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DARIER DISEASE: MORE THAN SKIN DEEP

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Karolinska Institutet

Stockholm 2021

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-353-8

DARIER DISEASE: MORE THAN SKIN DEEP

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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The thesis will be defended in public at the Lecture Hall L8:00, Center for Molecular Medicine, Visionsgatan 18, 17176 Stockholm, Sweden

Friday, 19 November 2021 at 09:00

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“You miss 100% of the shots you don’t take”

– Wayne Gretzky

POPULAR SCIENCE SUMMARY

Darier disease (DD) is an inherited skin disorder characterized by a severe rash that is often widespread. It causes major discomfort and reduced quality of life for the affected individual. DD is caused by mutations in a gene that is responsible for producing a calcium pump inside the cell. This pump, named SERCA2, has been found to be essential in the way the cell handles calcium. Dysfunction in the pump, like that seen in DD, has been observed as a mechanism in several diseases. SERCA2 is active in all cells of the body, meaning that individuals with DD might suffer from problems in other organs besides the skin, so-called comorbidities. Historically, DD has only been associated with disorders of the brain, such as bipolar disorder, depression, schizophrenia, psychosis and intellectual and learning disabilities. In this thesis, investigations into the associations between DD and comorbidities in several organs have been carried out.

Two main study designs were utilized in the studies included in this thesis. In study I, II and III, experimental case-control designs were used, where individuals with DD were compared with healthy matched control persons. In study II and IV, population-based cohort designs were used. Data on individuals with DD were taken from the National Patient Register and compared with matched individuals from the Total Population Register. Additionally, in study V, individual laboratory values in a small cohort of DD patients were examined and compared to the normal reference ranges.

Results show that DD is associated with a significant impairment in cognitive function, encompassing areas such as visual information processing, short-term memory and reaction time. There is also an association with heart disease, particularly heart failure, where patients exhibit a 59% increased risk of diagnosis. This association was most prominent in female DD patients. The heart disease diagnosis occurred on average almost seven years earlier among patients. Furthermore, individuals with DD exhibited a 74% risk increase for a diagnosis of type 1 diabetes. They were also shown to have a metabolic profile that is often seen during the development of type 2 diabetes. Additionally, there are signs that DD is associated with a possible defect in the immune system, which might help explain their recurring secondary bacterial and viral infections.

These findings paint a picture of DD as a disorder that is not just confined to the skin, but instead carry significant comorbidities in several organs. This is important to consider in routine medical care for these patients, since early discovery of, for example, heart disease and diabetes leads to timely and appropriate treatment and, consequently, better prognosis. The finding of impaired cognitive function has major ramifications in that special measures for the individual patient might be necessary in school or the workplace. Currently, there are no treatments for DD that directly target the disease mechanism, but in the future, the way we choose to treat DD may to some extent depend on its comorbidities.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Dariers sjukdom (DD) är en ärftlig hudåkomma med karaktäristiska svåra hudutslag som kan vara utbredda. Den orsakar obehag och sänkt livskvalitet för den drabbade individen. DD orsakas av mutationer i en gen som ansvarar för att producera en kalciumpump inuti cellen. Denna pump, SERCA2, har visats vara essentiell för hur cellen hanterar kalcium. Dysfunktion i pumpen, vilket ses hos patienter med DD, har observerats som en underliggande sjukdomsmekanism i flertalet olika åkommor. SERCA2 är aktiv i alla kroppens celler, vilket innebär att patienter med DD kan antas lida av besvär från andra organ än bara huden, så kallade komorbiditeter. Historiskt har DD endast visats vara associerad med sjukdomar relaterade till hjärnan, såsom bipolär sjukdom, depression, schizofreni, psykos och intellektuell funktionsnedsättning. I denna avhandling har kopplingarna mellan DD och komorbiditeter i flera olika organ undersökts.

Två huvudsakliga studieupplägg användes i delarbetena i denna avhandling. I studie I, II och III genomfördes experimentella fall-kontrollstudier, där individer med DD jämfördes med friska, matchade kontrollpersoner. I studie II och IV utfördes jämförelser på populationsnivå mellan individer med DD hämtade ur nationella patientregistret och friska jämförelseindivider hämtade ur registret över totalbefolkningen. I studie V undersöktes även värden på immunceller hos en liten grupp individer med DD jämfört med de normala referensintervallen.

Resultaten visar att DD är associerad med en signifikant sänkt kognitiv funktion, vilken innefattar områden såsom informationsbearbetning, korttidsminne och reaktionstid. DD är också associerad med en ökad risk för hjärtsjukdom i allmänhet och hjärtsvikt i synnerhet, där patienter löper en 59 % ökad risk att drabbas. Denna koppling var mest framträdande hos kvinnliga patienter. Dessutom fick patienterna sin diagnos på hjärtsjukdom i genomsnitt nästan sju år tidigare. Det föreligger även en 74 % ökad risk för en diagnos av typ 1-diabetes hos patienter med DD, och de uppvisar en profil i sin ämnesomsättning som ofta ses under utvecklingen av typ 2-diabetes. Därutöver finns tecken på att det kan förekomma defekter i immunförsvaret, vilket skulle kunna förklara de frekventa sekundärinfektioner med bakterier och virus som patienter med DD lider av.

Sammantaget ger dessa fynd bilden av DD som en sjukdom som inte bara drabbar huden, utan som istället är förknippad med betydande komorbiditeter i flera olika organ. Detta är av stor vikt att ta i beaktande i vården av dessa patienter, eftersom tidig upptäckt av till exempel hjärtsjukdom och diabetes leder till behandling i ett tidigare skede av sjukdomsförloppet, och i förlängningen en förbättrad prognos. Den nedsatta kognitiva funktionen som observerats har stor inverkan på den individuella patienten, varför särskilda anpassningar kan behöva göras under skolgången och i arbetslivet. Det finns ännu ingen behandling för DD som direkt påverkar sjukdomsmekanismen, men i framtiden kan sättet på vilket vi väljer att behandla DD i viss utsträckning bero på dess komorbiditeter.

ABSTRACT

Darier disease (DD) is a severe, inherited dermatological disorder with a characteristic clinical and histopathological appearance. It is caused by mutations in the *ATP2A2* gene, encoding a Ca^{2+} pump in the endoplasmic reticulum, Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase 2 (SERCA2), which has been found to be an essential regulator of cellular Ca^{2+} homeostasis. SERCA2 is expressed in all cells of the body, not just the skin, and has been implicated in physiological and pathophysiological mechanisms in several organs, thus rendering the occurrence of extra-dermal presentations plausible. DD has so far only been associated with brain-related disorders. The overall aim of this thesis was to investigate comorbidities associated with DD.

In study I, II and III, experimental, matched case-control designs were used to evaluate broad cognitive function, heart disease and diabetes in individuals with DD compared with matched healthy controls. Individuals with DD were found to exhibit a widespread, significant impairment in cognitive function assessed with the Cambridge Neuropsychological Test Automated Battery, evaluating general cognitive function. A possible defect in serum lipid handling was also seen, as well as evidence of pancreatic β-cell dysfunction indicating a metabolic state seen during the development of type 2 diabetes.

In study II and IV, a population-based cohort design, comparing data on individuals with DD taken from the National Patient Register with matched comparison subjects from the Total Population Register, was used. Individuals with DD displayed a 59% risk increase of heart failure (risk ratio [RR] 1.59, confidence interval [CI] 1.16-2.19) and a 32% risk increase of any heart diagnosis (RR 1.32, CI 1.02-1.70). Female DD patients had a 59% risk increase of any heart diagnosis (RR 1.59, CI 1.13-2.25). The first heart diagnosis also occurred almost seven years earlier in individuals with DD (70.0 *vs.* 76.9 years). A 74% increased risk of type 1 diabetes diagnosis was also seen (RR 1.74, CI 1.13-2.69).

In study V, immune cell markers were evaluated in a small cohort of DD patients. The values of CD19+ B cells were found to be lower than the normal laboratory range on average, which together with the evidence of the importance of SERCA2 in B cell development indicates a possible immune system defect. This finding can help explain the recurring secondary infections often troubling individuals with DD.

In conclusion, DD has been found to be associated with impaired cognitive function, heart disease and diabetes, as well as a possible defect in the immune system. The range of comorbidities associated with DD has been expanded to include several organs, resulting in the confident conclusion that DD should be viewed as a systemic disorder. This has several important implications, both for the individual patient and in routine care for this group of patients. Looking forward, it will quite possibly influence the way DD is treated.

LIST OF SCIENTIFIC PAPERS

I. **Philip Curman**, Johanna Bern, Linnea Sand, Martin Cederlöf, Etty Bachar-Wikström, Jakob D. Wikström. *Patients with Darier Disease Exhibit Cognitive Impairment while Patients with Hailey-Hailey Disease Do Not: An Experimental, Matched Case-control Study*. Acta Derm Venereol, 2021. 101(6).

II. Etty Bachar-Wikstrom, **Philip Curman**, Tara Ahanian, Ivone U.S. Leong, Henrik Larsson, Martin Cederlöf, Jakob D. Wikstrom. *Darier disease is associated with heart failure: a cross-sectional case-control and population based study*. Sci Rep, 2020. 10(1).

III. Tara Ahanian*, **Philip Curman***, Ivone U.S. Leong, Kerstin Brismar, Etty Bachar-Wikstrom, Martin Cederlöf, Jakob D. Wikstrom. *Metabolic phenotype in Darier disease: a cross-sectional clinical study*. Diabetol Metab Syndr, 2020. 12. ***Equal contribution**.

IV. Martin Cederlöf, **Philip Curman**, Tara Ahanian, Ivone U.S. Leong, Kerstin Brismar, Etty Bachar-Wikstrom, Jakob D. Wikstrom. *Darier disease is associated with type 1 diabetes: Findings from a population-based cohort study*. J Am Acad Dermatol, 2019. 81(6).

V. Chun-Chin Chen*, Bo-Ruei Chen*, Yinan Wang, **Philip Curman**, Helen A. Beilinson, Ryan M. Brecht, Catherine C. Liu, Ryan J. Farrell, Jaime de Juan-Sanz, Louis-Marie Charbonnier, Shingo Kajimura, Timothy A. Ryan, David G. Schatz, Talal A. Chatila, Jakob D. Wikstrom, Jessica K. Tyler, Barry P. Sleckman. *Sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) activity is required for V(D)J recombination*. J Exp Med, 2021. 218(8). *Equal contribution.

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I. **Philip Curman**, Anders Näsman, Hanna Brauner. *Trichodysplasia spinulosa: a comprehensive review of the disease and its treatment*. J Eur Acad Dermatol Venereol, 2021. 35(5).

II. **Philip Curman**, Jan Lapins, Niki Radros, Britta Krynitz, Jakob D Wikström. *Amelanotic melanoma concealed by psoriasis*. Acta Derm Venereol, 2020. 100(6).

III. Mireia Jasans-Barcélo*, **Philip Curman***, Lena Hagströmer, Jakob D Wikstrom, Darius Sairafi. *Improvement of Hailey-Hailey disease with low-dose naltrexone*. Br J Dermatol, 2020. 182(6). ***Equal contribution**.

