2677. https://doi.org/10.1128/MCB.16.6.2670

Foggia, L., Aronchik, I., Aberg, K., Brown, B., Hovnanian, A., & Mauro, T. M. (2006). Activity of the hSPCA1 Golgi Ca2+ pump is essential for Ca2+-mediated Ca2+ response and cell viability in Darier disease. *Journal of Cell Science*, *119*(Pt 4), 671–679. https://doi.org/10.1242/jcs.02781

Foggia, L., & Hovnanian, A. (2004). Calcium pump disorders of the skin. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *131C*(1), 20–31. https://doi.org/10.1002/ajmg.c.30031

Franke, W. W. (2009). Discovering the molecular components of intercellular junctions—A historical view. *Cold Spring Harbor Perspectives in Biology*, *1*(3), a003061. https://doi.org/10.1101/cshperspect.a003061

Freedberg, I. M., Tomic-Canic, M., Komine, M., & Blumenberg, M. (2001). Keratins and the keratinocyte activation cycle. *The Journal of Investigative Dermatology*, *116*(5), 633–640. https://doi.org/10.1046/j.1523-1747.2001.01327.x

Friedman, J. R., & Voeltz, G. K. (2011). The ER in 3D: A multifunctional dynamic membrane network. *Trends in Cell Biology*, *21*(12), 709–717. https://doi.org/10.1016/j.tcb.2011.07.004

Fuchs, E. (1990). Epidermal differentiation: The bare essentials. *The Journal of Cell Biology*, *111*(6 Pt 2), 2807–2814. https://doi.org/10.1083/jcb.111.6.2807

Fuchs, E., & Byrne, C. (1994). The epidermis: Rising to the surface. *Current Opinion in Genetics & Development*, *4*(5), 725–736. https://doi.org/10.1016/0959-437x(94)90140-x

Fuchs, E., & Raghavan, S. (2002). Getting under the skin of epidermal morphogenesis. *Nature Reviews. Genetics*, *3*(3), 199–209. https://doi.org/10.1038/nrg758

Fuchs, E., Tumbar, T., & Guasch, G. (2004). Socializing with the neighbors: Stem cells and their niche. *Cell*, *116*(6), 769–778. https://doi.org/10.1016/s0092-8674(04)00255-7

Fulton, J. E., Gross, P. R., Cornelius, C. E., & Kligman, A. M. (1968). Darier's disease. Treatment with topical vitamin A acid. *Archives of Dermatology*, *98*(4), 396–399. https://doi.org/10.1001/archderm.98.4.396

Gallucci, R. M., Sloan, D. K., Heck, J. M., Murray, A. R., & O'Dell, S. J. (2004). Interleukin 6 indirectly induces keratinocyte migration. *The Journal of Investigative Dermatology*, *122*(3), 764–772. https://doi.org/10.1111/j.0022-202X.2004.22323.x

Garrod, D., & Chidgey, M. (2008). Desmosome structure, composition and function. *Biochimica Et Biophysica Acta*, *1778*(3), 572–587. https://doi.org/10.1016/j.bbamem.2007.07.014

Gebhardt, A., Frye, M., Herold, S., Benitah, S. A., Braun, K., Samans, B., Watt, F. M., Elsässer, H.-P., & Eilers, M. (2006). Myc regulates keratinocyte adhesion and differentiation via complex

formation with Miz1. *The Journal of Cell Biology*, *172*(1), 139–149. https://doi.org/10.1083/jcb.200506057

Gélébart, P., Martin, V., Enouf, J., & Papp, B. (2003). Identification of a new SERCA2 splice variant regulated during monocytic differentiation. *Biochemical and Biophysical Research Communications*, *303*(2), 676–684. https://doi.org/10.1016/s0006-291x(03)00405-4

Gerhardt, D. M., Pajcini, K. V., D'altri, T., Tu, L., Jain, R., Xu, L., Chen, M. J., Rentschler, S., Shestova, O., Wertheim, G. B., Tobias, J. W., Kluk, M., Wood, A. W., Aster, J. C., Gimotty, P. A., Epstein, J. A., Speck, N., Bigas, A., & Pear, W. S. (2014). The Notch1 transcriptional activation domain is required for development and reveals a novel role for Notch1 signaling in fetal hematopoietic stem cells. *Genes & Development*, *28*(6), 576–593. https://doi.org/10.1101/gad.227496.113

Getsios, S., Huen, A. C., & Green, K. J. (2004). Working out the strength and flexibility of desmosomes. *Nature Reviews. Molecular Cell Biology*, *5*(4), 271–281. https://doi.org/10.1038/nrm1356

Goleva, E., Berdyshev, E., & Leung, D. Y. (2019). Epithelial barrier repair and prevention of allergy. *The Journal of Clinical Investigation*, *129*(4), 1463–1474. https://doi.org/10.1172/JCI124608

Gonzales, K. A. U., & Fuchs, E. (2017). Skin and Its Regenerative Powers: An Alliance between Stem Cells and Their Niche. *Developmental Cell*, *43*(4), 387–401. https://doi.org/10.1016/j.devcel.2017.10.001

Gordon, W. R., Vardar-Ulu, D., Histen, G., Sanchez-Irizarry, C., Aster, J. C., & Blacklow, S. C. (2007). Structural basis for autoinhibition of Notch. *Nature Structural & Molecular Biology*, *14*(4), 295–300. https://doi.org/10.1038/nsmb1227

Gorski, P. A., Ceholski, D. K., & Young, H. S. (2017). Structure-Function Relationship of the SERCA Pump and Its Regulation by Phospholamban and Sarcolipin. *Advances in Experimental Medicine and Biology*, *981*, 77–119. https://doi.org/10.1007/978-3-319-55858-5_5

Gratton, R., Tricarico, P. M., Moltrasio, C., Lima Estevão de Oliveira, A. S., Brandão, L., Marzano, A. V., Zupin, L., & Crovella, S. (2020). Pleiotropic Role of Notch Signaling in Human Skin Diseases. *International Journal of Molecular Sciences*, *21*(12), 4214. https://doi.org/10.3390/ijms21124214

Grbavec, D., & Stifani, S. (1996). Molecular interaction between TLE1 and the carboxyl-terminal domain of HES-1 containing the WRPW motif. *Biochemical and Biophysical Research Communications*, *223*(3), 701–705. https://doi.org/10.1006/bbrc.1996.0959

Green, K. J., & Simpson, C. L. (2007). Desmosomes: New perspectives on a classic. *The Journal of Investigative Dermatology*, *127*(11), 2499–2515. https://doi.org/10.1038/sj.jid.5701015

Green, K. N., Demuro, A., Akbari, Y., Hitt, B. D., Smith, I. F., Parker, I., & LaFerla, F. M. (2008). SERCA pump activity is physiologically regulated by presenilin and regulates amyloid beta production. *The Journal of Cell Biology*, *181*(7), 1107–1116. https://doi.org/10.1083/jcb.200706171

Guerini, D. (1998). The Ca2+ pumps and the Na+/Ca2+ exchangers. *Biometals: An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine*, *11*(4), 319–330. https://doi.org/10.1023/a:1009210001608

Hagino, T., Nakano, H., Saeki, H., & Kanda, N. (2022). A Case of Darier's Disease with a Novel Missense Mutation in ATP2A2 Successfully Treated with Calcipotriol/Betamethasone Dipropionate Two-Compound Ointment. *Clinical, Cosmetic and Investigational Dermatology*, *15*, 367–372. https://doi.org/10.2147/CCID.S354694

Haines, N., & Irvine, K. D. (2003). Glycosylation regulates Notch signalling. *Nature Reviews. Molecular Cell Biology*, *4*(10), 786–797. https://doi.org/10.1038/nrm1228

Hakuno, M., Shimizu, H., Akiyama, M., Amagai, M., Wahl, J. K., Wheelock, M. J., & Nishikawa, T. (2000). Dissociation of intra- and extracellular domains of desmosomal cadherins and E-cadherin in Hailey-Hailey disease and Darier's disease. *The British Journal of Dermatology*, *142*(4), 702–711. https://doi.org/10.1046/j.1365-2133.2000.03415.x

Hamanaka, R. B., & Chandel, N. S. (2013). Mitochondrial metabolism as a regulator of keratinocyte differentiation. *Cellular Logistics*, *3*(1), e25456. https://doi.org/10.4161/cl.25456

Hambleton, S., Valeyev, N. V., Muranyi, A., Knott, V., Werner, J. M., McMichael, A. J., Handford, P. A., & Downing, A. K. (2004). Structural and functional properties of the human notch-1 ligand binding region. *Structure (London, England: 1993)*, *12*(12), 2173–2183. https://doi.org/10.1016/j.str.2004.09.012

Hampton, R. Y. (2000). ER stress response: Getting the UPR hand on misfolded proteins. *Current Biology: CB*, *10*(14), R518-521. https://doi.org/10.1016/s0960-9822(00)00583-2

Hartmann, D., de Strooper, B., Serneels, L., Craessaerts, K., Herreman, A., Annaert, W., Umans, L., Lübke, T., Lena Illert, A., von Figura, K., & Saftig, P. (2002). The disintegrin/metalloprotease ADAM 10 is essential for Notch signalling but not for alpha-secretase activity in fibroblasts. *Human Molecular Genetics*, *11*(21), 2615–2624. https://doi.org/10.1093/hmg/11.21.2615

Hermeking, H., Rago, C., Schuhmacher, M., Li, Q., Barrett, J. F., Obaya, A. J., O'Connell, B. C., Mateyak, M. K., Tam, W., Kohlhuber, F., Dang, C. V., Sedivy, J. M., Eick, D., Vogelstein, B., & Kinzler, K. W. (2000). Identification of CDK4 as a target of c-MYC. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(5), 2229–2234. https://doi.org/10.1073/pnas.050586197

Hesser, B. A., Liang, X. H., Camenisch, G., Yang, S., Lewin, D. A., Scheller, R., Ferrara, N., & Gerber, H.-P. (2004). Down syndrome critical region protein 1 (DSCR1), a novel VEGF target gene that regulates expression of inflammatory markers on activated endothelial cells. *Blood*, *104*(1), 149–158. https://doi.org/10.1182/blood-2004-01-0273

Hirata, H., Ohtsuka, T., Bessho, Y., & Kageyama, R. (2000). Generation of structurally and functionally distinct factors from the basic helix-loop-helix gene Hes3 by alternative first exons. *The Journal of Biological Chemistry*, *275*(25), 19083–19089. https://doi.org/10.1074/jbc.M001075200

Hirata, H., Yoshiura, S., Ohtsuka, T., Bessho, Y., Harada, T., Yoshikawa, K., & Kageyama, R. (2002). Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop. *Science (New York, N.Y.)*, *298*(5594), 840–843. https://doi.org/10.1126/science.1074560

Hitomi, K. (2005). Transglutaminases in skin epidermis. *European Journal of Dermatology: EJD*, *15*(5), 313–319.