IV. **Philip Curman**, Martin Cederlöf, Etty Bachar-Wikstrom, Jakob D. Wikstrom. *Hailey-Hailey disease and diabetes: A population-based cohort study and a case series of HLA-DQ3 as a synergistic risk factor.* Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATF	Activating transcription factor
ATP	Adenosine triphosphate
Bcl-2	B cell lymphoma 2
BiP	Binding immunoglobulin protein
BMI	Body mass index
CANTAB	Cambridge Neuropsychological Test Automated Battery
CD	Cluster of differentiation
CDR	The Cause of Death Register
CHOP	CCAAT-enhancer-binding protein homologous protein
CI	Confidence interval
DD	Darier disease
Dsc	Desmocollin
Dsg	Desmoglein
ECG	Electrocardiography
eIF2α	Eukaryotic translation initiation factor 2α
ER	Endoplasmic reticulum
GRP78	Glucose-regulated protein 78
HDL	High-density lipoprotein
HF	Heart failure
HHD	Hailey-Hailey disease
HOMA	Homeostasis Model Assessment
HSV	Herpes simplex virus
ICD	International classification of diseases
IL	Interleukin
IRE1α	Inositol-requiring enzyme 1α
LDL	Low-density lipoprotein
MOT	Motor screening test
mRNA	Messenger ribonucleic acid
NBHW	National Board of Health and Welfare

NK cell	Natural killer cell
NPR	The National Patient Register
NT-proBNP	N-terminal-pro brain natriuretic peptide
OGTT	Oral glucose tolerance test
OMIM	Online Mendelian Inheritance in Man
OR	Odds ratio
PAL	Paired associates learning
PERK	Protein kinase-like ER kinase
RR	Risk ration, relative risk
RTI	Reaction time
RVP	Rapid visual information processing
SAMS	The Small Area Marketing Statistics Register
SD	Standard deviation
SERCA	Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase
SPCA	Secretory Pathway Ca^{2+}/Mn^{2+}-APTase
SR	Sarcoplasmic reticulum
ST2	Suppression of tumorigenicity 2
SWM	Spatial working memory
sXBP1	Spliced XBP1
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TNF	Tumor necrosis factor
TPR	The Total Population Register
UPR	Unfolded protein response
XBP1	X-box binding protein 1
YAG	Yttrium aluminum garnet

1 INTRODUCTION

Darier disease (DD) is a burdensome, hereditary disorder categorized as a dermatological disease, with a characteristic clinical presentation. It is well known by dermatologists for being potentially difficult to manage and treat, often relapsing and causing major distress and reduction in quality of life for the affected individual. It is caused by mutations in a gene that encodes a Ca^{2+} pump that is essential for cellular Ca^{2+} handling, and the mutation affects all cells of the body. Apart from the skin, patients have been found to be subjected to increased risk of several psychiatric disorders. Some efforts have previously been made to investigate other possible comorbidities, but without convincing results.

In this thesis the literature surrounding DD will be thoroughly reviewed, including a general background on the skin, cell-to-cell adhesion, Ca^{2+}, and the genetics and cellular mechanisms behind DD. In the sections that follow, the overarching research aims, methods, results and discussion of the studies included in this thesis will be explored in relation to the existing literature. Finally, concluding remarks and points of perspective/future outlooks, including the authors own conclusions, are discussed.

The main research aim was to decipher if, and to what extent, DD can be considered a systemic disorder not just confined to the skin, and, if so, what the possible ramifications for affected individuals might be.

2 LITERATURE REVIEW

2.1 THE SKIN

Tell anyone you are a dermatologist, and the chances are high that they will immediately reply "*Ah, well the skin is the largest organ of the body!*", which it indeed is. The skin (Latin: *Cutis*, Greek: *Derma*) is also the first essential barrier against external threats to the human body such as trauma, temperature, pathogens, irritants/chemicals, ultraviolet radiation, and other insults. Equally as important, it acts as a barrier in the other direction by keeping water and electrolytes from leaving the body in an unregulated manner. The skin both senses the *exterior* environment and reacts to it, and at the same time can convey the reactions and emotions of the *interior* through mechanisms such as sweating, flushing, blushing and creating goose bumps. The skin normally accounts for 1/6-1/7th of our total body weight, covers an average area of just under two m^2 and contains numerous cell types [1-5]. Diseases of the skin vary from mild to life-threatening, benign to malign, sporadic to life-long, unnoticeable to widespread and asymptomatic to severe. They often carry with them the potential to profoundly affect an individual's appearance, and, consequently, their lives.

In summary, proper functioning and integrity of the skin is essential for the survival and well-being for the human organism. Anatomically, the skin is comprised of three layers: the epidermis, dermis and subcutis (also called subcutaneous [adipose] tissue/fat or hypodermis) (***Figure 1***).

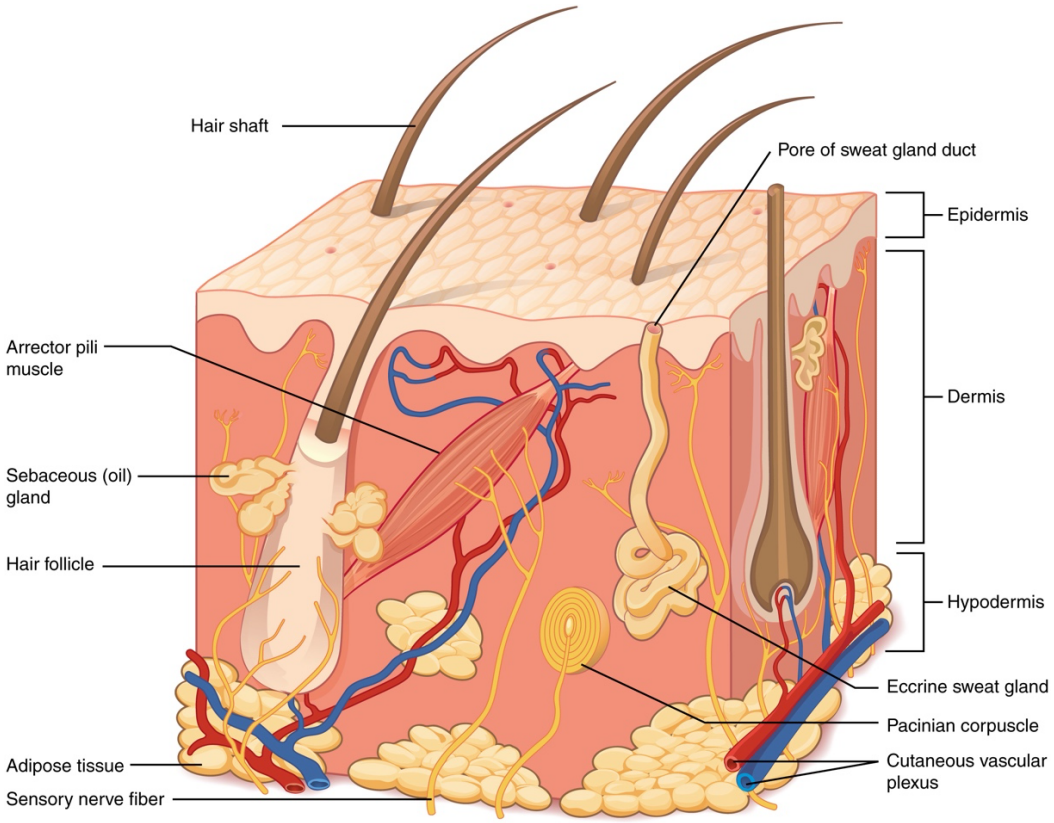


Figure 1. The structure of the skin.
OpenStax, Copyright© 2021, Creative Commons Attribution 4.0 International License. https://openstax.org/books/anatomy-and-physiology/pages/5-1-layers-of-the-skin.

2.1.1 The epidermis

If intact, the epidermis covers the entirety of our skin. The epidermis is the outermost layer, consisting of mainly keratinocytes (>90%), but also melanocytes, immune cells such as Langerhans cells and T-lymphocytes, and Merkel cells. The epidermis is itself composed of several layers, described below from deep to superficial (***Figure 2***).

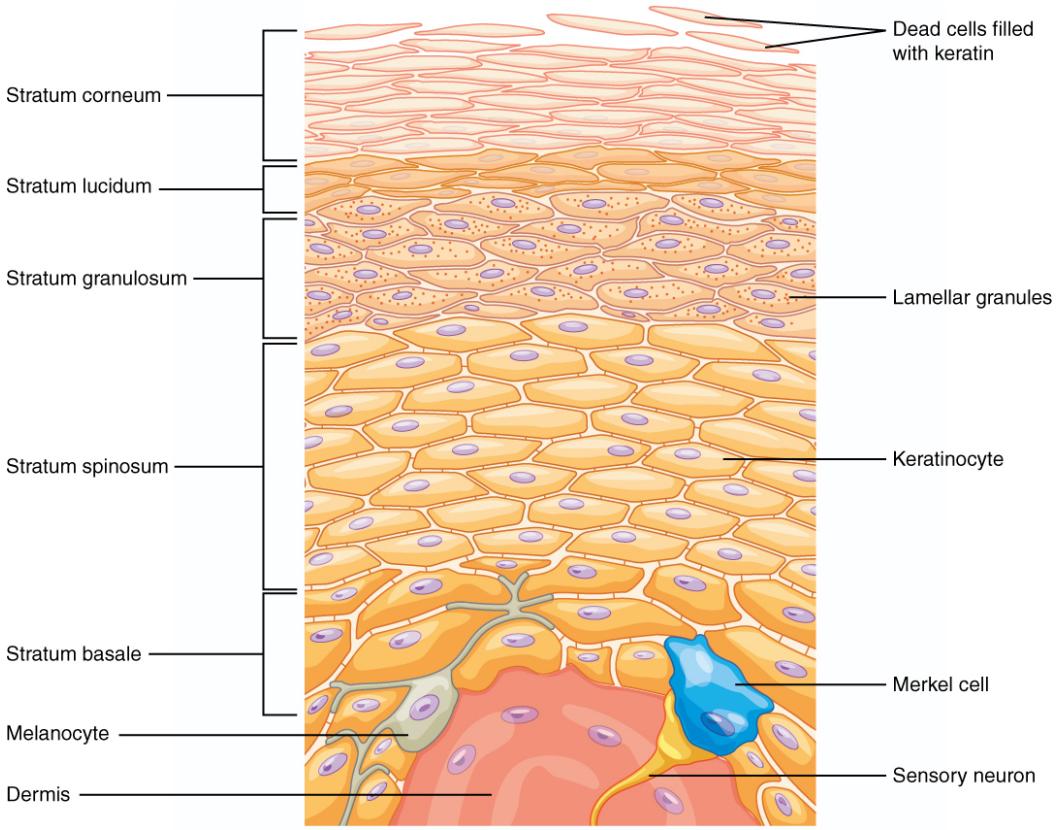


Figure 2. The epidermis.
OpenStax, Copyright© 2021, Creative Commons Attribution 4.0 International License. https://openstax.org/books/anatomy-and-physiology/pages/5-1-layers-of-the-skin.

Keratinocytes start their 4-6-week long journey of largely predetermined so-called *terminal differentiation* and proliferation as they leave the *stratum basale* (the basal layer). This is a single cell layer of cube-shaped keratinocyte progenitor cells responsible for most of the production of new epidermal cells. There are also progenitor cells associated with the hair follicle. *Keratin* constitutes the main protein of keratinocytes and give them their name. It is a key structural intermediate filament protein packed in different orders of magnitude that provides structural stability to the epidermis, hair and nails [6]. Keratin 5 and 14 expression are used as markers for basal layer keratinocytes [7].

Cells then move towards the surface through the *stratum spinosum* (the spiny layer). Comprised of an 8-10 cell layer thick compartment, cells here undergo mitosis in order to increase their numbers, regenerate and continue their journey upwards. They now display early differentiation-specific markers such as keratin 1 and 10.

Next, they enter the *stratum granulosum* (the granular layer). Here, keratinocytes display late differentiation-specific markers such as filaggrin, loricrin and involucrin. Cells become flattened and compressed as a sign of maturation, and their plasma membrane thickens.

On the volar sides of the hands and feet we next find the *stratum lucidum* (the clear layer). A part of the dermal hydrophobic barrier, this thin layer consists of defunct keratinocytes with a high content of a lipid-rich protein called eleidin that gives a clear, see-through appearance, consequently the name "the clear layer".

Finally, there is the *stratum corneum* (the horny layer), the most superficially located layer of the epidermis. Keratinocytes now undergo controlled cell death, lose their nuclei and go through the final steps of terminal differentiation in a process of keratinization to become so-called *corneocytes*. The endpoint of keratinocyte terminal differentiation is called the *cornified envelope*, which replaces the plasma membrane in corneocytes. Made up of crosslinked, protein-embedded keratin surrounded by a lipid envelope mainly consisting of ceramides and cholesterol, it represents the ultimate barrier property of the epidermis, and thus, the entire skin, offering protection from transepidermal water loss, insults from the environment, and harmful pathogens. Ultimately, the cells shed entirely from the skin through a process called desquamation [3, 8-18]. Around 0.5 billion dead skin cells, or 1,5 grams, is shed every day [19], and there are estimations that dead skin make up around 50% of total household dust.

2.1.2 The dermis

The dermis is the middle layer of the skin, located directly below the epidermis. It is composed of two distinct layers, the superficial *papillary dermis* and the deeper, thicker and denser *reticular dermis*. The dermis consists of extracellular matrix proteins such as collagen and elastin, which provides toughness and elasticity to the skin. It contains neuronal mechano- and thermoreceptors responsible for the sensation of touch and temperature, respectively. Blood vessels shoot through the dermis primarily in a vertical fashion. The dermis also accommodates the hair follicles, sweat glands and sebaceous glands. On the cellular level it is composed mainly of fibroblasts, as well as mast cells, macrophages and stem cells, to name a few [20, 21].

2.1.3 The subcutis

The subcutis, also called subcutaneous tissue/fat or hypodermis, is the deepest layer of the skin and consists primarily of adipose tissue. This is an ideal site for drug injections due to the relatively high degree of vascularization. Furthermore, it provides the skin with insulation and shock absorption properties. Adipocytes and fibroblasts make up the bulk of the cells in the subcutis, together with immune-related cells such as macrophages and monocytes [22, 23].

2.1.4 Cell-to-cell adhesion in the skin

Cell-to-cell adhesion between keratinocytes is fundamental to ensure proper functioning of the skin as a protective barrier for the human organism. In the epidermis, these adhesive complexes consist of desmosomes, adherens junctions and tight junctions.

2.1.4.1 Desmosomes

Desmosomes are large complexes specialized in the formation of strong mechanical intercellular adhesive strength between keratinocytes (***Figure 3***). They are formed by transmembrane glycoproteins in the Ca^{2+}-dependent cell adhesion protein family (cadherins), namely desmogleins (Dsg) and desmocollins (Dsc), which bind to each other extracellularly. Intracellularly, Dsg and Dsc complexes are linked to the keratin intermediate filament cytoskeleton via a complex array of proteins including plakoglobin, desmoplakin and plakophilin, thus providing adhesive strength [24-28]. Desmosomes are the main pillars of keratinocyte cell-to-cell adhesion, and also the source of many dermatological diseases, for example on the basis of gene mutations, e.g., striate palmoplantar keratoderma and skin fragility ectodermal dysplasia syndrome [29] or autoimmunity. e.g., pemphigus vulgaris and pemphigus foliaceus [30].

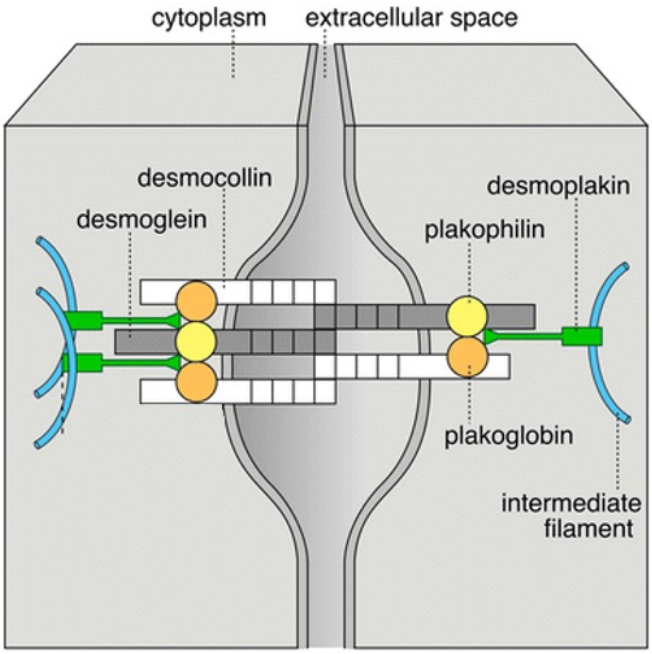


Figure 3. The desmosome.
*Adapted from Waschke, J., The Desmosome and Pemphigus. Histochem Cell Biol, 2008. **130**(1): p. 21-54.*

2.1.4.2 Adherens junctions

Adherens junctions, like desmosomes, are intercellular junctions composed of proteins from the cadherin family, namely E-cadherin, that form links in the intercellular space. Intracellularly they attach to the actin microfilament cytoskeleton by way of a protein complex consisting of α-catenin, β-catenin, p120 and plakoglobin (in the case of adherens junctions also referred to as γ-catenin) (***Figure 4***). Adherens junctions have been shown to play key roles in the mechanical permanence and barrier integrity of the epidermis, and possibly in the modulation of inflammatory responses in the epidermis [27, 31-33].

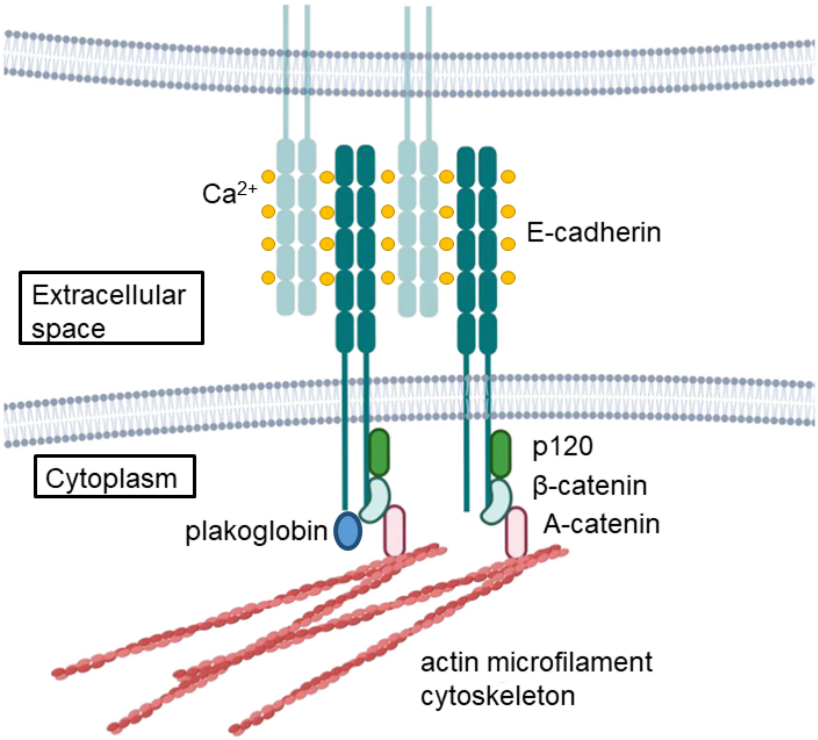


Figure 4. The adherens junction.
Adapted from Kim, H.N., et al., Cadherins, Selectins, and Integrins in CAM-DR in Leukemia. Front Oncol, 2020. ***10****.*

2.1.4.3 Tight junctions

Tight junctions (also called zonula occludens) are primarily located in the stratum granulosum keratinocytes, where they form a type of intercellular junction that seals, or occludes, the intercellular space. In this regard they form an essential barrier for water, other molecules and ions through the inhibition of passive free diffusion, as well as performing adhesive functions. Contrary to desmosomes and adherens junctions, the proteins located in the intercellular space do not belong to the cadherin family. Instead, they belong to either so-called "junctional adherens molecules", claudins or occludins. Intracellularly, they link to the actin cytoskeleton via the zonula occludens proteins [27, 32, 34, 35].

2.1.5 Calcium and the skin

Ca^{2+} is a regulator of a countless cellular activities in all cell types in the human body, including cell survival, cell death and numerous other functions [36-39]. Approximately 1.4 kg of Ca^{2+} exists in the human body, the vast majority of which is trapped in the skeleton and teeth, while only around 10 grams remain free to circulate in the body and perform cellular functions. Ca^{2+} inhabits the rather unique property of being an essential component of the direct electrophysiological functioning of all cells, while at the same time acting as a messenger in several signaling cascades [38, 40, 41].

As previously noted, the skin, and particularly the epidermis, performs many functions fundamental for the integrity of the human body. In order to operate properly, the processes of epidermal barrier formation and homeostasis, formation of intra-epidermal cohesion and keratinocyte differentiation are tightly regulated in the epidermis [42, 43]. Ca^{2+} plays important, if not essential, roles in all these processes.

2.1.5.1 Calcium and keratinocyte differentiation

The process by which keratinocytes differentiate is termed *terminal differentiation*, meaning the dismantling of cell cycle-related mechanisms and consequent loss of ability to divide, the ultimate step being the formation of the cornified envelope (see also the end of section 2.1.1) [44]. The induction of terminal differentiation is largely dependent on the level of Ca^{2+} in the extracellular space [11, 45-48], and it is well known that the skin keeps a distinctive extracellular Ca^{2+} gradient, with low levels in the basal layers which then increase gradually to peak in the stratum granulosum, after which it rapidly drops in the outermost stratum corneum [49-55].

An example of the specific influence of Ca^{2+} on keratinocyte differentiation are the actions of proteins from the protein kinase C family. These are Ca^{2+}-dependent and highly active in the keratinocyte differentiation shift from stratum spinosum to stratum granulosum by down-regulating keratin 1 and 10 gene expression while acting to promote gene expression for late stage differentiation [56].

2.1.5.2 Calcium and epidermal barrier function

Epidermal barrier integrity is largely dependent on the corneocytes, the cornified envelope and the structure of their immediate extracellular surroundings in the outermost layer of the epidermis, the stratum corneum. As discussed in the end of section 2.1.1, a complex structure of dead keratinocytes, extracellular matrix lipids and proteins, and cell-to-cell adhesion structures make up the actual barrier [13-18]. Disruption of barrier homeostasis causes severe alterations in Ca^{2+} level as a rapid response, which then normalize as the barrier homeostasis is regained [52, 53, 57, 58]. Conversely, inhibiting these reactive Ca^{2+} alterations

experimentally leads to impaired recovery of the barrier homeostasis [57, 59, 60]. Consequently, Ca^{2+} is an integral regulator of barrier homeostasis in the epidermis and even slight alterations in Ca^{2+} concentrations can cause disruptions in this core aspect of skin functioning.

2.1.5.3 Calcium and cell-to-cell adhesion

Not least, Ca^{2+} is a main determinant in the formation of cell-to-cell adhesion between keratinocytes, a key constituent of skin integrity and function consisting of desmosomes, adherens junctions and tight junctions, as mentioned in detail in section 2.1.4 [32, 35, 61]. The main structural proteins of desmosomes and adherens junctions, cadherins, receive their name from the fact that they are dependent on Ca^{2+} ("Ca^{2+}-dependent cell adhesion proteins"). It is the actual binding capacity and interaction between cadherins that require Ca^{2+} in order to function [62, 63] (see also ***Figure 4***). The importance can be illustrated by the fact that increases in extracellular Ca^{2+} more or less immediately causes keratinocytes to form adherens junctions [31] and desmosomes [24-26, 64]. Conversely, a low level of extracellular Ca^{2+} in keratinocyte cell culture halts the formation of cell-to-cell adhesion molecules and keeps it low over time. The influence of Ca^{2+} on the formation of adherens junctions and desmosomes is a process that is closely tied to the epidermal Ca^{2+} gradient and the terminal differentiation of keratinocytes [25, 31, 59, 61]. Cell-to-cell adhesion is discussed in more detail in section 2.1.4.