Hoffmann, M. H., & Griffiths, H. R. (2018). The dual role of Reactive Oxygen Species in autoimmune and inflammatory diseases: Evidence from preclinical models. *Free Radical Biology & Medicine*, *125*, 62–71. https://doi.org/10.1016/j.freeradbiomed.2018.03.016

Honeycutt, K. A., & Roop, D. R. (2004). C-Myc and epidermal stem cell fate determination. *The Journal of Dermatology*, *31*(5), 368–375. https://doi.org/10.1111/j.1346-8138.2004.tb00687.x

Hori, K., Sen, A., & Artavanis-Tsakonas, S. (2013). Notch signaling at a glance. *Journal of Cell Science*, *126*(Pt 10), 2135–2140. https://doi.org/10.1242/jcs.127308

Hovnanian, A. (2007). SERCA pumps and human diseases. *Sub-Cellular Biochemistry*, *45*, 337–363. https://doi.org/10.1007/978-1-4020-6191-2_12

Hovnanian, A. (2019). Acantholytic Disorders of the Skin. In S. Kang, M. Amagai, A. L. Bruckner, A. H. Enk, D. J. Margolis, A. J. McMichael, & J. S. Orringer (A c. Di), *Fitzpatrick's Dermatology* (9ª ed.). McGraw-Hill Education. accessmedicine.mhmedical.com/content.aspx?aid=1161331228

Hu, Y., Baud, V., Oga, T., Kim, K. I., Yoshida, K., & Karin, M. (2001). IKKalpha controls formation of the epidermis independently of NF-kappaB. *Nature*, *410*(6829), 710–714. https://doi.org/10.1038/35070605

Hunt, M., Torres, M., Bachar-Wikström, E., & Wikström, J. D. (2023). Multifaceted roles of mitochondria in wound healing and chronic wound pathogenesis. *Frontiers in Cell and Developmental Biology*, *11*, 1252318. https://doi.org/10.3389/fcell.2023.1252318

Hunt, M., Wang, N., Pupinyo, N., Curman, P., Torres, M., Jebril, W., Chatzinikolaou, M., Lorent, J., Silberberg, G., Bansal, R., Burner, T., Zhou, J., Kimeswenger, S., Hoetzenecker, W., Choate, K., Bachar-Wikstrom, E., & Wikstrom, J. D. (2024). Dantrolene corrects cellular disease features of Darier disease and may be a novel treatment. *EMBO Molecular Medicine*, *16*(9), 1986–2001. https://doi.org/10.1038/s44321-024-00104-3

Hunter, M. V., Willoughby, P. M., Bruce, A. E. E., & Fernandez-Gonzalez, R. (2018). Oxidative Stress Orchestrates Cell Polarity to Promote Embryonic Wound Healing. *Developmental Cell*, *47*(3), 377-387.e4. https://doi.org/10.1016/j.devcel.2018.10.013

Inoue, M., Sakuta, N., Watanabe, S., Zhang, Y., Yoshikaie, K., Tanaka, Y., Ushioda, R., Kato, Y., Takagi, J., Tsukazaki, T., Nagata, K., & Inaba, K. (2019). Structural Basis of Sarco/Endoplasmic Reticulum Ca2+-ATPase 2b Regulation via Transmembrane Helix Interplay. *Cell Reports*, *27*(4), 1221-1230.e3. https://doi.org/10.1016/j.celrep.2019.03.106

Ipponjima, S., Umino, Y., Nagayama, M., & Denda, M. (2020). Live imaging of alterations in cellular morphology and organelles during cornification using an epidermal equivalent model. *Scientific Reports*, *10*(1), 5515. https://doi.org/10.1038/s41598-020-62240-3

Irby, R. B., & Yeatman, T. J. (2000). Role of Src expression and activation in human cancer. *Oncogene*, *19*(49), 5636–5642. https://doi.org/10.1038/sj.onc.1203912

Iso, T., Kedes, L., & Hamamori, Y. (2003). HES and HERP families: Multiple effectors of the Notch signaling pathway. *Journal of Cellular Physiology*, *194*(3), 237–255. https://doi.org/10.1002/jcp.10208

Iso, T., Sartorelli, V., Poizat, C., Iezzi, S., Wu, H. Y., Chung, G., Kedes, L., & Hamamori, Y. (2001). HERP, a novel heterodimer partner of HES/E(spl) in Notch signaling. *Molecular and Cellular Biology*, *21*(17), 6080–6089. https://doi.org/10.1128/MCB.21.17.6080-6089.2001

Iwai, I., Han, H., den Hollander, L., Svensson, S., Ofverstedt, L.-G., Anwar, J., Brewer, J., Bloksgaard, M., Laloeuf, A., Nosek, D., Masich, S., Bagatolli, L. A., Skoglund, U., & Norlén, L. (2012). The human skin barrier is organized as stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety. *The Journal of Investigative Dermatology*, *132*(9), 2215–2225. https://doi.org/10.1038/jid.2012.43

Iwakoshi, N. N., Lee, A.-H., & Glimcher, L. H. (2003). The X-box binding protein-1 transcription factor is required for plasma cell differentiation and the unfolded protein response. *Immunological Reviews*, *194*, 29–38. https://doi.org/10.1034/j.1600-065x.2003.00057.x

Jackson, B., Tilli, C. M. L. J., Hardman, M. J., Avilion, A. A., MacLeod, M. C., Ashcroft, G. S., & Byrne, C. (2005). Late cornified envelope family in differentiating epithelia—Response to calcium and ultraviolet irradiation. *The Journal of Investigative Dermatology*, *124*(5), 1062–1070. https://doi.org/10.1111/j.0022-202X.2005.23699.x

Jamora, C., & Fuchs, E. (2002). Intercellular adhesion, signalling and the cytoskeleton. *Nature Cell Biology*, *4*(4), E101-108. https://doi.org/10.1038/ncb0402-e101

Jensen, A.-M. L., Sørensen, T. L.-M., Olesen, C., Møller, J. V., & Nissen, P. (2006). Modulatory and catalytic modes of ATP binding by the calcium pump. *The EMBO Journal*, *25*(11), 2305–2314. https://doi.org/10.1038/sj.emboj.7601135

Ji, W. B., Joung, S.-Y., Min, B.-W., & Um, J. W. (2018). Surgical excision for non-familial hypertrophic Darier's disease. *ANZ Journal of Surgery*, *88*(1–2), E77–E78. https://doi.org/10.1111/ans.13255

Kageyama, R., & Nakanishi, S. (1997). Helix-loop-helix factors in growth and differentiation of the vertebrate nervous system. *Current Opinion in Genetics & Development*, *7*(5), 659–665. https://doi.org/10.1016/s0959-437x(97)80014-7

Kageyama, R., Ohtsuka, T., & Kobayashi, T. (2007). The Hes gene family: Repressors and oscillators that orchestrate embryogenesis. *Development (Cambridge, England)*, *134*(7), 1243–1251. https://doi.org/10.1242/dev.000786

Kageyama, R., Ohtsuka, T., & Tomita, K. (2000). The bHLH gene Hes1 regulates differentiation of multiple cell types. *Molecules and Cells*, *10*(1), 1–7. https://doi.org/10.1007/s10059-000-0001-0

Kalinin, A. E., Kajava, A. V., & Steinert, P. M. (2002). Epithelial barrier function: Assembly and structural features of the cornified cell envelope. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, *24*(9), 789–800. https://doi.org/10.1002/bies.10144

Kaneko, M., Niinuma, Y., & Nomura, Y. (2003). Activation signal of nuclear factor-kappa B in response to endoplasmic reticulum stress is transduced via IRE1 and tumor necrosis factor receptor-

associated factor 2. *Biological & Pharmaceutical Bulletin*, *26*(7), 931–935. https://doi.org/10.1248/bpb.26.931

Kang, S. A., Seol, J. H., & Kim, J. (2005). The conserved WRPW motif of Hes6 mediates proteasomal degradation. *Biochemical and Biophysical Research Communications*, *332*(1), 33–36. https://doi.org/10.1016/j.bbrc.2005.04.089

Kannan-Thulasiraman, P., Seachrist, D. D., Mahabeleshwar, G. H., Jain, M. K., & Noy, N. (2010). Fatty acid-binding protein 5 and PPARbeta/delta are critical mediators of epidermal growth factor receptor-induced carcinoma cell growth. *The Journal of Biological Chemistry*, *285*(25), 19106–19115. https://doi.org/10.1074/jbc.M109.099770

Kaufman, C. K., & Fuchs, E. (2000). It's got you covered. NF-kappaB in the epidermis. *The Journal of Cell Biology*, *149*(5), 999–1004. https://doi.org/10.1083/jcb.149.5.999

Kawai, T., & Akira, S. (2009). The roles of TLRs, RLRs and NLRs in pathogen recognition. *International Immunology*, *21*(4), 317–337. https://doi.org/10.1093/intimm/dxp017

Kershaw, E. E., & Flier, J. S. (2004). Adipose tissue as an endocrine organ. *The Journal of Clinical Endocrinology and Metabolism*, *89*(6), 2548–2556. https://doi.org/10.1210/jc.2004-0395

Kim, B.-H., Shenoy, A. R., Kumar, P., Bradfield, C. J., & MacMicking, J. D. (2012). IFN-inducible GTPases in host cell defense. *Cell Host & Microbe*, *12*(4), 432–444. https://doi.org/10.1016/j.chom.2012.09.007

Kim, Y.-G., Kamada, N., Shaw, M. H., Warner, N., Chen, G. Y., Franchi, L., & Núñez, G. (2011). The Nod2 sensor promotes intestinal pathogen eradication via the chemokine CCL2-dependent recruitment of inflammatory monocytes. *Immunity*, *34*(5), 769–780. https://doi.org/10.1016/j.immuni.2011.04.013

Klar, J., Schweiger, M., Zimmerman, R., Zechner, R., Li, H., Törmä, H., Vahlquist, A., Bouadjar, B., Dahl, N., & Fischer, J. (2009). Mutations in the fatty acid transport protein 4 gene cause the ichthyosis prematurity syndrome. *American Journal of Human Genetics*, *85*(2), 248–253. https://doi.org/10.1016/j.ajhg.2009.06.021

Knust, E., Schrons, H., Grawe, F., & Campos-Ortega, J. A. (1992). Seven genes of the Enhancer of split complex of Drosophila melanogaster encode helix-loop-helix proteins. *Genetics*, *132*(2), 505–518. https://doi.org/10.1093/genetics/132.2.505

Kobe, B., & Kajava, A. V. (2001). The leucine-rich repeat as a protein recognition motif. *Current Opinion in Structural Biology*, *11*(6), 725–732. https://doi.org/10.1016/s0959-440x(01)00266-4

Kolly, C., Suter, M. M., & Müller, E. J. (2005). Proliferation, cell cycle exit, and onset of terminal differentiation in cultured keratinocytes: Pre-programmed pathways in control of C-Myc and Notch1 prevail over extracellular calcium signals. *The Journal of Investigative Dermatology*, *124*(5), 1014–1025. https://doi.org/10.1111/j.0022-202X.2005.23655.x

Kopan, R., & Ilagan, M. X. G. (2009). The canonical Notch signaling pathway: Unfolding the activation mechanism. *Cell*, *137*(2), 216–233. https://doi.org/10.1016/j.cell.2009.03.045

Kovács, T., Felföldi, F., Papp, B., Pászty, K., Bredoux, R., Enyedi A, null, & Enouf, J. (2001). All three splice variants of the human sarco/endoplasmic reticulum Ca2+-ATPase 3 gene are translated to proteins: A study of their co-expression in platelets and lymphoid cells. *The Biochemical Journal*, *358*(Pt 3), 559–568. https://doi.org/10.1042/0264-6021:3580559

Kowalczyk, A. P., Bornslaeger, E. A., Norvell, S. M., Palka, H. L., & Green, K. J. (1999). Desmosomes: Intercellular adhesive junctions specialized for attachment of intermediate filaments. *International Review of Cytology*, *185*, 237–302. https://doi.org/10.1016/s0074-7696(08)60153-9

Kragballe, K., Steijlen, P. M., Ibsen, H. H., van de Kerkhof, P. C., Esmann, J., Sorensen, L. H., & Axelsen, M. B. (1995). Efficacy, tolerability, and safety of calcipotriol ointment in disorders of keratinization. Results of a randomized, double-blind, vehicle-controlled, right/left comparative study. *Archives of Dermatology*, *131*(5), 556–560.

Krampert, M., Bloch, W., Sasaki, T., Bugnon, P., Rülicke, T., Wolf, E., Aumailley, M., Parks, W. C., & Werner, S. (2004). Activities of the matrix metalloproteinase stromelysin-2 (MMP-10) in matrix

degradation and keratinocyte organization in wounded skin. *Molecular Biology of the Cell*, *15*(12), 5242–5254. https://doi.org/10.1091/mbc.e04-02-0109

Krebs, J., Agellon, L. B., & Michalak, M. (2015). Ca(2+) homeostasis and endoplasmic reticulum (ER) stress: An integrated view of calcium signaling. *Biochemical and Biophysical Research Communications*, *460*(1), 114–121. https://doi.org/10.1016/j.bbrc.2015.02.004

Kuenzli, S., & Saurat, J.-H. (2003). Peroxisome proliferator-activated receptors in cutaneous biology. *The British Journal of Dermatology*, *149*(2), 229–236. https://doi.org/10.1046/j.1365-2133.2003.05532.x

LaFoya, B., Munroe, J. A., Pu, X., & Albig, A. R. (2018). Src kinase phosphorylates Notch1 to inhibit MAML binding. *Scientific Reports*, *8*(1), 15515. https://doi.org/10.1038/s41598-018-33920-y

Lai, E. C. (2004). Notch signaling: Control of cell communication and cell fate. *Development (Cambridge, England)*, *131*(5), 965–973. https://doi.org/10.1242/dev.01074

Lavrijsen, A. P., Oestmann, E., Hermans, J., Boddé, H. E., Vermeer, B. J., & Ponec, M. (1993). Barrier function parameters in various keratinization disorders: Transepidermal water loss and vascular response to hexyl nicotinate. *The British Journal of Dermatology*, *129*(5), 547–553. https://doi.org/10.1111/j.1365-2133.1993.tb00482.x

Lee, E. H., Kim, S. S., & Seo, S. R. (2017). Pyrrolidine dithiocarbamate (PDTC) inhibits inflammatory signaling via expression of regulator of calcineurin activity 1 (RCAN1): Anti-inflammatory mechanism of PDTC through RCAN1 induction. *Biochemical Pharmacology*, *143*, 107–117. https://doi.org/10.1016/j.bcp.2017.07.011

Lee, J. E., Morrison, W., & Hollien, J. (2018). Hairy and enhancer of split 1 (HES1) protects cells from endoplasmic reticulum stress-induced apoptosis through repression of GADD34. *The Journal of Biological Chemistry*, *293*(16), 5947–5955. https://doi.org/10.1074/jbc.RA118.002124

Lee, S. E., & Lee, S. H. (2018). Skin Barrier and Calcium. *Annals of Dermatology*, *30*(3), 265–275. https://doi.org/10.5021/ad.2018.30.3.265

Lee, S.-E., Kang, Y. C., Kim, Y., Kim, S., Yu, S.-H., Park, J. H., Kim, I.-H., Kim, H.-Y., Han, K., Lee, H. K., Kim, S.-H., & Kim, C.-H. (2022). Preferred Migration of Mitochondria toward Cells and Tissues with Mitochondrial Damage. *International Journal of Molecular Sciences*, *23*(24), 15734. https://doi.org/10.3390/ijms232415734

Lefèvre-Utile, A., Saichi, M., Oláh, P., Delord, M., Homey, B., Soumelis, V., & MAARS consortium. (2022). Transcriptome-based identification of novel endotypes in adult atopic dermatitis. *Allergy*, *77*(5), 1486–1498. https://doi.org/10.1111/all.15150

Lefort, K., & Dotto, G. P. (2004). Notch signaling in the integrated control of keratinocyte growth/differentiation and tumor suppression. *Seminars in Cancer Biology*, *14*(5), 374–386. https://doi.org/10.1016/j.semcancer.2004.04.017

Legrand, A., Darrigade, A.-S., Taieb, A., Milpied, B., & Seneschal, J. (2020). Response to low-dose intravenous immunoglobulin in a case of recalcitrant Darier disease. *JAAD Case Reports*, *6*(3), 189–191. https://doi.org/10.1016/j.jdcr.2020.01.007

Leinonen, P. T., Myllylä, R. M., Hägg, P. M., Tuukkanen, J., Koivunen, J., Peltonen, S., Oikarinen, A., Korkiamäki, T., & Peltonen, J. (2005). Keratinocytes cultured from patients with Hailey-Hailey disease and Darier disease display distinct patterns of calcium regulation. *The British Journal of Dermatology*, *153*(1), 113–117. https://doi.org/10.1111/j.1365-2133.2005.06623.x

Letulé, V., Herzinger, T., Ruzicka, T., & Molin, S. (2013). Treatment of Darier disease with oral alitretinoin. *Clinical and Experimental Dermatology*, *38*(5), 523–525. https://doi.org/10.1111/ced.12078

Li, L., Tucker, R. W., Hennings, H., & Yuspa, S. H. (1995a). Chelation of intracellular Ca2+ inhibits murine keratinocyte differentiation in vitro. *Journal of Cellular Physiology*, *163*(1), 105–114. https://doi.org/10.1002/jcp.1041630112

Li, L., Tucker, R. W., Hennings, H., & Yuspa, S. H. (1995b). Inhibitors of the intracellular Ca(2+)-ATPase in cultured mouse keratinocytes reveal components of terminal differentiation that are regulated by distinct intracellular Ca2+ compartments. *Cell Growth & Differentiation: The Molecular Biology Journal of the American Association for Cancer Research*, *6*(9), 1171–1184.

Li, N., Park, M., Xiao, S., Liu, Z., & Diaz, L. A. (2017). ER-to-Golgi blockade of nascent desmosomal cadherins in SERCA2-inhibited keratinocytes: Implications for Darier's disease. *Traffic (Copenhagen, Denmark)*, *18*(4), 232–241. https://doi.org/10.1111/tra.12470

Li, Q., Lu, Q., Hwang, J. Y., Büscher, D., Lee, K. F., Izpisua-Belmonte, J. C., & Verma, I. M. (1999). IKK1-deficient mice exhibit abnormal development of skin and skeleton. *Genes & Development*, *13*(10), 1322–1328. https://doi.org/10.1101/gad.13.10.1322

Liu, N., Matsumura, H., Kato, T., Ichinose, S., Takada, A., Namiki, T., Asakawa, K., Morinaga, H., Mohri, Y., De Arcangelis, A., Geroges-Labouesse, E., Nanba, D., & Nishimura, E. K. (2019). Stem cell competition orchestrates skin homeostasis and ageing. *Nature*, *568*(7752), 344–350. https://doi.org/10.1038/s41586-019-1085-7

Logeat, F., Bessia, C., Brou, C., LeBail, O., Jarriault, S., Seidah, N. G., & Israël, A. (1998). The Notch1 receptor is cleaved constitutively by a furin-like convertase. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(14), 8108–8112. https://doi.org/10.1073/pnas.95.14.8108

Luo, Y., & Haltiwanger, R. S. (2005). O-fucosylation of notch occurs in the endoplasmic reticulum. *The Journal of Biological Chemistry*, *280*(12), 11289–11294. https://doi.org/10.1074/jbc.M414574200

Lytton, J., & MacLennan, D. H. (1988). Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca2+-ATPase gene. *The Journal of Biological Chemistry*, *263*(29), 15024–15031.

Lytton, J., Westlin, M., Burk, S. E., Shull, G. E., & MacLennan, D. H. (1992). Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *The Journal of Biological Chemistry*, *267*(20), 14483–14489.

MacLennan, D. H., Brandl, C. J., Korczak, B., & Green, N. M. (1985). Amino-acid sequence of a Ca2+ + Mg2+-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence. *Nature*, *316*(6030), 696–700. https://doi.org/10.1038/316696a0

Malumbres, M. (2014). Cyclin-dependent kinases. *Genome Biology*, *15*(6), 122. https://doi.org/10.1186/gb4184

Manning, D., Dart, C., & Evans, R. L. (2022). Store-operated calcium channels in skin. *Frontiers in Physiology*, *13*, 1033528. https://doi.org/10.3389/fphys.2022.1033528

Marchi, S., Patergnani, S., Missiroli, S., Morciano, G., Rimessi, A., Wieckowski, M. R., Giorgi, C., & Pinton, P. (2018). Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. *Cell Calcium*, *69*, 62–72. https://doi.org/10.1016/j.ceca.2017.05.003

Maretzky, T., Scholz, F., Köten, B., Proksch, E., Saftig, P., & Reiss, K. (2008). ADAM10-mediated E-cadherin release is regulated by proinflammatory cytokines and modulates keratinocyte cohesion in eczematous dermatitis. *The Journal of Investigative Dermatology*, *128*(7), 1737–1746. https://doi.org/10.1038/sj.jid.5701242

Marino, M., Stoilova, T., Giorgi, C., Bachi, A., Cattaneo, A., Auricchio, A., Pinton, P., & Zito, E. (2015). SEPN1, an endoplasmic reticulum-localized selenoprotein linked to skeletal muscle pathology, counteracts hyperoxidation by means of redox-regulating SERCA2 pump activity. *Human Molecular Genetics*, *24*(7), 1843–1855. https://doi.org/10.1093/hmg/ddu602

Martin, V., Bredoux, R., Corvazier, E., Van Gorp, R., Kovacs, T., Gelebart, P., & Enouf, J. (2002). Three novel sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) 3 isoforms. Expression, regulation, and function of the membranes of the SERCA3 family. *The Journal of Biological Chemistry*, *277*(27), 24442–24452. https://doi.org/10.1074/jbc.M202011200

Mašek, J., & Andersson, E. R. (2017). The developmental biology of genetic Notch disorders. *Development (Cambridge, England)*, *144*(10), 1743–1763. https://doi.org/10.1242/dev.148007

Massi, D., & Panelos, J. (2012). Notch signaling and the developing skin epidermis. *Advances in Experimental Medicine and Biology*, *727*, 131–141. https://doi.org/10.1007/978-1-4614-0899-4_10

Matsuyoshi, N., Tanaka, T., Toda, K., Okamoto, H., Furukawa, F., & Imamura, S. (1995). Soluble E-cadherin: A novel cutaneous disease marker. *The British Journal of Dermatology*, *132*(5), 745–749. https://doi.org/10.1111/j.1365-2133.1995.tb00720.x

Mauro, T., Bench, G., Sidderas-Haddad, E., Feingold, K., Elias, P., & Cullander, C. (1998). Acute barrier perturbation abolishes the Ca2+ and K+ gradients in murine epidermis: Quantitative measurement using PIXE. *The Journal of Investigative Dermatology*, *111*(6), 1198–1201. https://doi.org/10.1046/j.1523-1747.1998.00421.x

McKenna, K. E., Walsh, M. Y., & Burrows, D. (1999). Treatment of unilateral Darier's disease with topical isotretinoin. *Clinical and Experimental Dermatology*, *24*(5), 425–427. https://doi.org/10.1046/j.1365-2230.1999.00519.x

Meldolesi, J., & Pozzan, T. (1998). The endoplasmic reticulum Ca2+ store: A view from the lumen. *Trends in Biochemical Sciences*, *23*(1), 10–14. https://doi.org/10.1016/s0968-0004(97)01143-2

Mellem, D., Sattler, M., Pagel-Wolff, S., Jaspers, S., Wenck, H., Rübhausen, M. A., & Fischer, F. (2017). Fragmentation of the mitochondrial network in skin in vivo. *PloS One*, *12*(6), e0174469. https://doi.org/10.1371/journal.pone.0174469

Menon, G. K., & Elias, P. M. (1991). Ultrastructural localization of calcium in psoriatic and normal human epidermis. *Archives of Dermatology*, *127*(1), 57–63.