2.2 DARIER DISEASE

Darier disease (DD, Online Mendelian Inheritance in Man [OMIM] catalog of human genes and genetic disorders #124200), also called Darier-White disease, keratosis follicularis and dyskeratosis follicularis, is a rare, severe, hereditary dermatological disease with an estimated prevalence of around 1:50,000 individuals [65-71]. Thus, it is estimated that approximately 200 individuals with DD exist in Sweden, and roughly 150,000 worldwide. DD is caused by autosomal dominant mutations in the *ATP2A2* gene. DD is incurable and follows a chronically relapsing course, often causing significant negative impact on the affected individual's life.

2.2.1 History

In the year 1889 the first clinical descriptions of individuals with DD were reported independently by both French dermatologist Jean Darier at the Hôpital Saint-Louis in Paris and American dermatologist James C White at Harvard University in Cambridge, Massachusetts [72, 73]. They both described a clinical presentation of brownish, partly crusted lesions with a foul odor, following a predominantly follicular distribution. The initial investigations raised suspicions of a parasitic protozoa infestation, but attempts failed to prove this. Early histological examinations showed signs of abnormal keratinization, and later the specific

follicular involvement could be refuted. The hereditary characteristics of the disease was soon postulated by White when the daughter of the subject in the initial case study developed similar lesions [74].

2.2.2 Epidemiology

The overall prevalence of DD is thought to be around 1:50,000 individuals on average, with studies ranging from 1:30,000 in the west of Scotland [68] and 1:36,000 in north-east England [66], to 1:55,000 in Oxfordshire [71], and 1:100,000 in Denmark [75]. Men and women are equally affected. DD has a very high penetrance, variable degree of symptoms, and follows an autosomal dominant inheritance pattern, meaning that, on average, 50% of a patients' offspring are affected [66]. A majority of individuals with DD experience a disease onset between the ages of 6 and 20, peaking in the middle of this interval (11-15 years of age) [76].

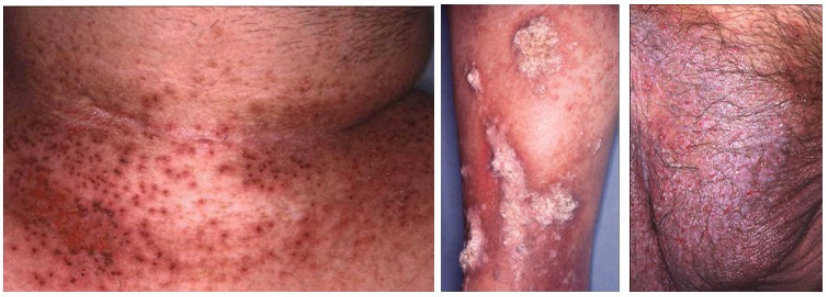
2.2.3 Clinical features

2.2.3.1 Appearance and distribution pattern

DD is characterized by widespread and chronically relapsing oily, hyperkeratotic papules and coalescing plaques with a predilection to seborrheic areas of the skin (***Figure 5A***), as well as nail deformities and mucous membrane involvement [76-79]. Originally thought to be a disease of the follicles, DD is often found outside follicular regions. A recent study found that the hands and nails were the most commonly affected body sites, and that most patients exhibit a combination of several distribution patterns such as acral, flexural and seborrheic [80].

Initially consisting of red-brown keratotic papules, lesions tend to coalesce and form hyperkeratotic plaques over time if the disease gets more severe, which it often does. Another distinguishing feature is the occurrence of small hypomelanotic macules that often can be seen intermixed with the other rashes. Acrally located wart-like lesions are common, and cases of vesiculo-bullous DD has been described [81]. Nail changes consist of a typical V-shaped distal indentation and longitudinally oriented red and white streaks (***Figure 5B***). Mucosal lesions consisting of red and white plaques or white cobblestone patterns also commonly occurs in DD and predominantly affect the hard palate, but also other parts of the oral cavity [82] (***Figure 5C***). Most individuals with DD experience an itch that is most often mild to moderate. An unfortunate characteristic of DD is the presence of malodor frequently emerging from affected skin [76, 78, 80]. Individuals with DD exhibit a significantly reduced quality of life which also correlates to disease severity [83].

A



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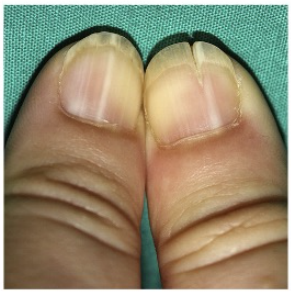

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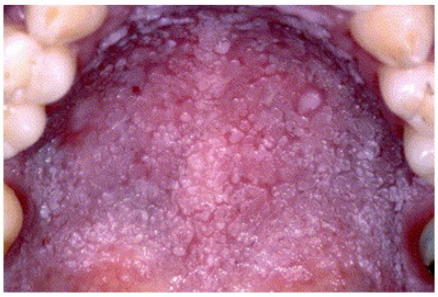

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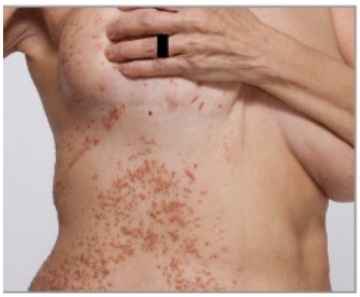

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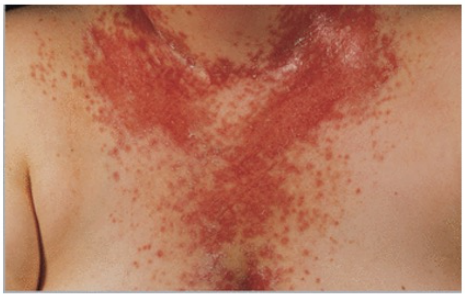

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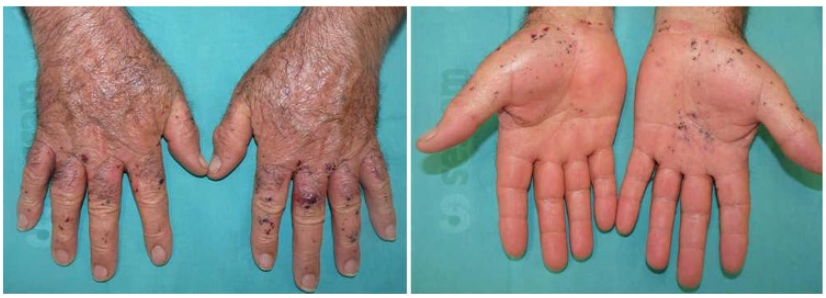

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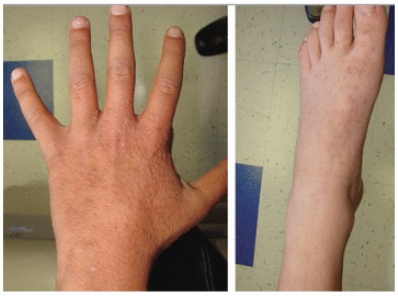

H

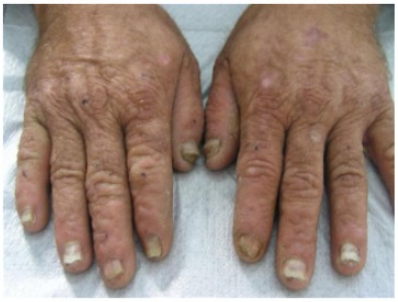

Figure 5. Overview of clinical features in Darier disease (DD) and its subtypes. **A**: Common presentations of classical DD. **B**: Typical nail changes in DD. **C**: DD in the hard palate. **D**: Type 1 segmental DD. **E**: Type 2 segmental DD. **F**: Acral hemorrhagic DD. **G-H**: Acrokeratosis verruciformis of Hopf.
*Images adapted from: Takagi, A., et al., Darier disease. J Dermatol, 2016. **43**(3): p. 275-9 (**A**); Engin, B., et al., Darier disease: A fold (intertriginous) dermatosis. Clin Dermatol, 2015. **33**(4): p. 448-51 (**B**); Jalil, A.A., et al., Darier disease: a case report. Br J Oral Maxillofac Surg, 2005. **43**(4): p. 336-8 (**C**); Sartori-Valinotti, J.C., et al., Segmental type 1 Darier disease: a case series highlighting late-onset disease. Br J Dermatol, 2015. **173**(2): p. 587-9 (**D**); Fölster-Holst, R., et al., Molecular genetic support for the rule of dichotomy in type 2 segmental Darier disease. Br J Dermatol, 2012. **166**(2): p. 464-6 (**E**); Flores-Terry, M., et al., Acral Hemorrhagic Darier Disease. Actas Dermosifiliogr, 2017. **108**(7): p. e49-e52 (**F**); Berk, D.R., et al., A sporadic patient with acrokeratosis verruciformis of Hopf and a novel ATP2A2 mutation. Br J Dermatol, 2010. **163**(3): p. 653-4 (**G**); Rallis, E., et al., Acrokeratosis verruciformis of Hopf (Hopf disease): case report and review of the literature. Dermatol Online J, 2005. **11**(2): p. 10 (**H**).*

2.2.3.2 Type 1 and 2 segmental Darier disease

Type 1 and 2 segmental DD, also called linear DD, mosaic DD and zosteriform DD, are rare subtypes of the disease.

Type 1 segmental DD is caused by postzygotic mutation in the disease-causing *ATP2A2* gene. This leads to a localized phenotype of DD following the lines of Blaschko, which represent pathways of epidermal migration during fetal development. Patients experience the same age of onset, clinical course and histopathological findings as classical generalized DD, but in a mosaic, localized distribution. Background skin is completely unaffected [84-89] (***Figure 5D***). One case of linear comedonal DD has also been descried [90].

Type 2 segmental DD is an even rarer form of DD than type 1. Here, patients have both a germline *ATP2A2* mutation and a postzygotic deactivating so-called "second hit" mutation in the other allele of the same gene, an example of "loss of heterozygosity". This leads to a mosaic distribution of a much more severe rash following the lines of Blaschko, while the background skin exhibits a classical DD phenotype [91-93] (***Figure 5E***). Only a few cases of type 2 segmental DD have been described.

2.2.3.3 Acral hemorrhagic Darier disease

A rare subtype of DD, the acral hemorrhagic form display asymmetrical red to black small macules on the volar aspects of the hands and feet as well as the dorsal hands (***Figure 5F***). They have been found to consist of bleeding into vesicles formed as part of the DD rash. A specific mutation on exon 15 of the disease-causing *ATP2A2* gene has been described in several unrelated cases. Besides this particular clinical feature, acral hemorrhagic DD is identical to classical DD [76, 94-97].

2.2.3.4 Acrokeratosis verruciformis of Hopf

Acrokeratosis verruciformis of Hopf, first described by German dermatologist Gustav Hopf in 1931 [98], is sometimes considered a subtype of DD, sometimes as a separate disease entity. Affected individuals carry mutations affecting the same gene, *ATP2A2*, and an acral spectrum of clinical features including the nail changes and palmoplantar hyperkeratotic papules and plaques often seen in DD, albeit without involvement of any other sites (***Figure 5G-H***). The histopathological appearance differs completely however, as the signature features of DD are not found. Interestingly, there are families where some individuals only show signs of Acrokeratosis verruciformis of Hopf and others have all the typical signs of DD, and cases that appear to solely have acral changes only to much later develop signs of widespread DD. To further confuse things, the term "acrokeratosis verruciformis" is sometimes used to describe the wart-like keratotic papules often seen on the dorsal hands of individuals with DD. Thus, there is significant terminological, etiological and clinical overlap [99-103].

2.2.4 Histopathology

The hallmark features of DD histopathology are *acantholysis*, meaning the loss of cohesion between epidermal keratinocytes, and *dyskeratosis*, meaning the abnormal, premature or defective keratinization of keratinocytes [104-106].

The acantholysis seen in DD has been found to be largely caused by the loss of, or abnormal functioning of, desmosomes, large complexes responsible for strong cell-to-cell adhesion between keratinocytes [107-110]. The functioning of adherens junctions has also been shown to be compromised [111]. An early ultrastructure study found that desmosomes in DD lose their connection to the keratin intermediate filament cytoskeleton [112]. Desmosomes are discussed in detail in section 2.1.4.1.

The dyskeratosis seen in DD is ultimately due to the apoptosis of individual keratinocytes, giving rise to two distinct histopathological signs. "Corps ronds" are enlarged, acantholytic keratinocytes with that stain darker than normal and exhibit a contracted and fragmented nucleus with a perinuclear cytoplasmic clearing. "Grains" correspond to small cells in the stratum corneum that stain strongly eosinophilic in the cytoplasm due to clumps of collapsed keratin together with parakeratotic remains of the nuclei [104-106, 113].

Together, acantholysis and dyskeratosis form specific histopathological features that an experienced dermatohistopathologist will recognize as DD.

2.2.5 Diagnostic methods

The diagnosis of DD is traditionally made based on:

1. Clinical features,
2. Histopathology, and
3. Family history [66, 76, 77, 79].

It is not uncommon for patients with DD to experience significant patient's and doctor's delay, or to be misdiagnosed with a different dermatological affliction, a common problem in rare diseases [114, 115].

DD could also be diagnosed by performing genetic testing on the disease-causing *ATP2A2* gene. While this is not commonly performed in standard clinical practice, it can be an option under different circumstances such as when presented with cases that are difficult to diagnose, in the process of genetic counseling or for the purposes of scientific research. As genetic testing is becoming more accessible and affordable, it is plausible that it will become standard practice as a means to diagnose DD and other genetic disorders.

2.2.6 Management and treatment

To date, no targeted, curative or long-term tolerable treatment for DD exists, rendering all treatment attempts symptomatic. Patients must be aware of general measures for reducing the risk of exacerbations, and both topical treatments (e.g., salves and creams), systemic drugs and surgical treatment modalities are employed. As with many disorders lacking favorable treatment options, many experimental therapies have been explored. All treatments for DD explored in this review are summarized in ***Table 1***.

2.2.6.1 General measures

The mainstay of general care for DD consists of avoidance of exacerbating factors such as heat, sweating, friction, direct sunlight and stress. Patients are encouraged to wear light clothing to avoid unnecessary sweating and chafing. Emollients and sun protection creams are used liberally as needed. Good general personal hygiene with frequent skin cleansing is usually beneficial [116, 117]. Recurring secondary bacterial and viral infections are treated with oral antibiotics and antivirals, respectively.

2.2.6.2 Topical treatments

Several topical treatment modalities have been evaluated. Emollients, exfoliants and corticosteroids are very common treatments for DD, all unfortunately with poor effect [76, 77, 116]. Calcipotriol, a synthetic vitamin D analogue, led to worsening of the disease in a majority

of patients in one trial [118]. There are some reports on the effectiveness of topical retinoids as monotherapy, primarily in localized disease [119, 120]. The successful treatment of DD with other agents in topical form such as 5-fluorouracil [121], pimecrolimus [122], cyclooxygenase-2 inhibitors [123] and diclofenac sodium 1 and 3% [124, 125] have been described. Unfortunately, these are reports of individual cases and thus carry low scientific value as a basis for treatment recommendations. In the author's own experience, usage of the desiccating and antimicrobial treatment modality of topical potassium permanganate solution can also be beneficial in some cases.

2.2.6.3 Systemic treatments

Severe cases of DD are most often treated with oral retinoids such as isotretinoin, alitretinoin and acitretin, where the latter is most commonly used. Oral retinoids have been reported to be efficacious in most cases, but with low to non-existent long-term patient acceptability due to adverse side-effects. Also, patients experience relapse upon cessation of treatment [76-78, 116, 126, 127]. There are reports of successful treatment with the retinoid etretinate, which yields acitretin as its active metabolite, in cases where acitretin treatment was ineffective [128]. However, a longer-term follow-up study highlights the strong negative side-effects etretinate, causing a majority of patients in one study to discontinue the treatment within nine months [129]. In summary, oral retinoids are efficacious, but carry with them significant negative impact for the patient and are thus not feasible in the long term.

There are reports of ciclosporin as a secondary option for treating severe DD, for example in patients not responding to or not tolerating oral retinoids [130-132]. As with oral retinoids, caution needs to be taken as to potentially severe side-effects, and long-term treatment is not recommended.

2.2.6.4 Surgical treatments

Surgical or locally destructive treatments have been evaluated for the treatment of severe and intractable localized lesions. Successful laser modalities include regular and fractional CO_2 laser [133, 134], erbium:YAG laser [135] and pulsed-dye laser [136]. Allograft skin transplantation has also been found effective in one case [137]. Several different cases of surgical excision has also been performed on lesions located on the breasts, in the perianal region and on the nails [117]. Additionally, dermabrasion has been utilized in a total of 12 DD patients at one center with good long-term results [138, 139].

These treatments must be performed with utmost care and only in select, refractory cases due to the high risk of negative side-effects such as scarring and keloid formation.

General measures	Avoid exacerbating factors (e.g., heat, sweating, friction) Good general skin hygiene Wear light, cool clothing Emollients Sun protection creams Treat secondary bacterial and viral infections
Topical treatments	Glucocorticosteroids Exfoliants (e.g., salicylic acid creams) Retinoids (e.g., adapalene, retinol, tretinoin, tazarotene) Calcipotriol 5-fluorouracil Pimecrolimus Cyclooxygenase-2 inhibitors Diclofenac sodium 1 and 3% Potassium permanganate solution
Systemic treatments	Retinoids (acitretin, isotretinoin, alitretinoin, etretinate) Ciclosporin
Surgical treatments	Surgical excision Fractional CO_2 laser Erbium:YAG laser Pulsed-dye laser Allograft skin transplantation Dermabrasion
Other/experimental treatments	Botulinum toxin injections Photodynamic therapy Radiation therapy Low-dose naltrexone Oral doxycycline Oral magnesium Oral vitamin A Low-dose intravenous immunoglobulin

Table 1. A summary of all treatments for DD included in this review. Please note that many of these therapies might be ineffective or even lead to worsening of the disease. See the text for more in-depth descriptions. YAG: yttrium aluminum garnet.

2.2.6.5 Other/experimental treatments

Over the years, several therapeutic attempts of a more experimental kind have been reported. These include botulinum toxin injections [140, 141], photodynamic therapy [142], radiation therapy [143-145], low-dose naltrexone [146], oral doxycycline [147], oral magnesium [148], oral vitamin A [149], and low-dose intravenous immunoglobulin [150]. One case series (n = 14) reported the effectiveness of dietary supplementation with fatty acids [151], leaning on indications from a previous case-control study with DD patients (n = 13) which showed an

abnormal metabolism of fatty acids in individuals with DD compared with control subjects [152]. Most patients improved, but the results have never been replicated.

While being announced successful in many of the published reports, a high degree of caution should be employed in relation to these therapies. The data are limited to case reports and small case series, and none of the treatments target the disease mechanism. Even in the case of a fortunate treatment attempt, the chronically relapsing nature of DD means it will most certainly exacerbate eventually. Patients also tend to seek healthcare when their symptoms are at their worst and would probably improve spontaneously even without treatment. In randomized controlled trials, this "regression to the mean" effect is accounted for in the placebo group [153], but not in these case reports of experimental treatments. Thus, the risks of the treatment must have been thoroughly thought through beforehand and communicated to the patient.

2.2.7 Prognosis

DD follows a chronic, non-remitting, continuously relapsing course. Some patients experience worsening with age, while others see an improvement over time. Most, if not all, patients exhibit recurring secondary bacterial and viral infections that require treatment, and it is not uncommon for patients to require in-patient ward treatment intermittently [67, 76, 77, 79]. To date, no curative treatment for DD exists.

2.3 GENETICS OF DARIER DISEASE

Shortly after being first described in 1889 [72, 73], White proposed the fact that, and the fashion in which, DD is inherited [74]. Thus, DD has long been known to follow an autosomal dominant inheritance pattern [154]. In 1992 the first linkage analysis study was performed on two large DD kindreds, mapping the disease gene to the so-called "Duffy blood locus" on chromosome 1p13 [155]. This was found to be incorrect [156], and further linkage analyses could narrow down the genetic mapping to a locus on chromosome 12q, specifically 12q23-q24.1 [157-159]. The size of the interval of the disease-causing gene locus was further reduced over the following few years [160-166]. Ultimately, in 1999 the disease-causing gene was identified as *ATP2A2*, mapped to chromosome 12q24.11 using positional cloning [69]. *ATP2A2* encodes the Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase 2 (SERCA2), a Ca^{2+} pump responsible for the transportation of Ca^{2+} from the cytosol into the endoplasmic reticulum (ER) lumen [69, 167].

2.3.1 The ATP2A2 gene

The *ATP2A2* gene belongs to a family of three genes, namely *ATP2A1*, *ATP2A2* and *ATP2A3*. They all encode SERCA proteins. The transcript of the *ATP2A2* gene gives two main isoforms, a and b, by alternative splicing, which consist of 21 and 20 exons, respectively [168, 169].

Isoforms c and d also exist by means of alternative splicing (see section 2.4.3). Interestingly, not long before the locus for the disease-causing gene for DD was found, the *ATP2A2* gene itself underwent a similar process of mapping to chromosome 12q23-q24.1 [170-172]. Therefore, researchers knew that the locus housing the disease-causing indeed contained the *ATP2A2* gene.

2.3.2 ATP2A2 mutations

In the time following shortly after the initial discovery of heterozygote *ATP2A2* mutations as the cause of DD, several more descriptions of novel mutations were reported [84, 94, 173-177]. A 2002 review of the so far 103 described mutations concluded that mutations were widespread in the gene [178]. Currently, there are 437 total mutation variants reported of which 299 are unique [Leiden Open Variation Database; www.lovd.nl/ATP2A2, accessed 2021-10-11] [167]. They consist of mostly missense/nonsense mutations, thereafter deletions, splice-site mutations and insertions. No mutational hotspot has been identified, and mutations have been found in all exons [167, 174, 178-180]. Most mutations display a familial congregation, being unique to the affected family, and only in a minority of cases do the same mutation affect different families [180].

2.3.2.1 Darier disease lacking ATP2A2 mutations

Interestingly, several studies have found that on average 26±14% of individuals with DD lack detectable *ATP2A2* mutations [167, 173, 174, 179-185]. There could be several explanations for this, one being that there are other, undetected, mutations in genes affecting proteins generating a similar cellular phenotype like that seen in DD [167]. This would be an example of what is called “locus heterogeneity”, meaning that mutations on different genes or loci can give rise to similar or identical phenotypes. As a side note, the opposite phenomenon is termed “allelic heterogeneity”, where mutations in the same gene can give rise to several different phenotypes.

Another possible explanation for the lack of detectable *ATP2A2* mutations in patients with DD is that there could be potential disease-causing mutations in non-sequenced regions of the gene, such as in the promoter, intronic or untranslated regions, or even large insertions or deletions undetectable by sequencing methods that only examine the exons and bordering intronic regions [179]. Despite the plausibility of these theories there currently exists a gap in our understanding of how and why DD can occur without detectable *ATP2A2* mutations.