Menon, G. K., Elias, P. M., Lee, S. H., & Feingold, K. R. (1992). Localization of calcium in murine epidermis following disruption and repair of the permeability barrier. *Cell and Tissue Research*, *270*(3), 503–512. https://doi.org/10.1007/BF00645052

Michalak, M., Groenendyk, J., Szabo, E., Gold, L. I., & Opas, M. (2009). Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. *The Biochemical Journal*, *417*(3), 651–666. https://doi.org/10.1042/BJ20081847

Millán-Parrilla, F., Rodrigo-Nicolás, B., Molés-Poveda, P., Armengot-Carbó, M., Quecedo-Estébanez, E., & Gimeno-Carpio, E. (2014). Improvement of Darier disease with diclofenac sodium 3% gel. *Journal of the American Academy of Dermatology*, *70*(4), e89–e90. https://doi.org/10.1016/j.jaad.2013.11.033

Mills, K. H. G. (2023). IL-17 and IL-17-producing cells in protection versus pathology. *Nature Reviews. Immunology*, *23*(1), 38–54. https://doi.org/10.1038/s41577-022-00746-9

Miyauchi, Y., Daiho, T., Yamasaki, K., Takahashi, H., Ishida-Yamamoto, A., Danko, S., Suzuki, H., & Iizuka, H. (2006). Comprehensive analysis of expression and function of 51 sarco(endo)plasmic reticulum Ca2+-ATPase mutants associated with Darier disease. *The Journal of Biological Chemistry*, *281*(32), 22882–22895. https://doi.org/10.1074/jbc.M601966200

Mizuguchi, S., Gotoh, K., Nakashima, Y., Setoyama, D., Takata, Y., Ohga, S., & Kang, D. (2021). Mitochondrial Reactive Oxygen Species Are Essential for the Development of Psoriatic Inflammation. *Frontiers in Immunology*, *12*, 714897. https://doi.org/10.3389/fimmu.2021.714897

Møller, J. V., Nissen, P., Sørensen, T. L.-M., & le Maire, M. (2005). Transport mechanism of the sarcoplasmic reticulum Ca2+ -ATPase pump. *Current Opinion in Structural Biology*, *15*(4), 387–393. https://doi.org/10.1016/j.sbi.2005.06.005

Møller, J. V., Olesen, C., Jensen, A.-M. L., & Nissen, P. (2005). The structural basis for coupling of Ca2+ transport to ATP hydrolysis by the sarcoplasmic reticulum Ca2+-ATPase. *Journal of Bioenergetics and Biomembranes*, *37*(6), 359–364. https://doi.org/10.1007/s10863-005-9471-2

Moore, S. M., Holt, V. V., Malpass, L. R., Hines, I. N., & Wheeler, M. D. (2015). Fatty acid-binding protein 5 limits the anti-inflammatory response in murine macrophages. *Molecular Immunology*, *67*(2 Pt B), 265–275. https://doi.org/10.1016/j.molimm.2015.06.001

Moreci, R. S., & Lechler, T. (2020). Epidermal structure and differentiation. *Current Biology: CB*, *30*(4), R144–R149. https://doi.org/10.1016/j.cub.2020.01.004

Moriyama, M., Durham, A.-D., Moriyama, H., Hasegawa, K., Nishikawa, S., Radtke, F., & Osawa, M. (2008). Multiple roles of Notch signaling in the regulation of epidermal development. *Developmental Cell*, *14*(4), 594–604. https://doi.org/10.1016/j.devcel.2008.01.017

Moriyama, M., Moriyama, H., Uda, J., Kubo, H., Nakajima, Y., Goto, A., Morita, T., & Hayakawa, T. (2017). BNIP3 upregulation via stimulation of ERK and JNK activity is required for the protection of keratinocytes from UVB-induced apoptosis. *Cell Death & Disease*, *8*(2), e2576. https://doi.org/10.1038/cddis.2017.4

Moriyama, M., Moriyama, H., Uda, J., Matsuyama, A., Osawa, M., & Hayakawa, T. (2014). BNIP3 plays crucial roles in the differentiation and maintenance of epidermal keratinocytes. *The Journal of Investigative Dermatology*, *134*(6), 1627–1635. https://doi.org/10.1038/jid.2014.11

Müller, E. J., Caldelari, R., Kolly, C., Williamson, L., Baumann, D., Richard, G., Jensen, P., Girling, P., Delprincipe, F., Wyder, M., Balmer, V., & Suter, M. M. (2006). Consequences of depleted SERCA2-gated calcium stores in the skin. *The Journal of Investigative Dermatology*, *126*(4), 721–731. https://doi.org/10.1038/sj.jid.5700091

Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Tian, X., Pan, D. J., Ray, W. J., & Kopan, R. (2000). A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. *Molecular Cell*, *5*(2), 197–206. https://doi.org/10.1016/s1097-2765(00)80416-5

Mundt, K. E., Golsteyn, R. M., Lane, H. A., & Nigg, E. A. (1997). On the regulation and function of human polo-like kinase 1 (PLK1): Effects of overexpression on cell cycle progression. *Biochemical and Biophysical Research Communications*, *239*(2), 377–385. https://doi.org/10.1006/bbrc.1997.7378

Munro, C. S. (1992). The phenotype of Darier's disease: Penetrance and expressivity in adults and children. *The British Journal of Dermatology*, *127*(2), 126–130. https://doi.org/10.1111/j.1365-2133.1992.tb08044.x

Nam, Y., Sliz, P., Song, L., Aster, J. C., & Blacklow, S. C. (2006). Structural basis for cooperativity in recruitment of MAML coactivators to Notch transcription complexes. *Cell*, *124*(5), 973–983. https://doi.org/10.1016/j.cell.2005.12.037

Nellen, R. G. L., Steijlen, P. M., van Steensel, M. A. M., Vreeburg, M., European Professional Contributors, Frank, J., & van Geel, M. (2017). Mendelian Disorders of Cornification Caused by Defects in Intracellular Calcium Pumps: Mutation Update and Database for Variants in ATP2A2 and ATP2C1 Associated with Darier Disease and Hailey-Hailey Disease. *Human Mutation*, *38*(4), 343–356. https://doi.org/10.1002/humu.23164

Nestle, F. O., Di Meglio, P., Qin, J.-Z., & Nickoloff, B. J. (2009). Skin immune sentinels in health and disease. *Nature Reviews. Immunology*, *9*(10), 679–691. https://doi.org/10.1038/nri2622

Ng, D. C., Shafaee, S., Lee, D., & Bikle, D. D. (2000). Requirement of an AP-1 site in the calcium response region of the involucrin promoter. *The Journal of Biological Chemistry*, *275*(31), 24080–24088. https://doi.org/10.1074/jbc.M002508200

Ni, X., & Lai, Y. (2020). Keratinocyte: A trigger or an executor of psoriasis? *Journal of Leukocyte Biology*, *108*(2), 485–491. https://doi.org/10.1002/JLB.5MR0120-439R

Nickoloff, B. J., Qin, J.-Z., Chaturvedi, V., Denning, M. F., Bonish, B., & Miele, L. (2002). Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-kappaB and PPARgamma. *Cell Death and Differentiation*, *9*(8), 842–855. https://doi.org/10.1038/sj.cdd.4401036

Nicolas, M., Wolfer, A., Raj, K., Kummer, J. A., Mill, P., van Noort, M., Hui, C., Clevers, H., Dotto, G. P., & Radtke, F. (2003). Notch1 functions as a tumor suppressor in mouse skin. *Nature Genetics*, *33*(3), 416–421. https://doi.org/10.1038/ng1099

Niessen, C. (2010). Regulation of cell and tissue polarity: Implications for skin homeostasis and disease. *Expert Review of Dermatology*.

https://www.academia.edu/116424752/Regulation_of_cell_and_tissue_polarity_implications_for_skin_homeostasis_and_disease

Niessen, C. M. (2007). Tight junctions/adherens junctions: Basic structure and function. *The Journal of Investigative Dermatology*, *127*(11), 2525–2532. https://doi.org/10.1038/sj.jid.5700865

Niessen, C. M., & Gottardi, C. J. (2008). Molecular components of the adherens junction. *Biochimica Et Biophysica Acta*, *1778*(3), 562–571. https://doi.org/10.1016/j.bbamem.2007.12.015

Nopriyati, Suherman, A. L., Yahya, Y. F., & Devi, M. (2022). The Role of Calcium in the Skin Barrier. *Bioscientia Medicina : Journal of Biomedicine and Translational Research*, *6*(7), Articolo 7. https://doi.org/10.37275/bsm.v6i7.543

Nowell, C., & Radtke, F. (2013). Cutaneous Notch signaling in health and disease. *Cold Spring Harbor Perspectives in Medicine*, *3*(12), a017772. https://doi.org/10.1101/cshperspect.a017772

Ntziachristos, P., Lim, J. S., Sage, J., & Aifantis, I. (2014). From fly wings to targeted cancer therapies: A centennial for notch signaling. *Cancer Cell*, *25*(3), 318–334. https://doi.org/10.1016/j.ccr.2014.02.018

Nürnberger, T., Brunner, F., Kemmerling, B., & Piater, L. (2004). Innate immunity in plants and animals: Striking similarities and obvious differences. *Immunological Reviews*, *198*, 249–266. https://doi.org/10.1111/j.0105-2896.2004.0119.x

Oblong, J. E., Bowman, A., Rovito, H. A., Jarrold, B. B., Sherrill, J. D., Black, M. R., Nelson, G., Kimball, A. B., & Birch-Machin, M. A. (2020). Metabolic dysfunction in human skin: Restoration of mitochondrial integrity and metabolic output by nicotinamide (niacinamide) in primary dermal fibroblasts from older aged donors. *Aging Cell*, *19*(10), e13248. https://doi.org/10.1111/acel.13248

O'Brien, K. F., Fricke, M. A., Kent, R. A., & DeKlotz, C. M. C. (2020). Laser Treatment of Darier Disease: Report of Two Cases and Systematic Review of the Literature. *Journal of Lasers in Medical Sciences*, *11*(4), 395–404. https://doi.org/10.34172/jlms.2020.63

Oeckinghaus, A., & Ghosh, S. (2009). The NF-kappaB family of transcription factors and its regulation. *Cold Spring Harbor Perspectives in Biology*, *1*(4), a000034. https://doi.org/10.1101/cshperspect.a000034

Ogawa, E., Owada, Y., Ikawa, S., Adachi, Y., Egawa, T., Nemoto, K., Suzuki, K., Hishinuma, T., Kawashima, H., Kondo, H., Muto, M., Aiba, S., & Okuyama, R. (2011). Epidermal FABP (FABP5) regulates keratinocyte differentiation by 13(S)-HODE-mediated activation of the NF-κB signaling pathway. *The Journal of Investigative Dermatology*, *131*(3), 604–612. https://doi.org/10.1038/jid.2010.342

Ohsako, S., Hyer, J., Panganiban, G., Oliver, I., & Caudy, M. (1994). Hairy function as a DNA-binding helix-loop-helix repressor of Drosophila sensory organ formation. *Genes & Development*, *8*(22), 2743–2755. https://doi.org/10.1101/gad.8.22.2743

Okajima, T., & Irvine, K. D. (2002). Regulation of notch signaling by o-linked fucose. *Cell*, *111*(6), 893–904. https://doi.org/10.1016/s0092-8674(02)01114-5

Olesen, C., Picard, M., Winther, A.-M. L., Gyrup, C., Morth, J. P., Oxvig, C., Møller, J. V., & Nissen, P. (2007). The structural basis of calcium transport by the calcium pump. *Nature*, *450*(7172), 1036–1042. https://doi.org/10.1038/nature06418

Orihuela, E., Tyring, S. K., Pow-Sang, M., Dozier, S., Cirelli, R., Arany, I., Rady, P., & Sanchez, R. (1995). Development of human papillomavirus type 16 associated squamous cell carcinoma of the scrotum in a patient with Darier's disease treated with systemic isotretinoin. *The Journal of Urology*, *153*(6), 1940–1943.