2.4 SERCA

The gene construct of *ATP2A2* is Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase 2 (SERCA2), a ubiquitously expressed protein that is also considered a “housekeeping” protein, meaning a

protein considered to carry out a function essential for the maintenance of basal functions in the cell. The disease phenotype of DD is caused by SERCA2 *haploinsufficiency*, meaning that the healthy allele of the *ATP2A2* gene is unable to compensate for the loss of function in the mutated allele. This in turn leads to the production of less than adequate amounts of SERCA2 necessary for normal cell functioning [69, 186-192]. Homozygote loss-of-function mutations are believed to be lethal. A review of protein turnover rates has found that the half-life of SERCA2 in immune cells (B cells, natural killer [NK] cells and monocytes), hepatocytes and mouse embryonic neurons on average vary between 40 and 196 hours [193].

2.4.1 SERCA structure

The evolutionarily highly conserved family of SERCA pumps are encoded by three genes, *ATP2A1*, *ATP2A2* and *ATP2A3*. Via relatively tissue-specific alternative splicing mechanisms, a total of 12 isoforms has been found [194]. They all consist of roughly 1000 amino acid-long single polypeptide chains weighing around 110 kDa (***Table 2***) [169, 195, 196]. The folded protein resides in the ER membrane and consists of 10 transmembrane helices (M1-M10), a cytosolic stalk domain (S1-S5) and three cytosolic domains: the actuator (A), nucleotide adenosine triphosphate (ATP)-binding (N) and phosphorylation (P) domain. The M2, M5, M6 and M8 transmembrane helices are essential in forming the Ca^{2+} channel while M4-M6 facilitate the transportation of Ca^{2+} across the ER membrane [197-201].

2.4.2 SERCA function

SERCAs are characterized as *P-type ion-motive ATPases*, transporting two Ca^{2+} ions from the cytosol into the ER at the expense of one molecule of ATP [202, 203]. They are the only active transporters of Ca^{2+} from the cytosol to the ER lumen, where Ca^{2+} is stored at a much higher concentration, around 1000-10,000-fold, than in the cytosol [204, 205] (***Figure 6***).

SERCAs are the most important Ca^{2+} transporters in the ER, which in turn is the largest Ca^{2+} deposit in the cell [206-208]. Intracellular Ca^{2+} stores has been found to be the main constituents of the epidermal Ca^{2+} gradient [209-212], and since SERCA is the main Ca^{2+} pump in the ER, it has a strong, direct influence on not only intracellular Ca^{2+}, but also the entire Ca^{2+} gradient in the epidermis and consequently also epidermal keratinocyte differentiation [213-215]. Taken together, SERCA is in itself the single most important regulator of cellular Ca^{2+} homeostasis [36].

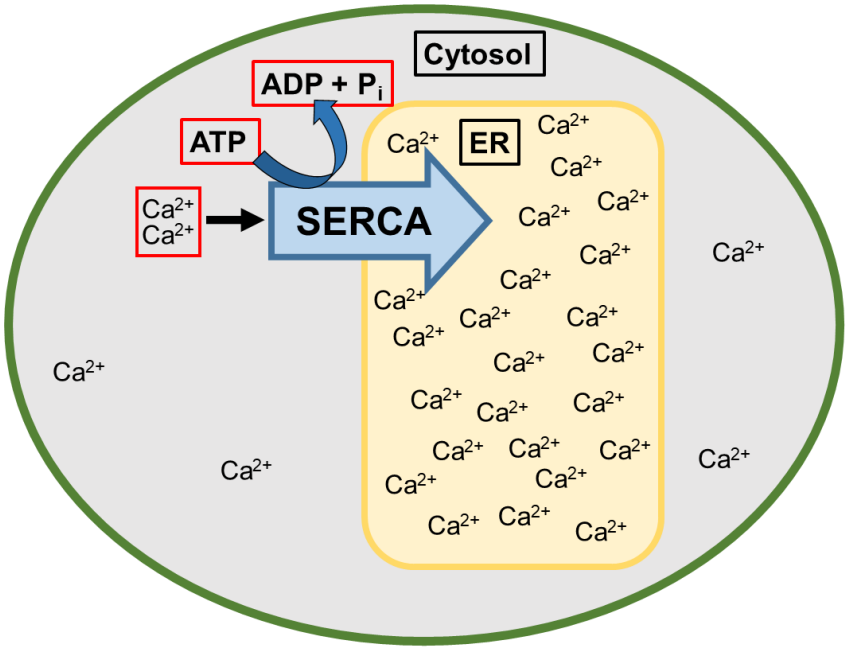

Figure 6. SERCA transports two Ca^{2+} ions from the cytosol into the ER at the expense of one molecule of ATP, against a high Ca^{2+} gradient. ADP: adenosine diphosphate; ATP: adenosine triphosphate; ER: endoplasmic reticulum; P_i: inorganic phosphate; SERCA: Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase.

2.4.3 SERCA isoforms

Currently, 12 known isoforms of SERCA have been identified so far, produced via relatively tissue-specific alternative splicing: two isoforms of SERCA1 (SERCA1a-b), four isoforms of SERCA2 (SERCA2a-d) and six isoforms of SERCA3 (SERCA3a-f) (***Table 2***). They are comparable in size and weight, while differing considerably in their tissue expression patterns, and their affinity for Ca^{2+} may also vary [216, 217].

2.4.3.1 SERCA1

SERCA1 has two known isoforms, encoded by the *ATP2A1* gene. In contrast to the other SERCAs, SERCA1 exhibits a temporal expression pattern. SERCA1a is predominantly expressed in adults, while SERCA2b is mostly expressed in the neonatal period. SERCA1 has been found to be expressed in primarily fast-twitch skeletal muscle [218].

2.4.3.2 SERCA2

SERCA2 has four known isoforms, encoded by the *ATP2A2* gene, which is mutated in DD [69]. SERCA2a is expressed mainly in slow-twitch and cardiac muscle [219, 220]. It has also been found in low levels in different cells of the brain [221] Functional studies have found that the SERCA2a and SERCA1a isoforms appear to have comparable affinities for Ca^{2+} [222, 223]. SERCA2b is ubiquitously expressed [217], has the highest affinity for Ca^{2+} of all SERCA

isoforms, and will be discussed at length in section 2.4.4. SERCA2c has been found in epithelial, mesenchymal, hematopoietic cells and to a lesser degree in the brain [195, 224, 225]. It is thought to derive from the incorporation of a short coding sequence between exon 20 and 21 in the SERCA2a isoform [225] and has the lowest affinity for Ca^{2+} of the four SERCA2 isoforms [195]. SERCA2d messenger ribonucleic acid (mRNA) has so far only been identified in skeletal muscle, and no protein isoform equivalent has yet been discovered [226].

SERCA isoform	Protein size & weight	Expression pattern
SERCA1a	994 aa, 109.3 kDa	Fast-twitch skeletal muscle, adult
SERCA1b	1001 aa, 110.5 kDa	Fast-twitch skeletal muscle, neonatal
SERCA2a	997 aa, 109.7 kDa	Slow-twitch and cardiac muscle, brain, (~skin)
SERCA2b	1042 aa, 114.8 kDa	Ubiquitous
SERCA2c	999 aa, 108.9 kDa	Epithelial, mesenchymal and hematopoietic cells, brain
SERCA2d	1007 aa, 110.6 kDa	Skeletal muscle
SERCA3a	999 aa, 109.2 kDa	Brain, heart, pancreas, immune cells, lung, liver, placenta
SERCA3b	1043 aa, 113.9 kDa	Brain, heart, pancreas, immune cells, lung, liver, placenta
SERCA3c	1029 aa, 112.4 kDa	Platelets, T-lymphocytes
SERCA3d	1044 aa, 114.1 kDa	Brain, heart, pancreas, immune cells, lung, liver, placenta, skeletal muscle
SERCA3e	1052 aa, 114.9 kDa	Pancreas, lung
SERCA3f	1033 aa, 112.6 kDa	Ubiquitous

Table 2. Protein size and expression pattern of all 12 known isoforms of SERCA. aa: amino acids; kDa: kilodalton; SERCA: Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase.
Partly adapted from Britzolaki, A., et al., A Role for SERCA Pumps in the Neurobiology of Neuropsychiatric and Neurodegenerative Disorders. Adv Exp Med Biol, 2020. ***1131****: p. 131-161.*

2.4.3.3 SERCA3

SERCA3 has six known isoforms, encoded by the *ATP2A3* gene. SERCA3a and SERCA3b have been found to be expressed in a variety of tissues including brain, heart, pancreas, immune cells, lung, liver and placenta [227, 228]. SERCA3c has been detected in platelets and immortalized T-lymphocytes (so-called "Jurkat cells") [228]. SERCA3d displays a similar expression pattern as SERCA3a and b, as well as skeletal muscle, while SERCA3e seems to be limited to pancreatic and lung tissue [227]. SERCA3f, the final known isoform of SERCA3, appear to be expressed in all human tissues and cell types [229]. The affinity for Ca^{2+} is similar for all SERCA3 isoforms, is comparable to that of SERCA2a and evidently lower than that of SERCA2b [230].

2.4.4 SERCA2b and the skin

SERCA2b is the SERCA isoform of main interest in skin. Still, DD patients' *ATP2A2* mutations most commonly affect all isoforms [215]. It has a ubiquitous expression pattern and is considered a "housekeeping" protein, being one of the most critical determinants of not only ER Ca^{2+} homeostasis, but indeed for the Ca^{2+} homeostasis of the entire cell [36, 168, 186, 219, 231]. SERCA2b has a uniquely long C-terminus that can localize to the ER membrane, giving rise to an eleventh transmembrane helix (M11, also called "2b-tail") [222, 223, 232, 233]. The M11 helix seems to contribute to making SERCA2b the isoform with the highest affinity for Ca^{2+}, which has been found to be twofold that of SERCA2a and SERCA1a [186, 222, 233, 234].

Interestingly, *ATP2A2* mutations have been described in the M11 helix, thus making them specific for SERCA2b and resulting in a premature termination codon, yielding the DD phenotype [181, 235]. This can be seen as proof of concept that SERCA2b is the most important isoform in skin, as other SERCAs, which are unaffected in these individuals, would otherwise be able to compensate for this loss-of-function in SERCA2b. Taken together, the skin appears to be a particularly sensitive tissue type for SERCA2b loss-of-function, which is clearly seen in the way DD presents clinically [94, 236, 237].

2.5 ER STRESS

The principal functions of the ER concerns protein processing and synthesis, lipid synthesis, and Ca^{2+} storage and management [205, 208, 238-240]. It is estimated that around one third of all proteins in the cell undergo post-translational processing in the ER [241]. There are several cellular stress conditions where the ER is subjected to an accumulation of unfolded and/or misfolded proteins in the ER lumen, which causes *ER stress* [242-248]. ER stress in turn prompts the *unfolded protein response* (UPR), a multifactorial cellular response system that initially acts to alleviate the ER stress.

When the ER stress is chronic, such as in DD [111], or when it becomes ineffective or reaches an irreversible level, the UPR shifts and becomes pro-apoptotic [242, 244, 245, 249] or possibly even pro-cancerous [250-252]. The apoptotic response is largely caused by well-known regulators of cell death belonging to the B cell lymphoma 2 (Bcl-2) family [253-257]. ER stress has also been shown to have purely physiological functions in keratinocyte differentiation [258, 259].

2.5.1 Unfolded protein response

3 sensor proteins in the ER membrane are responsible for the UPR: Inositol-requiring enzyme 1α (*IRE1α*), protein kinase-like ER kinase (*PERK*) and activating transcription factor 6 (*ATF6*). During unstressed cellular states, the ER resident chaperone glucose-regulated protein 78

(*GRP78*, also called binding immunoglobulin protein [BiP]) is bound to each of the three ER transmembrane sensor proteins. The induction of the UPR occurs when GRP78 dissociates from them as a response to the accumulation of unfolded and/or misfolded proteins in the ER [246, 260, 261].