Oriss, T. B., Krishnamoorthy, N., Ray, P., & Ray, A. (2014). Dendritic cell c-kit signaling and adaptive immunity: Implications for the upper airways. *Current Opinion in Allergy and Clinical Immunology*, *14*(1), 7–12. https://doi.org/10.1097/ACI.0000000000000019

Osterland, C. K., Wilkinson, R. D., & St Louis, E. A. (1990). Expression of c-myc protein in skin and synovium in psoriasis and psoriatic arthritis. *Clinical and Experimental Rheumatology*, *8*(2), 145–150.

Otto, T., & Sicinski, P. (2017). Cell cycle proteins as promising targets in cancer therapy. *Nature Reviews. Cancer*, *17*(2), 93–115. https://doi.org/10.1038/nrc.2016.138

Owada, Y., Suzuki, I., Noda, T., & Kondo, H. (2002). Analysis on the phenotype of E-FABP-gene knockout mice. *Molecular and Cellular Biochemistry*, *239*(1–2), 83–86.

Pagliaro, L., Marchesini, M., & Roti, G. (2021). Targeting oncogenic Notch signaling with SERCA inhibitors. *Journal of Hematology & Oncology*, *14*(1), 8. https://doi.org/10.1186/s13045-020-01015-9

Paillusson, S., Stoica, R., Gomez-Suaga, P., Lau, D. H. W., Mueller, S., Miller, T., & Miller, C. C. J. (2016). There's Something Wrong with my MAM; the ER-Mitochondria Axis and Neurodegenerative Diseases. *Trends in Neurosciences*, *39*(3), 146–157. https://doi.org/10.1016/j.tins.2016.01.008

Palacios-Álvarez, I., Andrés-Ramos, I., Silva, M. Y., & Simal, G. (2017). Treatment of Darier's disease with diclofenac sodium 3% gel. *Dermatologic Therapy*, *30*(3). https://doi.org/10.1111/dth.12478

Pang, C. Y., Lee, H. C., Yang, J. H., & Wei, Y. H. (1994). Human skin mitochondrial DNA deletions associated with light exposure. *Archives of Biochemistry and Biophysics*, *312*(2), 534–538. https://doi.org/10.1006/abbi.1994.1342

Paroush, Z., Finley, R. L., Kidd, T., Wainwright, S. M., Ingham, P. W., Brent, R., & Ish-Horowicz, D. (1994). Groucho is required for Drosophila neurogenesis, segmentation, and sex determination and interacts directly with hairy-related bHLH proteins. *Cell*, *79*(5), 805–815. https://doi.org/10.1016/0092-8674(94)90070-1

Pasparakis, M., Haase, I., & Nestle, F. O. (2014). Mechanisms regulating skin immunity and inflammation. *Nature Reviews. Immunology*, *14*(5), 289–301. https://doi.org/10.1038/nri3646

Pastar, I., Stojadinovic, O., Yin, N. C., Ramirez, H., Nusbaum, A. G., Sawaya, A., Patel, S. B., Khalid, L., Isseroff, R. R., & Tomic-Canic, M. (2014). Epithelialization in Wound Healing: A Comprehensive Review. *Advances in Wound Care*, *3*(7), 445–464. https://doi.org/10.1089/wound.2013.0473

PELS, I. R., & GOODMAN, M. H. (1939). CRITERIA FOR THE HISTOLOGIC DIAGNOSIS OF KERATOSIS FOLLICULARIS (DARIER): REPORT OF A CASE WITH VESICULATION. *Archives of Dermatology and Syphilology*, *39*(3), 438–455. https://doi.org/10.1001/archderm.1939.01480210053006

Pérez-Carmona, L., Fleta-Asín, B., Moreno-García-Del-Real, C., & Jaén-Olasolo, P. (2011). Successful treatment of Darier's disease with topical pimecrolimus. *European Journal of Dermatology: EJD*, *21*(2), 301–302. https://doi.org/10.1684/ejd.2011.1309

Perez-Moreno, M., Jamora, C., & Fuchs, E. (2003). Sticky business: Orchestrating cellular signals at adherens junctions. *Cell*, *112*(4), 535–548. https://doi.org/10.1016/s0092-8674(03)00108-9

Periz, G., & Fortini, M. E. (1999). Ca(2+)-ATPase function is required for intracellular trafficking of the Notch receptor in Drosophila. *The EMBO Journal*, *18*(21), 5983–5993. https://doi.org/10.1093/emboj/18.21.5983

Pettit, C., Ulman, C. A., Spohn, G., & Kaffenberger, J. (2018). A case of segmental Darier disease treated with doxycycline monotherapy. *Dermatology Online Journal*, *24*(3), 13030/qt2827h6qq.

Picard, M., & Shirihai, O. S. (2022). Mitochondrial signal transduction. *Cell Metabolism*, *34*(11), 1620–1653. https://doi.org/10.1016/j.cmet.2022.10.008

Pickering, C. R., Zhou, J. H., Lee, J. J., Drummond, J. A., Peng, S. A., Saade, R. E., Tsai, K. Y., Curry, J. L., Tetzlaff, M. T., Lai, S. Y., Yu, J., Muzny, D. M., Doddapaneni, H., Shinbrot, E., Covington, K. R., Zhang, J., Seth, S., Caulin, C., Clayman, G. L., … Frederick, M. J. (2014). Mutational landscape of aggressive cutaneous squamous cell carcinoma. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *20*(24), 6582–6592. https://doi.org/10.1158/1078-0432.CCR-14-1768

Plager, D. A., Leontovich, A. A., Henke, S. A., Davis, M. D. P., McEvoy, M. T., Sciallis, G. F., & Pittelkow, M. R. (2007). Early cutaneous gene transcription changes in adult atopic dermatitis and potential clinical implications. *Experimental Dermatology*, *16*(1), 28–36. https://doi.org/10.1111/j.1600-0625.2006.00504.x

Plikus, M. V., Gay, D. L., Treffeisen, E., Wang, A., Supapannachart, R. J., & Cotsarelis, G. (2012). Epithelial stem cells and implications for wound repair. *Seminars in Cell & Developmental Biology*, *23*(9), 946–953. https://doi.org/10.1016/j.semcdb.2012.10.001

Prasad, V., Boivin, G. P., Miller, M. L., Liu, L. H., Erwin, C. R., Warner, B. W., & Shull, G. E. (2005). Haploinsufficiency of Atp2a2, encoding the sarco(endo)plasmic reticulum Ca2+-ATPase isoform 2 Ca2+ pump, predisposes mice to squamous cell tumors via a novel mode of cancer susceptibility. *Cancer Research*, *65*(19), 8655–8661. https://doi.org/10.1158/0008-5472.CAN-05-0026

Presland, R. B., Bassuk, J. A., Kimball, J. R., & Dale, B. A. (1995). Characterization of two distinct calcium-binding sites in the amino-terminus of human profilaggrin. *The Journal of Investigative Dermatology*, *104*(2), 218–223. https://doi.org/10.1111/1523-1747.ep12612770

Proksch, E., Brandner, J. M., & Jensen, J.-M. (2008). The skin: An indispensable barrier. *Experimental Dermatology*, *17*(12), 1063–1072. https://doi.org/10.1111/j.1600-0625.2008.00786.x

Prost-Squarcioni, C., Fraitag, S., Heller, M., & Boehm, N. (2008). [Functional histology of dermis]. *Annales De Dermatologie Et De Venereologie*, *135*(1 Pt 2), 1S5-20. https://doi.org/10.1016/S0151-9638(08)70206-0

Quaranta, M., Knapp, B., Garzorz, N., Mattii, M., Pullabhatla, V., Pennino, D., Andres, C., Traidl-Hoffmann, C., Cavani, A., Theis, F. J., Ring, J., Schmidt-Weber, C. B., Eyerich, S., & Eyerich, K. (2014). Intraindividual genome expression analysis reveals a specific molecular signature of psoriasis and eczema. *Science Translational Medicine*, *6*(244), 244ra90. https://doi.org/10.1126/scitranslmed.3008946

Quondamatteo, F., Reinhardt, D. P., Charbonneau, N. L., Pophal, G., Sakai, L. Y., & Herken, R. (2002). Fibrillin-1 and fibrillin-2 in human embryonic and early fetal development. *Matrix Biology: Journal of the International Society for Matrix Biology*, *21*(8), 637–646. https://doi.org/10.1016/s0945-053x(02)00100-2

Rand, M. D., Lindblom, A., Carlson, J., Villoutreix, B. O., & Stenflo, J. (1997). Calcium binding to tandem repeats of EGF-like modules. Expression and characterization of the EGF-like modules of human Notch-1 implicated in receptor-ligand interactions. *Protein Science: A Publication of the Protein Society*, *6*(10), 2059–2071. https://doi.org/10.1002/pro.5560061002

Rangarajan, A., Talora, C., Okuyama, R., Nicolas, M., Mammucari, C., Oh, H., Aster, J. C., Krishna, S., Metzger, D., Chambon, P., Miele, L., Aguet, M., Radtke, F., & Dotto, G. P. (2001). Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *The EMBO Journal*, *20*(13), 3427–3436. https://doi.org/10.1093/emboj/20.13.3427

Ray, W. J., Yao, M., Nowotny, P., Mumm, J., Zhang, W., Wu, J. Y., Kopan, R., & Goate, A. M. (1999). Evidence for a physical interaction between presenilin and Notch. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(6), 3263–3268. https://doi.org/10.1073/pnas.96.6.3263

Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, L., Cherbas, P., & Artavanis-Tsakonas, S. (1991). Specific EGF repeats of Notch mediate interactions with Delta and Serrate: Implications for Notch as a multifunctional receptor. *Cell*, *67*(4), 687–699. https://doi.org/10.1016/0092-8674(91)90064-6

Rimessi, A., Previati, M., Nigro, F., Wieckowski, M. R., & Pinton, P. (2016). Mitochondrial reactive oxygen species and inflammation: Molecular mechanisms, diseases and promising therapies. *The International Journal of Biochemistry & Cell Biology*, *81*(Pt B), 281–293. https://doi.org/10.1016/j.biocel.2016.06.015

Ringpfeil, F., Raus, A., DiGiovanna, J. J., Korge, B., Harth, W., Mazzanti, C., Uitto, J., Bale, S. J., & Richard, G. (2001). Darier disease—Novel mutations in ATP2A2 and genotype-phenotype correlation. *Experimental Dermatology*, *10*(1), 19–27. https://doi.org/10.1034/j.1600-0625.2001.100103.x

Rivier, M., Safonova, I., Lebrun, P., Griffiths, C. E., Ailhaud, G., & Michel, S. (1998). Differential expression of peroxisome proliferator-activated receptor subtypes during the differentiation of human

keratinocytes. *The Journal of Investigative Dermatology*, *111*(6), 1116–1121. https://doi.org/10.1046/j.1523-1747.1998.00439.x

Rodriguez, L. M., Kazemi, T., Cheng, C. E., Kang, J. J., Beron, P. J., Kozma, B. D., & Gee, S. N. (2018). Focal multimodality radiation therapy: A promising treatment for recalcitrant Darier disease. *Dermatologic Therapy*, *31*(4), e12641. https://doi.org/10.1111/dth.12641

Roti, G., Carlton, A., Ross, K. N., Markstein, M., Pajcini, K., Su, A. H., Perrimon, N., Pear, W. S., Kung, A. L., Blacklow, S. C., Aster, J. C., & Stegmaier, K. (2013). Complementary genomic screens identify SERCA as a therapeutic target in NOTCH1 mutated cancer. *Cancer Cell*, *23*(3), 390–405. https://doi.org/10.1016/j.ccr.2013.01.015

Roti, G., & Stegmaier, K. (2014). New Approaches to Target T-ALL. *Frontiers in Oncology*, *4*, 170. https://doi.org/10.3389/fonc.2014.00170

Rousselle, P., Braye, F., & Dayan, G. (2019). Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. *Advanced Drug Delivery Reviews*, *146*, 344–365. https://doi.org/10.1016/j.addr.2018.06.019

Rowland, A. A., & Voeltz, G. K. (2012). Endoplasmic reticulum-mitochondria contacts: Function of the junction. *Nature Reviews. Molecular Cell Biology*, *13*(10), 607–625. https://doi.org/10.1038/nrm3440

Rozpedek, W., Pytel, D., Mucha, B., Leszczynska, H., Diehl, J. A., & Majsterek, I. (2016). The Role of the PERK/eIF2α/ATF4/CHOP Signaling Pathway in Tumor Progression During Endoplasmic Reticulum Stress. *Current Molecular Medicine*, *16*(6), 533–544. https://doi.org/10.2174/1566524016666160523143937

Ruiz-Perez, V. L., Carter, S. A., Healy, E., Todd, C., Rees, J. L., Steijlen, P. M., Carmichael, A. J., Lewis, H. M., Hohl, D., Itin, P., Vahlquist, A., Gobello, T., Mazzanti, C., Reggazini, R., Nagy, G., Munro, C. S., & Strachan, T. (1999). ATP2A2 mutations in Darier's disease: Variant cutaneous phenotypes are associated with missense mutations, but neuropsychiatric features are independent of mutation class. *Human Molecular Genetics*, *8*(9), 1621–1630. https://doi.org/10.1093/hmg/8.9.1621

Ryan, K. M., & Birnie, G. D. (1996). Myc oncogenes: The enigmatic family. *The Biochemical Journal*, *314 (Pt 3)*(Pt 3), 713–721. https://doi.org/10.1042/bj3140713

Sakamuro, D., & Prendergast, G. C. (1999). New Myc-interacting proteins: A second Myc network emerges. *Oncogene*, *18*(19), 2942–2954. https://doi.org/10.1038/sj.onc.1202725

Sakuntabhai, A., Burge, S., Monk, S., & Hovnanian, A. (1999). Spectrum of novel ATP2A2 mutations in patients with Darier's disease. *Human Molecular Genetics*, *8*(9), 1611–1619. https://doi.org/10.1093/hmg/8.9.1611

Sakuntabhai, A., Ruiz-Perez, V., Carter, S., Jacobsen, N., Burge, S., Monk, S., Smith, M., Munro, C. S., O'Donovan, M., Craddock, N., Kucherlapati, R., Rees, J. L., Owen, M., Lathrop, G. M., Monaco, A. P., Strachan, T., & Hovnanian, A. (1999). Mutations in ATP2A2, encoding a Ca2+ pump, cause Darier disease. *Nature Genetics*, *21*(3), 271–277. https://doi.org/10.1038/6784

Salvador, J. M., Brown-Clay, J. D., & Fornace, A. J. (2013). Gadd45 in stress signaling, cell cycle control, and apoptosis. *Advances in Experimental Medicine and Biology*, *793*, 1–19. https://doi.org/10.1007/978-1-4614-8289-5_1

Sanchez-Irizarry, C., Carpenter, A. C., Weng, A. P., Pear, W. S., Aster, J. C., & Blacklow, S. C. (2004). Notch subunit heterodimerization and prevention of ligand-independent proteolytic activation depend, respectively, on a novel domain and the LNR repeats. *Molecular and Cellular Biology*, *24*(21), 9265–9273. https://doi.org/10.1128/MCB.24.21.9265-9273.2004

Sasai, Y., Kageyama, R., Tagawa, Y., Shigemoto, R., & Nakanishi, S. (1992). Two mammalian helix-loop-helix factors structurally related to Drosophila hairy and Enhancer of split. *Genes & Development*, *6*(12B), 2620–2634. https://doi.org/10.1101/gad.6.12b.2620

Sato, K., Yamasaki, K., Daiho, T., Miyauchi, Y., Takahashi, H., Ishida-Yamamoto, A., Nakamura, S., Iizuka, H., & Suzuki, H. (2004). Distinct types of abnormality in kinetic properties of three Darier disease-causing sarco(endo)plasmic reticulum Ca2+-ATPase mutants that exhibit normal

expression and high Ca2+ transport activity. *The Journal of Biological Chemistry*, *279*(34), 35595–35603. https://doi.org/10.1074/jbc.M404887200

Savignac, M., Edir, A., Simon, M., & Hovnanian, A. (2011). Darier disease: A disease model of impaired calcium homeostasis in the skin. *Biochimica Et Biophysica Acta*, *1813*(5), 1111–1117. https://doi.org/10.1016/j.bbamcr.2010.12.006

Schmidt, A., & Koch, P. J. (2007). Desmosomes: Just cell adhesion or is there more? *Cell Adhesion & Migration*, *1*(1), 28–32. https://doi.org/10.4161/cam.1.1.4204

Schmidt, E. V. (1999). The role of c-myc in cellular growth control. *Oncogene*, *18*(19), 2988–2996. https://doi.org/10.1038/sj.onc.1202751

Schroeter, E. H., Kisslinger, J. A., & Kopan, R. (1998). Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature*, *393*(6683), 382–386. https://doi.org/10.1038/30756

Schwingen, J., Kaplan, M., & Kurschus, F. C. (2020). Review-Current Concepts in Inflammatory Skin Diseases Evolved by Transcriptome Analysis: In-Depth Analysis of Atopic Dermatitis and Psoriasis. *International Journal of Molecular Sciences*, *21*(3), 699. https://doi.org/10.3390/ijms21030699

Segal, B. H., Grimm, M. J., Khan, A. N. H., Han, W., & Blackwell, T. S. (2012). Regulation of innate immunity by NADPH oxidase. *Free Radical Biology & Medicine*, *53*(1), 72–80. https://doi.org/10.1016/j.freeradbiomed.2012.04.022

Segre, J. (2003). Complex redundancy to build a simple epidermal permeability barrier. *Current Opinion in Cell Biology*, *15*(6), 776–782. https://doi.org/10.1016/j.ceb.2003.10.001

Segre, J. A. (2006). Epidermal barrier formation and recovery in skin disorders. *The Journal of Clinical Investigation*, *116*(5), 1150–1158. https://doi.org/10.1172/JCI28521

Seidah, N. G., & Chrétien, M. (1997). Eukaryotic protein processing: Endoproteolysis of precursor proteins. *Current Opinion in Biotechnology*, *8*(5), 602–607. https://doi.org/10.1016/s0958-1669(97)80036-5

Senoo, M., Pinto, F., Crum, C. P., & McKeon, F. (2007). P63 Is essential for the proliferative potential of stem cells in stratified epithelia. *Cell*, *129*(3), 523–536. https://doi.org/10.1016/j.cell.2007.02.045

Serrels, B., Serrels, A., Mason, S. M., Baldeschi, C., Ashton, G. H., Canel, M., Mackintosh, L. J., Doyle, B., Green, T. P., Frame, M. C., Sansom, O. J., & Brunton, V. G. (2009). A novel Src kinase inhibitor reduces tumour formation in a skin carcinogenesis model. *Carcinogenesis*, *30*(2), 249–257. https://doi.org/10.1093/carcin/bgn278

Sfecci, A., Orion, C., Darrieux, L., Tisseau, L., & Safa, G. (2015). Extensive Darier Disease Successfully Treated with Doxycycline Monotherapy. *Case Reports in Dermatology*, *7*(3), 311–315. https://doi.org/10.1159/000441467

Shahidullah, H., Humphreys, F., & Beveridge, G. W. (1994). Darier's disease: Severe eczematization successfully treated with cyclosporin. *The British Journal of Dermatology*, *131*(5), 713–716. https://doi.org/10.1111/j.1365-2133.1994.tb04990.x

Shen, K., Pender, C. L., Bar-Ziv, R., Zhang, H., Wickham, K., Willey, E., Durieux, J., Ahmad, Q., & Dillin, A. (2022). Mitochondria as Cellular and Organismal Signaling Hubs. *Annual Review of Cell and Developmental Biology*, *38*, 179–218. https://doi.org/10.1146/annurev-cellbio-120420-015303

Shen, T., & Huang, S. (2012). The role of Cdc25A in the regulation of cell proliferation and apoptosis. *Anti-Cancer Agents in Medicinal Chemistry*, *12*(6), 631–639. https://doi.org/10.2174/187152012800617678

Shenoy, A. R., Wellington, D. A., Kumar, P., Kassa, H., Booth, C. J., Cresswell, P., & MacMicking, J. D. (2012). GBP5 promotes NLRP3 inflammasome assembly and immunity in mammals. *Science (New York, N.Y.)*, *336*(6080), 481–485. https://doi.org/10.1126/science.1217141

Simpson, C. L., Patel, D. M., & Green, K. J. (2011). Deconstructing the skin: Cytoarchitectural determinants of epidermal morphogenesis. *Nature Reviews. Molecular Cell Biology*, *12*(9), 565–580. https://doi.org/10.1038/nrm3175

Simpson, C. L., Tokito, M. K., Uppala, R., Sarkar, M. K., Gudjonsson, J. E., & Holzbaur, E. L. F. (2021). NIX initiates mitochondrial fragmentation via DRP1 to drive epidermal differentiation. *Cell Reports*, *34*(5), 108689. https://doi.org/10.1016/j.celrep.2021.108689

Singh, R. (2022). Basal Cells in the Epidermis and Epidermal Differentiation. *Stem Cell Reviews and Reports*, *18*(6), 1883–1891. https://doi.org/10.1007/s12015-021-10256-1

So, J.-S. (2018). Roles of Endoplasmic Reticulum Stress in Immune Responses. *Molecules and Cells*, *41*(8), 705–716. https://doi.org/10.14348/molcells.2018.0241

Sondhi, M., Vashist, S., & Mahajan, V. K. (2020). Darier's Disease - Response to Oral Vitamin A: Report of a Case and Brief Review. *Indian Dermatology Online Journal*, *11*(1), 72–78. https://doi.org/10.4103/idoj.IDOJ_85_19

Song, Y., Li, L., Ou, Y., Gao, Z., Li, E., Li, X., Zhang, W., Wang, J., Xu, L., Zhou, Y., Ma, X., Liu, L., Zhao, Z., Huang, X., Fan, J., Dong, L., Chen, G., Ma, L., Yang, J., … Zhan, Q. (2014). Identification of genomic alterations in oesophageal squamous cell cancer. *Nature*, *509*(7498), 91–95. https://doi.org/10.1038/nature13176

Sørensen, T. L.-M., Møller, J. V., & Nissen, P. (2004). Phosphoryl transfer and calcium ion occlusion in the calcium pump. *Science (New York, N.Y.)*, *304*(5677), 1672–1675. https://doi.org/10.1126/science.1099366

South, A. P., Purdie, K. J., Watt, S. A., Haldenby, S., den Breems, N., Dimon, M., Arron, S. T., Kluk, M. J., Aster, J. C., McHugh, A., Xue, D. J., Dayal, J. H., Robinson, K. S., Rizvi, S. H., Proby, C. M., Harwood, C. A., & Leigh, I. M. (2014). NOTCH1 mutations occur early during cutaneous squamous cell carcinogenesis. *The Journal of Investigative Dermatology*, *134*(10), 2630–2638. https://doi.org/10.1038/jid.2014.154

Sreedhar, A., Aguilera-Aguirre, L., & Singh, K. K. (2020). Mitochondria in skin health, aging, and disease. *Cell Death & Disease*, *11*(6), 444. https://doi.org/10.1038/s41419-020-2649-z

Sriburi, R., Jackowski, S., Mori, K., & Brewer, J. W. (2004). XBP1: A link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. *The Journal of Cell Biology*, *167*(1), 35–41. https://doi.org/10.1083/jcb.200406136

Srivastava, S. S., Alam, H., Patil, S. J., Shrinivasan, R., Raikundalia, S., Chaudhari, P. R., & Vaidya, M. M. (2018). Keratin 5/14-mediated cell differentiation and transformation are regulated by TAp63 and Notch-1 in oral squamous cell carcinoma-derived cells. *Oncology Reports*, *39*(5), 2393–2401. https://doi.org/10.3892/or.2018.6298