2.5.1.1 IRE1α signaling

IRE1α signaling leads to the splicing of X-box binding protein 1 (*XBP1*) mRNA, yielding spliced XBP1 (sXBP1). sXBP1 is a potent and wide-acting transcription factor targeting a multitude of genes with activity pertaining to the ER, immune system, inflammatory responses, differentiation and lipid metabolism [262-267].

2.5.1.2 PERK signaling

PERK signaling leads to the phosphorylation of eukaryotic translation initiation factor 2α (*eIF2α*), resulting in the decrease of protein synthesis to alleviate ER working load, as well as activation of *ATF4* which regulates the transcription of genes associated with ER protective functions. In contrast, too much PERK activation gives rise to the activation of pro-apoptotic pathways through the CCAAT-enhancer-binding protein homologous protein (*CHOP*) [245, 257, 268, 269].

2.5.1.3 ATF6 signaling

ATF6 is in itself a potent transcription factor, and upon activation by dissociation of GRP78 (like IRE1α and PERK) it briefly translocates to the Golgi apparatus where it is activated by protease cleavage to be able to exert its effect as an activator of several genes associated with protective and reviving functions for the ER [261, 264, 270, 271].

2.6 CELLULAR PHENOTYPE OF DARIER DISEASE

The cellular phenotype of DD patient-derived keratinocytes has attracted a lot of attention due to their unique, naturally occurring loss-of-function in SERCA2, which enables the study of cellular and ER Ca^{2+} and the effect on numerous elements such as ER stress, cell-to-cell adhesion and epidermal differentiation.

As one might expect, DD keratinocytes have been shown to have depleted stores of ER Ca^{2+} and elevated resting levels of intracellular Ca^{2+} [272-275], leading to a constitutive high level of ER stress [111, 276]. One study even found that the mutant protein SERCA2 is itself a significant initiator of ER stress in DD [277]. The level of resting intracellular Ca^{2+} varied greatly among keratinocytes cultured from different individuals with DD, illustrative of the

variable cellular phenotype of DD [273]. Further, in normal keratinocyte cell culture, a shift to high extracellular Ca^{2+} led to a significant increase in the gene expression of *ATP2A2*, indicating a complex regulatory mechanism involving SERCA2 in response to Ca^{2+} in the extracellular space [278].

The entire epidermal Ca^{2+} gradient described in section 2.1.5.1 has been found to largely depend on the Ca^{2+} stores in the ER and Golgi apparatus [209-212, 215], leading to abnormal keratinocyte differentiation in DD because of the lower levels of ER Ca^{2+} [262, 279-282]. This also underlies the histopathological dyskeratosis seen in biopsies from individuals with DD. Furthermore, lesional DD skin has been shown to display lower SERCA2a and SERCA2b staining intensity compared with non-lesional skin in within-patient comparisons. However, no difference was found between non-lesional DD skin compared to non-patient skin [283], implying a possible local downregulation of SERCA2 in lesional DD skin.

The main hallmark feature of DD histopathology is acantholysis, or the loss of cell-to-cell adhesion, as discussed in section 2.2.4. DD keratinocytes have been found to have altered, immature or otherwise abnormally functioning desmosomal and adherens junction components [107, 109, 111, 262, 284-286]. The same is seen in experimental settings when mimicking the DD cellular phenotype [108, 213, 280, 287, 288].

2.7 COMORBIDITIES OF DARIER DISEASE

The comorbidities associated with DD will be discussed at length in the results and discussion sections, since this topic encompasses the main research aims of this thesis.

2.8 HAILEY-HAILEY DISEASE

In study I, two patient groups were included, namely individuals with DD and individuals with Hailey-Hailey disease (HHD). Therefore, a brief background is presented here.

HHD is an acantholytic heterozygote autosomal dominant skin disorder with similar pathophysiology and phenotype as DD due to mutations in the *ATP2C1* gene, encoding a Ca^{2+} and manganese (Mn^{2+}) pump in the Golgi apparatus: Secretory Pathway Ca^{2+}/Mn^{2+}-APTase 1 (SPCA1) [167, 289-291]. Patients exhibit erythematous, blistering, erosive and often painful plaques primarily in the flexural areas of the skin in addition to sites of friction [291-293]. Due to the similar cellular phenotype between HHD and DD, and the fact that no evaluation of cognition in HHD had previously been performed, individuals with HHD were included in study I.

3 RESEARCH AIMS

The overall aim of this thesis was to investigate the presence of comorbidities in individuals with Darier disease (DD), and thus to decipher if, and to what extent, DD is a systemic condition with extra-dermal engagement. Specifically, the individual studies aimed to:

1. Experimentally investigate the existence of a cognitive impairment in individuals with DD and Hailey-Hailey disease (study I).
2. Examine the occurrence of heart disease in individuals with DD, both in an experimental setting and on the population level (study II).
3. Experimentally assess the metabolic phenotype in individuals with DD to explore the association between DD and diabetes (study III).
4. Investigate the association between DD and diabetes on the population level (study IV).
5. Evaluate immunological markers in individuals with DD, and explore the scientific rationale behind an association between DD and a deficit related to the immune system (study V).

4 MATERIALS AND METHODS

4.1 SUMMARY OF MATERIALS AND METHODS

A summary of the study designs, main research aims/factors analyzed, data sources, inclusion and exclusion criteria, and ethical approvals for the studies included in this thesis are presented in ***Table 3***.

4.2 DATA SOURCES

4.2.1 Swedish national registers

Sweden has a long history of record keeping for the population, dating all the way back to the late 17th century when the Swedish church started keeping registers of its members. In 1749 Sweden started collecting nationwide data on its population, and in 1947 the personal identity number (Swedish: *personnummer*) was introduced [294]. The aim of the national registers is to obtain data that completely reflect the Swedish population to allow the government and other organizations, e.g., research institutions, to use such data in decision making, scientific research etc. The registers are kept by Statistics Sweden (Swedish: *Statistiska centralbyrån*), the National Board of Health and Welfare (NBHW, Swedish: *Socialstyrelsen*) and several other Swedish government agencies [295]. There is a large number of Swedish national and health care-related registers (> 30) and linkage between them is made possible in large part by the personal identity numbers, which are used in coded/unidentifiable form [294]. The linkage is done through Statistics Sweden.

4.2.1.1 The Total Population Register

The Total Population Register (TPR, Swedish: *Registret över totalbefolkningen*) started in 1968, with some data available since 1961. It is held by Statistics Sweden and contains demographic data for all residents born after 1932. The data is updated monthly, quarterly and yearly. Only the annual data are used for purposes such as the selection of comparison subjects in research. Data kept includes birth date, gender, county and country of birth, data on immigration and emigration, civil status, and residential address for all Swedish residents alive at the end of the year [295].

Study	I	II*		III	IV	V**
Study design	Experimental, matched case-control study	Population-based cohort study	Experimental, matched case-control study	Experimental, matched case-control study	Population-based cohort study	Cohort study/case series
Main factors analyzed	The association between Darier and Hailey-Hailey disease, and cognitive impairment	The association between Darier disease and heart disease		The association between Darier disease and diabetes	The association between Darier disease and diabetes	The association between Darier disease and deficits in the immune system
Data sources	Patient cohort and matched controls, data collected prospectively	The NPR, TPR, CDR and SAMS	Patient cohort and matched controls, data collected prospectively	Patient cohort and matched controls, data collected prospectively	The NPR, TPR, CDR and SAMS	Patient cohort, data collected prospectively
Inclusion criteria	Typical skin lesions in combination with typical histopathology and/or family history	Diagnosis of Darier disease Q82.8E (ICD-10) and 75.7D (ICD-9). Diagnosis of heart failure I50, myocardial infarction I21 and arrhythmia I49 (ICD-10)	Typical skin lesions in combination with typical histopathology and/or family history.	Typical skin lesions in combination with typical histopathology and/or family history	Diagnosis of Darier disease Q82.8E (ICD-10) and 75.7D (ICD-9). Diagnosis of diabetes type 1 E10 and diabetes type 2 E11 (ICD-10)	Clinical phenotype of Darier disease and pathogenic *ATP2A2* mutation
Exclusion criteria	Age < 18 years, significant ongoing psychiatric disorders, neurotropic or psychotropic medication, active substance abuse, dementia, acute illness during the past four weeks, pregnancy	None	Oral corticosteroid treatment, active substance abuse, severe kidney or liver disease, acute illness during the past four weeks, pregnancy	Oral corticosteroid treatment, active substance abuse, severe kidney or liver disease, acute illness during the past four weeks, pregnancy	None	Age < 18 years, ongoing disorder or medication affecting the immune system, active substance abuse, acute illness during the past four weeks, pregnancy
Ethical approval	2015/1798-31	2015/1798-31 and 2009/939-31/5		2015/1798-31	2009/939-31/5	2015/1798-31

Table 3. Summary of materials and methods in the studies included in this thesis. *Study II is henceforth discussed as two separate studies, a register-based cohort study (sometimes referred to as a population-based study) and an experimental, matched case-control study (sometimes referred to as a clinical study). **For study V, solely a description of the contributions relevant to the doctoral student are included. CDR: The Cause of Death Register; ICD: International Classification of Diseases; NPR: The National Patient Register; SAMS: The Small Area Marketing Statistics Register; TPR: The Total Population Register.

4.2.1.2 The National Patient Register

The National Patient Register (NPR) was started in 1964, with complete coverage from 1987. The NPR is held by the NBHW. Initially, it was limited to data on inpatient and hospital discharge diagnoses as part of the Swedish National Inpatient Register. Psychiatric diagnoses are included since 1973. Starting in 1997, data on surgical day care procedures are included, and since 2001 all outpatient data from both public and private caregivers are included in the NPR [296], however with incomplete coverage until 2006.

The NPR contains diagnoses based on the International Classification of Diseases (ICD). The diagnoses are assigned by the responsible physician, and the coverage is close to 100% for inpatient diagnoses and 80% for outpatient diagnoses in total and almost 100% for outpatient diagnoses derived from public caregivers. An external validation study concludes positive predictive values of 85-95% for a large number of diagnoses in the NPR [296].

4.2.1.3 The Cause of Death Register

The Swedish Cause of Death Register (CDR) includes the cause and date of death for all Swedish residents, with full coverage since 1952, including residents dying abroad. The CDR is almost 100% complete and highly reliable. The register is maintained by the NBHW [297].

4.2.1.4 The Small Area Marketing Statistics Register

The Small Area Marketing Statistics (SAMS) Register is a geographical classification system dividing Sweden into more than 9,000 areas of residence. Through their residential addresses, Swedish residents have been assigned into SAMS areas since 1982. The register is maintained by Statistics Sweden [298].

4.2.2 Measures

4.2.2.1 ICD codes

The World Health Organization's ICD codes is a medical classification system and the international standard for the reporting of diseases and related health conditions [299, 300]. In Sweden, three versions of the ICD have been in use since 1969: ICD-8 (1969 to 1986), ICD-9 (1987 to 1996) and ICD-10 (1997 to present).

In the register-based studies (II and IV), Darier disease (DD) was defined by a diagnosis of Q82.8E (ICD-10) or 75.7D (ICD-9) in the NPR. Unfortunately, the ICD-8 code for DD is unspecific since it includes several other dermatological genetic disorders and could thus not be included. The ICD-codes used are found in ***Table 4***.