Staller, P., Peukert, K., Kiermaier, A., Seoane, J., Lukas, J., Karsunky, H., Möröy, T., Bartek, J., Massagué, J., Hänel, F., & Eilers, M. (2001). Repression of p15INK4b expression by Myc through association with Miz-1. *Nature Cell Biology*, *3*(4), 392–399. https://doi.org/10.1038/35070076

Steijlen, P. M., Happle, R., van Muijen, G. N., & van de Kerkhof, P. C. (1991). Topical treatment with 13-cis-retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. *Dermatologica*, *182*(3), 178–183. https://doi.org/10.1159/000247778

Steinert, P. M. (1993). Structure, function, and dynamics of keratin intermediate filaments. *The Journal of Investigative Dermatology*, *100*(6), 729–734. https://doi.org/10.1111/1523-1747.ep12475665

Stout, R., & Birch-Machin, M. (2019). Mitochondria's Role in Skin Ageing. *Biology*, *8*(2), 29. https://doi.org/10.3390/biology8020029

Stransky, N., Egloff, A. M., Tward, A. D., Kostic, A. D., Cibulskis, K., Sivachenko, A., Kryukov, G. V., Lawrence, M. S., Sougnez, C., McKenna, A., Shefler, E., Ramos, A. H., Stojanov, P., Carter, S. L., Voet, D., Cortés, M. L., Auclair, D., Berger, M. F., Saksena, G., … Grandis, J. R. (2011). The mutational landscape of head and neck squamous cell carcinoma. *Science (New York, N.Y.)*, *333*(6046), 1157–1160. https://doi.org/10.1126/science.1208130

Stuart, R. O., Sun, A., Bush, K. T., & Nigam, S. K. (1996). Dependence of epithelial intercellular junction biogenesis on thapsigargin-sensitive intracellular calcium stores. *The Journal of Biological Chemistry*, *271*(23), 13636–13641. https://doi.org/10.1074/jbc.271.23.13636

Stüttgen, G., Ippen, H., & Mahrle, G. (1977). Oral vitamin A acid in treatment of dermatoses with pathologic keratinization. *International Journal of Dermatology*, *16*(6), 500–502. https://doi.org/10.1111/j.1365-4362.1977.tb01863.x

Subramaniam, T., Fauzi, M. B., Lokanathan, Y., & Law, J. X. (2021). The Role of Calcium in Wound Healing. *International Journal of Molecular Sciences*, *22*(12), 6486. https://doi.org/10.3390/ijms22126486

Sugiura, K., Muro, Y., Futamura, K., Matsumoto, K., Hashimoto, N., Nishizawa, Y., Nagasaka, T., Saito, H., Tomita, Y., & Usukura, J. (2009). The unfolded protein response is activated in differentiating epidermal keratinocytes. *The Journal of Investigative Dermatology*, *129*(9), 2126–2135. https://doi.org/10.1038/jid.2009.51

Sun, X., Wu, Y., Herculano, B., & Song, W. (2014). RCAN1 overexpression exacerbates calcium overloading-induced neuronal apoptosis. *PloS One*, *9*(4), e95471. https://doi.org/10.1371/journal.pone.0095471

Szabat, M., Kalynyak, T. B., Lim, G. E., Chu, K. Y., Yang, Y. H., Asadi, A., Gage, B. K., Ao, Z., Warnock, G. L., Piret, J. M., Kieffer, T. J., & Johnson, J. D. (2011). Musashi expression in β-cells coordinates insulin expression, apoptosis and proliferation in response to endoplasmic reticulum stress in diabetes. *Cell Death & Disease*, *2*(11), e232. https://doi.org/10.1038/cddis.2011.119

Taelman, V., Van Wayenbergh, R., Sölter, M., Pichon, B., Pieler, T., Christophe, D., & Bellefroid, E. J. (2004). Sequences downstream of the bHLH domain of the Xenopus hairy-related transcription factor-1 act as an extended dimerization domain that contributes to the selection of the partners. *Developmental Biology*, *276*(1), 47–63. https://doi.org/10.1016/j.ydbio.2004.08.019

Takagi, A., Kamijo, M., & Ikeda, S. (2016). Darier disease. *The Journal of Dermatology*, *43*(3), 275–279. https://doi.org/10.1111/1346-8138.13230

Takebayashi, K., Sasai, Y., Sakai, Y., Watanabe, T., Nakanishi, S., & Kageyama, R. (1994). Structure, chromosomal locus, and promoter analysis of the gene encoding the mouse helix-loop-helix factor HES-1. Negative autoregulation through the multiple N box elements. *The Journal of Biological Chemistry*, *269*(7), 5150–5156.

Takeda, K., Takeuchi, O., Tsujimura, T., Itami, S., Adachi, O., Kawai, T., Sanjo, H., Yoshikawa, K., Terada, N., & Akira, S. (1999). Limb and skin abnormalities in mice lacking IKKalpha. *Science (New York, N.Y.)*, *284*(5412), 313–316. https://doi.org/10.1126/science.284.5412.313

Takeuchi, H., Kantharia, J., Sethi, M. K., Bakker, H., & Haltiwanger, R. S. (2012). Site-specific O-glucosylation of the epidermal growth factor-like (EGF) repeats of notch: Efficiency of glycosylation is affected by proper folding and amino acid sequence of individual EGF repeats. *The Journal of Biological Chemistry*, *287*(41), 33934–33944. https://doi.org/10.1074/jbc.M112.401315

Tavadia, S., Authi, K. S., Hodgins, M. B., & Munro, C. S. (2004). Expression of the sarco/endoplasmic reticulum calcium ATPase type 2 and 3 isoforms in normal skin and Darier's disease. *The British Journal of Dermatology*, *151*(2), 440–445. https://doi.org/10.1111/j.1365-2133.2004.06130.x

Thomas, J. R., Cooke, J. P., & Winkelmann, R. K. (1982). High-dose vitamin A therapy for Darier's disease. *Archives of Dermatology*, *118*(11), 891–894.

Thomas, S. M., & Brugge, J. S. (1997). Cellular functions regulated by Src family kinases. *Annual Review of Cell and Developmental Biology*, *13*, 513–609. https://doi.org/10.1146/annurev.cellbio.13.1.513

Tinkle, C. L., Lechler, T., Pasolli, H. A., & Fuchs, E. (2004). Conditional targeting of E-cadherin in skin: Insights into hyperproliferative and degenerative responses. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(2), 552–557. https://doi.org/10.1073/pnas.0307437100

Toyoshima, C. (2007). Ion Pumping by Calcium ATPase of Sarcoplasmic Reticulum. In S. Ebashi & I. Ohtsuki (A c. Di), *Regulatory Mechanisms of Striated Muscle Contraction* (pp. 295–303). Springer Japan. https://doi.org/10.1007/978-4-431-38453-3_25

Toyoshima, C. (2008). Structural aspects of ion pumping by Ca2+-ATPase of sarcoplasmic reticulum. *Archives of Biochemistry and Biophysics*, *476*(1), 3–11. https://doi.org/10.1016/j.abb.2008.04.017

Toyoshima, C. (2009). How Ca2+-ATPase pumps ions across the sarcoplasmic reticulum membrane. *Biochimica Et Biophysica Acta*, *1793*(6), 941–946. https://doi.org/10.1016/j.bbamcr.2008.10.008

Toyoshima, C., Nakasako, M., Nomura, H., & Ogawa, H. (2000). Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 A resolution. *Nature*, *405*(6787), 647–655. https://doi.org/10.1038/35015017

Travers, K. J., Patil, C. K., Wodicka, L., Lockhart, D. J., Weissman, J. S., & Walter, P. (2000). Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell*, *101*(3), 249–258. https://doi.org/10.1016/s0092-8674(00)80835-1

Tu, C.-L., & Bikle, D. D. (2013). Role of the calcium-sensing receptor in calcium regulation of epidermal differentiation and function. *Best Practice & Research. Clinical Endocrinology & Metabolism*, *27*(3), 415–427. https://doi.org/10.1016/j.beem.2013.03.002

Tu, C.-L., Celli, A., Mauro, T., & Chang, W. (2019). Calcium-Sensing Receptor Regulates Epidermal Intracellular Ca2+ Signaling and Re-Epithelialization after Wounding. *The Journal of Investigative Dermatology*, *139*(4), 919–929. https://doi.org/10.1016/j.jid.2018.09.033

Uchida, Y., & Holleran, W. M. (2008). Omega-O-acylceramide, a lipid essential for mammalian survival. *Journal of Dermatological Science*, *51*(2), 77–87. https://doi.org/10.1016/j.jdermsci.2008.01.002

Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P., & Ron, D. (2000). Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science (New York, N.Y.)*, *287*(5453), 664–666. https://doi.org/10.1126/science.287.5453.664

van der Vliet, A., & Janssen-Heininger, Y. M. W. (2014). Hydrogen peroxide as a damage signal in tissue injury and inflammation: Murderer, mediator, or messenger? *Journal of Cellular Biochemistry*, *115*(3), 427–435. https://doi.org/10.1002/jcb.24683

van Dooren-Greebe, R. J., van de Kerkhof, P. C., & Happle, R. (1989). Acitretin monotherapy in Darier's disease. *The British Journal of Dermatology*, *121*(3), 375–379. https://doi.org/10.1111/j.1365-2133.1989.tb01432.x

Vandecaetsbeek, I., Trekels, M., De Maeyer, M., Ceulemans, H., Lescrinier, E., Raeymaekers, L., Wuytack, F., & Vangheluwe, P. (2009). Structural basis for the high Ca2+ affinity of the ubiquitous SERCA2b Ca2+ pump. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(44), 18533–18538. https://doi.org/10.1073/pnas.0906797106

Varga, T., Czimmerer, Z., & Nagy, L. (2011). PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochimica Et Biophysica Acta*, *1812*(8), 1007–1022. https://doi.org/10.1016/j.bbadis.2011.02.014

Vasileiou, P. V. S., Evangelou, K., Vlasis, K., Fildisis, G., Panayiotidis, M. I., Chronopoulos, E., Passias, P.-G., Kouloukoussa, M., Gorgoulis, V. G., & Havaki, S. (2019). Mitochondrial Homeostasis and Cellular Senescence. *Cells*, *8*(7), 686. https://doi.org/10.3390/cells8070686

Vauclair, S., Majo, F., Durham, A.-D., Ghyselinck, N. B., Barrandon, Y., & Radtke, F. (2007). Corneal epithelial cell fate is maintained during repair by Notch1 signaling via the regulation of vitamin A metabolism. *Developmental Cell*, *13*(2), 242–253. https://doi.org/10.1016/j.devcel.2007.06.012

Vázquez-Carrera, M., & Wahli, W. (2022). PPARs as Key Mediators in the Regulation of Metabolism and Inflammation. *International Journal of Molecular Sciences*, *23*(9), 5025. https://doi.org/10.3390/ijms23095025

Verboomen, H., Mertens, L., Eggermont, J., Wuytack, F., & Van Den Bosch, L. (1995). Modulation of SERCA2 activity: Regulated splicing and interaction with phospholamban. *Bioscience Reports*, *15*(5), 307. https://doi.org/10.1007/BF01788363

Verboomen, H., Wuytack, F., De Smedt, H., Himpens, B., & Casteels, R. (1992). Functional difference between SERCA2a and SERCA2b Ca2+ pumps and their modulation by phospholamban. *The Biochemical Journal*, *286 (Pt 2)*(Pt 2), 591–595. https://doi.org/10.1042/bj2860591

Verboomen, H., Wuytack, F., Van den Bosch, L., Mertens, L., & Casteels, R. (1994). The functional importance of the extreme C-terminal tail in the gene 2 organellar Ca(2+)-transport ATPase (SERCA2a/b). *The Biochemical Journal*, *303 (Pt 3)*(Pt 3), 979–984. https://doi.org/10.1042/bj3030979

Vestal, D. J., & Jeyaratnam, J. A. (2011). The guanylate-binding proteins: Emerging insights into the biochemical properties and functions of this family of large interferon-induced guanosine triphosphatase. *Journal of Interferon & Cytokine Research: The Official Journal of the International Society for Interferon and Cytokine Research*, *31*(1), 89–97. https://doi.org/10.1089/jir.2010.0102

Vidali, S., Feichtinger, R. G., Emberger, M., Brunner, S. M., Gaisbauer, S., Blatt, T., Smiles, W. J., Kreutzer, C., Weise, J. M., & Kofler, B. (2023). Ageing is associated with a reduction in markers of mitochondrial energy metabolism in the human epidermis. *Experimental Dermatology*, *32*(6), 900–905. https://doi.org/10.1111/exd.14778

Vieira, M. L., de Paula Samorano, L., da Matta Rivitti-Machado, M. C., & de Oliveira, Z. N. P. (2020). Darier disease: Long-term treatment with systemic retinoids at a tertiary hospital. *Journal Der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology: JDDG*, *18*(6), 628–630. https://doi.org/10.1111/ddg.14113

Walter, P., & Ron, D. (2011). The unfolded protein response: From stress pathway to homeostatic regulation. *Science (New York, N.Y.)*, *334*(6059), 1081–1086. https://doi.org/10.1126/science.1209038

Wang, F., Flanagan, J., Su, N., Wang, L.-C., Bui, S., Nielson, A., Wu, X., Vo, H.-T., Ma, X.-J., & Luo, Y. (2012). RNAscope: A novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *The Journal of Molecular Diagnostics: JMD*, *14*(1), 22–29. https://doi.org/10.1016/j.jmoldx.2011.08.002

Wang, Q., Wang, X., Liang, Q., Wang, S., Xiwen, L., Pan, F., Chen, H., & Li, D. (2018). Distinct prognostic value of mRNA expression of guanylate-binding protein genes in skin cutaneous melanoma. *Oncology Letters*, *15*(5), 7914–7922. https://doi.org/10.3892/ol.2018.8306

Wang, Y., Bruce, A. T., Tu, C., Ma, K., Zeng, L., Zheng, P., Liu, Y., & Liu, Y. (2011). Protein aggregation of SERCA2 mutants associated with Darier disease elicits ER stress and apoptosis in keratinocytes. *Journal of Cell Science*, *124*(Pt 21), 3568–3580. https://doi.org/10.1242/jcs.084053

Wang, Y., Shao, L., Shi, S., Harris, R. J., Spellman, M. W., Stanley, P., & Haltiwanger, R. S. (2001). Modification of epidermal growth factor-like repeats with O-fucose. Molecular cloning and expression of a novel GDP-fucose protein O-fucosyltransferase. *The Journal of Biological Chemistry*, *276*(43), 40338–40345. https://doi.org/10.1074/jbc.M107849200

Wang, Z., Wang, Y., Bradbury, N., Gonzales Bravo, C., Schnabl, B., & Di Nardo, A. (2020). Skin wound closure delay in metabolic syndrome correlates with SCF deficiency in keratinocytes. *Scientific Reports*, *10*(1), 21732. https://doi.org/10.1038/s41598-020-78244-y

Watt, F. M. (2002). Role of integrins in regulating epidermal adhesion, growth and differentiation. *The EMBO Journal*, *21*(15), 3919–3926. https://doi.org/10.1093/emboj/cdf399

Watt, F. M., Estrach, S., & Ambler, C. A. (2008). Epidermal Notch signalling: Differentiation, cancer and adhesion. *Current Opinion in Cell Biology*, *20*(2), 171–179. https://doi.org/10.1016/j.ceb.2008.01.010

Watt, F. M., Frye, M., & Benitah, S. A. (2008). MYC in mammalian epidermis: How can an oncogene stimulate differentiation? *Nature Reviews. Cancer*, *8*(3), 234–242. https://doi.org/10.1038/nrc2328

Watt, F. M., Lo Celso, C., & Silva-Vargas, V. (2006). Epidermal stem cells: An update. *Current Opinion in Genetics & Development*, *16*(5), 518–524. https://doi.org/10.1016/j.gde.2006.08.006

Werner, A., Amann, E., Schnitzius, V., Habermeier, A., Luckner-Minden, C., Leuchtner, N., Rupp, J., Closs, E. I., & Munder, M. (2016). Induced arginine transport via cationic amino acid transporter-1 is necessary for human T-cell proliferation. *European Journal of Immunology*, *46*(1), 92–103. https://doi.org/10.1002/eji.201546047

Wertz, P. W. (2013). Current understanding of skin biology pertinent to skin penetration: Skin biochemistry. *Skin Pharmacology and Physiology*, *26*(4–6), 217–226. https://doi.org/10.1159/000351949

Westergaard, M., Henningsen, J., Johansen, C., Rasmussen, S., Svendsen, M. L., Jensen, U. B., Schrøder, H. D., Staels, B., Iversen, L., Bolund, L., Kragballe, K., & Kristiansen, K. (2003). Expression and localization of peroxisome proliferator-activated receptors and nuclear factor kappaB in normal and lesional psoriatic skin. *The Journal of Investigative Dermatology*, *121*(5), 1104–1117. https://doi.org/10.1046/j.1523-1747.2003.12536.x

Wheeland, R. G., & Gilmore, W. A. (1985). The surgical treatment of hypertrophic Darier's disease. *The Journal of Dermatologic Surgery and Oncology*, *11*(4), 420–423. https://doi.org/10.1111/j.1524-4725.1985.tb01294.x

Wolfsberg, T. G., Primakoff, P., Myles, D. G., & White, J. M. (1995). ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease domain: Multipotential functions in cell-cell and cell-matrix interactions. *The Journal of Cell Biology*, *131*(2), 275–278. https://doi.org/10.1083/jcb.131.2.275

Wu, L., Aster, J. C., Blacklow, S. C., Lake, R., Artavanis-Tsakonas, S., & Griffin, J. D. (2000). MAML1, a human homologue of Drosophila mastermind, is a transcriptional co-activator for NOTCH receptors. *Nature Genetics*, *26*(4), 484–489. https://doi.org/10.1038/82644

Wuytack, F., Kanmura, Y., Eggermont, J. A., Raeymaekers, L., Verbist, J., Hartweg, D., Gietzen, K., & Casteels, R. (1989). Smooth muscle expresses a cardiac/slow muscle isoform of the Ca2+-transport ATPase in its endoplasmic reticulum. *The Biochemical Journal*, *257*(1), 117–123. https://doi.org/10.1042/bj2570117

Xiang, Y., Ye, Y., Zhang, Z., & Han, L. (2018). Maximizing the Utility of Cancer Transcriptomic Data. *Trends in Cancer*, *4*(12), 823–837. https://doi.org/10.1016/j.trecan.2018.09.009

Xiao, J.-J., Liu, Q., Li, Y., Peng, F.-F., Wang, S., Zhang, Z., Liu, H., Yu, H., Tao, S., & Zhang, B.-F. (2022). Regulator of calcineurin 1 deletion attenuates mitochondrial dysfunction and apoptosis in acute kidney injury through JNK/Mff signaling pathway. *Cell Death & Disease*, *13*(9), 774. https://doi.org/10.1038/s41419-022-05220-x

Xie, Y., Wu, N., Tang, S., Zhou, Z., Chen, J., Li, J., Wu, F., Xu, M., Xu, X., Liu, Y., & Ma, X. (2024). Endoplasmic Reticulum Dysfunction: An Emerging Mechanism of Vitiligo Pathogenesis. *Clinical, Cosmetic and Investigational Dermatology*, *17*, 1133–1144. https://doi.org/10.2147/CCID.S459070

Xin, Y., Lu, Q., & Li, Q. (2011). IKK1 control of epidermal differentiation is modulated by notch signaling. *The American Journal of Pathology*, *178*(4), 1568–1577. https://doi.org/10.1016/j.ajpath.2010.12.021

Xue, X., Yu, J., Li, C., Wang, F., Guo, Y., Li, Y., & Shi, H. (2022). Full-Length Transcriptome Sequencing Analysis of Differentially Expressed Genes and Pathways After Treatment of Psoriasis With Oxymatrine. *Frontiers in Pharmacology*, *13*, 889493. https://doi.org/10.3389/fphar.2022.889493

Yap, A. S., Brieher, W. M., & Gumbiner, B. M. (1997). Molecular and functional analysis of cadherin-based adherens junctions. *Annual Review of Cell and Developmental Biology*, *13*, 119–146. https://doi.org/10.1146/annurev.cellbio.13.1.119

Yap, L., Tay, H. G., Nguyen, M. T. X., Tjin, M. S., & Tryggvason, K. (2019). Laminins in Cellular Differentiation. *Trends in Cell Biology*, *29*(12), 987–1000. https://doi.org/10.1016/j.tcb.2019.10.001

Yeshurun, A., Ziv, M., Cohen-Barak, E., Vered, S., Rozenman, D., Sah, M., Khayat, M., Polyakov, O., Amichai, B., Zlotogorski, A., Shalev, S., & Dodiuk-Gad, R. P. (2021). An Update on the Cutaneous Manifestations of Darier Disease. *Journal of Cutaneous Medicine and Surgery*, *25*(5), 498–503. https://doi.org/10.1177/1203475421999331

Yoshida, H. (2007). ER stress and diseases. *The FEBS Journal*, *274*(3), 630–658. https://doi.org/10.1111/j.1742-4658.2007.05639.x

Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., & Mori, K. (2001). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell*, *107*(7), 881–891. https://doi.org/10.1016/s0092-8674(01)00611-0

Yoshihara, N., Ueno, T., Takagi, A., Oliva Trejo, J. A., Haruna, K., Suga, Y., Komatsu, M., Tanaka, K., & Ikeda, S. (2015). The significant role of autophagy in the granular layer in normal skin differentiation and hair growth. *Archives of Dermatological Research*, *307*(2), 159–169. https://doi.org/10.1007/s00403-014-1508-0

Yuki, T., Haratake, A., Koishikawa, H., Morita, K., Miyachi, Y., & Inoue, S. (2007). Tight junction proteins in keratinocytes: Localization and contribution to barrier function. *Experimental Dermatology*, *16*(4), 324–330. https://doi.org/10.1111/j.1600-0625.2006.00539.x

Yuspa, S. H., Kilkenny, A. E., Steinert, P. M., & Roop, D. R. (1989). Expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations in vitro. *The Journal of Cell Biology*, *109*(3), 1207–1217. https://doi.org/10.1083/jcb.109.3.1207

Zavattaro, E., Celasco, M., Delrosso, G., Ferri, S., Bornacina, C., Valente, G., Veronese, F., Fusco, N., Gariglio, M., & Colombo, E. (2014). Acitretin-induced acral hemorrhagic lesions in Darier-White disease. *Cutis*, *94*(6), E1-5.

Zhang, P., Toyoshima, C., Yonekura, K., Green, N. M., & Stokes, D. L. (1998). Structure of the calcium pump from sarcoplasmic reticulum at 8-A resolution. *Nature*, *392*(6678), 835–839. https://doi.org/10.1038/33959

Zhang, X.-B., Luo, Q., Li, C.-X., He, Y.-Q., & Xu, X. (2008). Clinical investigation of acitretin in children with severe inherited keratinization disorders in China. *The Journal of Dermatological Treatment*, *19*(4), 221–228. https://doi.org/10.1080/09546630801955150

Zhang, Y., Li, Q., Rao, E., Sun, Y., Grossmann, M. E., Morris, R. J., Cleary, M. P., & Li, B. (2015). Epidermal Fatty Acid binding protein promotes skin inflammation induced by high-fat diet. *Immunity*, *42*(5), 953–964. https://doi.org/10.1016/j.immuni.2015.04.016