Study	Diagnosis	ICD-10	ICD-9
	Darier disease	Q82.8E	75.7D
Study II	Heart failure	I50	
	Myocardial infarction	I21	
	Arrhythmia	I49	
	Alcohol misuse	F10	
RR[2]	Tobacco use	Z72.0 and Z72.0W	
RR[3]	Smoking	Z72.0A	
	Essential hypertension	I10.9	
	Secondary hypertension	I15.9	
	Type 1 diabetes	E10	
	Type 2 diabetes	E11	
	Hyperlipidemia	E78.5	
	Pure hypercholesterolemia	E78.0	
	Pure hypertriglyceridemia	E78.1	
	Mixed hyperlipidemia	E78.2 and E78.2X	
	Familial hypercholesterolemia	E78.0A	
	Obesity	E66.0, E66.8 and E66.9	
Study IV	Type 1 diabetes	E10	
	Type 2 diabetes	E11	

Table 4. All ICD codes used in the register-based studies in this thesis. ICD: International Classification of Diseases; RR: risk ratio/relative risk (RR^2 and RR^3 refer to the adjusted RRs included in study II, see ***Table 13***).

4.2.2.2 Clinical samples

Patient blood was collected in study II, III and V. The laboratory analyses performed are summarized in ***Table 5***.

Patient height and weight were measured in order to calculate body mass index (BMI) in study I, II and III. Electrocardiography (ECG) was performed in study II. Research subjects underwent oral glucose tolerance testing (OGTT) in study III. In study II and III, individuals with DD were sub-grouped into pathogenic *vs.* benign mutation status and acitretin *vs.* no acitretin treatment. The genetic testing conducted on the *ATP2A2* gene in the DD cohort had previously been performed outside the scope of the studies included in this thesis. Mutation status, i.e., pathogenic *vs.* benign, was determined by *in silico* prediction programs as described in Leong et al., 2017 [179].

Study	Laboratory parameter	Unit
Study II	NT-proBNP	ng/L
	ST2	µg/mL
	Galectin-3	µg/L
	Troponin T	ng/L
	Triglycerides	mmol/L
	Cholesterol	mmol/L
	HDL	mmol/L
	LDL	mmol/L
Study III	Fasting plasma glucose	mmol/L
	2-h plasma glucose	mmol/L
	HbA1c	mmol/mol
	Insulin	mIE/L
	Proinsulin	pmol/L
	C-peptide	nmol/L
Study V*	**B cells**	
	CD19+ B cells	%
	CD20+ B cells	%
	CD21- CD38- B cells	%
	T cells	
	CD3+ T cells	%
	CD3+ CD4+ T cells	%
	CD3+ CD8+ T cells	%
	CD4+ CD25+ CD127- regulatory T cells	%
	CD4+ naive T cells (CD45RA+/- CCR7+/-)	%
	CD4+ Th cells (Th1, Th2, Th17)	%
	CD8+ naive T cells (CD45RA+/- CCR7+/-)	%
	CD4+ HLA-DR+ CD38+/- activated T cells	%
	CD8+ HLA-DR+ CD38+/- activated T cells	%
	Other	
	CD16+ CD56+ CD3- NK cells	%

Table 5. All laboratory analyses performed in the experimental and cohort/case series studies in this thesis. CCR: C-C chemokine receptor; CD: cluster of differentiation; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; HLA: human leukocyte antigen; L: liter; LDL: low-density lipoprotein; mL: milliliter; mmol: millimole; NK: natural killer; NT-proBNP: N-terminal-pro brain natriuretic peptide; ng: nanogram; nmol: nanomole; pmol: picomole; ST2: suppression of tumorigenicity 2; Th: T helper; µg: microgram. *For the overall purposes of study V, only CD19+ B cells were included in the final publication.

4.2.2.3 Cognitive assessment

Cognitive assessment in study I was conducted using the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cognitive assessment software, Cambridge Cognition Ltd, Cambridge, UK) [301]. The test is valid, reliable and takes possible cultural biases into account [302-304].

4.3 STUDY DESIGN

4.3.1 Population-based cohort studies (register-based studies, study II and IV)

The overall aims were to investigate the association between DD and heart disease (study II) and diabetes (study IV). Register-based cohort studies on the Swedish nationwide population level were performed by linking data between the TPR and the NPR. The CDR was used to verify that participants were alive while included in the study and not accidentally participating after having passed away. The SAMS was used for participants' residential addresses to enable geographical matching.

ICD diagnoses used in the data searches are described in ***Table 4***. Diagnoses were assigned by the responsible physician at discharge from inpatient care or during outpatient care visits. In study II and IV, a total of 935 and 770 individuals with DD were included, respectively. In study IV, a total of 18 individuals with DD were excluded due to emigration and the resulting removal from the registers. Each individual with DD was matched to up to 100 comparison individuals from the general population in the TPR. The final study populations included 93,487 and 76,987 comparison subjects in study II and IV, respectively.

Matching accounted for gender, birth year and county of residence at the time of the first diagnosis of the matched DD individual. Thus, possible environmental confounding factors were taken into consideration. The study period included all DD individuals with a diagnosis included in the registers between the year ICD-9 was implemented, i.e., 1987, until 2013.

4.3.2 Experimental, matched case-control studies (clinical studies)

4.3.2.1 Study I

The overall aim of study I was to investigate the association between DD and cognitive impairment.

The study population consisted of individuals with DD (n = 29), individuals with HHD (n = 25) and comparison subjects in a 1:1 ratio to each patient. Matching was executed for age, gender and level of education. Age was matched in ± 5-year intervals. Level of education was graded in six categories according to the highest achieved level of education and matched in ± 1-level intervals: (*i*) disrupted education before high school; (*ii*) disrupted high school; (*iii*)

high school education; (*iv*) university education three years; (*v*) university education master's degree; and (*vi*) PhD.

Patients were recruited at the dermatology department at Karolinska University Hospital (Stockholm, Sweden), and from family members of the participating patients, as DD often runs in the family. Control subjects were primarily recruited through their participation as controls in previous scientific research at the department, as well as through advertisements and a local primary healthcare clinic. Control subjects received a monetary compensation of 200 SEK for their participation.

Inclusion criteria for individuals with DD and HHD were typical skin lesions evaluated by a dermatologist in combination with typical histopathological findings and/or family history. Family history was defined as a diagnosis of DD or HHD assigned at a dermatology clinic for a first- or second-degree relative. Of note, most participating patients had also performed genetic testing prior to participation, further validating the diagnoses of DD and HHD. Exclusion criteria were age < 18 years, significant ongoing psychiatric disorders, neurotropic or psychotropic medication, active substance abuse, dementia, acute illness during the past four weeks, and pregnancy.

Participants first underwent thorough history-taking and evaluation of skin symptoms. Height and weight were measured. The CANTAB test runs on an iPad computer tablet (see also section 4.2.2.3). Testing was performed under identical circumstances for all participants, namely in a closed, quiet room together with the study leader under undisturbed conditions. The test battery takes approximately 30 minutes and consists of five consecutive tests that yields a total of 10 key measurements: (*i*) motor screening test (MOT) (one key measurement); (*ii*) paired associates learning (PAL), measuring episodic memory (two key measurements); (*iii*) reaction time (RTI) (two key measurements); (*iv*) spatial working memory (SWM), measuring assumptions, planning and strategy (two key measurements); and (*v*) rapid visual information processing (RVP) (three key measurements). In brief, the tests consist of symbols, shapes and numbers appearing on the screen, with simple tasks having to be performed under time- and precision-based circumstances as accurately as possible. The individual tests and the cognitive functions assessed are described in further detail in ***Table 6***.

4.3.2.2 Study II and III

The overall aims of study II and III were to examine the association between DD and heart disease, and DD and diabetes, respectively.

The study population consisted of individuals with DD (n = 25), and comparison subjects in a 1:1 ratio. Controls were matched for age, gender and body mass index (BMI). Age was matched in ± five-year intervals. BMI was matched in four different categories: < 18.5, 18.5–24.99, 25–29.99 and > 30. Patients were primarily defined as one group, but also sub-grouped for pathogenic *vs.* benign mutation and acitretin *vs.* no acitretin treatment.

Test	Test description	Cognitive functions assessed
MOT	The mean latency time to correctly respond to a visual stimulus on the screen. Measured in milliseconds.	Sensorimotor skill and comprehension
PAL 1	The number of times a subject chose the correct box on their first attempt when recalling the pattern locations after initially viewing them on the screen.	Visual memory and rapidly learning novel skills
PAL 2	The number of times a subject chose the incorrect box on the screen, plus an adjustment for the estimated number of errors made in the array of trials, attempts and recalls they did not reach.	
RTI 1	The median time taken to release the response button and select the target stimulus after it flashes yellow on the screen. Measured in milliseconds.	Motor and mental response speed, impulsivity
RTI 2	The median duration taken to release the response button after the presentation of a target stimulus. Measured in milliseconds.	
SWM 1	The number of times a subject incorrectly revisits a box in which a token has previously been found, which is against the test instruction.	Retention and manipulation of visuospatial information, planning, strategy handling and working memory
SWM 2	The number of times a subject begins a new search pattern from the same box they started with previously, which is against the test instruction. If subjects always begin a search from the same starting point, we infer that the subject is employing a planned strategy for finding the tokens.	
RVP 1	The signal detection measure of a subject's sensitivity to the target sequence (string of three numbers), regardless of response tendency. In essence, this metric is a measure of how good the subject is at detecting target sequences.	Information processing and sustained attention
RVP 2	The median response latency on the trials where the subject responded correctly. Calculated across all assessed trials.	
RVP 3	The number of sequence presentations that were false alarms (i.e., trying to fool the subject) divided by the number of sequence presentations that were false alarms plus the number of sequence presentations that were correct rejections. This yields a probability score for rejecting false alarms.	

Table 6. Detailed test descriptions and the cognitive functions they assess. MOT: motor screening test; PAL: paired associates learning; RTI: reaction time; RVP: rapid visual information processing; SWM: spatial working memory.

Participants were recruited as in study I (see previous section). At the time of the studies, all patients but one had previously performed genetic testing for *ATP2A2* by whole exome and Sanger sequencing. Control subjects received a monetary compensation of 500 SEK for their participation.

Inclusion criteria for individuals with DD were identical to study I. Exclusion criteria were oral corticosteroid treatment, active substance abuse, severe kidney or liver disease, acute illness during the past four weeks, and pregnancy.

Participants arrived at the study visit the morning after an overnight fast starting at 10 pm, with no smoking, use of snuff or caffeine intake during the fast. Possible morning medications were postponed until after the visit. Firstly, blood was sampled. Participants then performed an oral glucose tolerance test (ingesting 75 grams of glucose within a 5-minute time period). During the 2-hour resting period that followed, comprehensive history-taking and physical evaluation of skin symptoms was performed, participants were photographed (patients only), height and weight were measured, and an electrocardiography (ECG) was carried out. After the 2-hour resting period, another plasma glucose blood sample was taken, after which the visit ended.

For participants taking oral acitretin, a seven-day washout period preceded the visit. Acitretin has a half-life of approximately 50 hours and is known to influence blood lipids such as triglycerides and cholesterol [305-307]. A longer period of discontinued medication was considered unethical.

In study III, one of the main outcome measures was the Homeostasis Model Assessment (HOMA), a computer model for evaluating pancreatic β-cell function (HOMA2-%B) and insulin resistance (or insulin sensitivity, HOMA2-%S). The model assesses fasting plasma glucose and insulin or c-peptide in patient-sampled blood as a percentage compared to a healthy reference population, and is widely used in diabetes research [308]. All laboratory parameters sampled are summarized in ***Table 5***.

4.3.3 Cohort study/case series (study V)

The overall aim of study V was to investigate the association between DD and deficits in the immune system.

Only the contributions relevant to the doctoral student is discussed in this thesis. The bulk of study V consists of pre-clinical laboratory work, to which there has been no direct contribution.

The study group consisted of a cohort of individuals with DD (n = 16). Inclusion criteria were identical to study I, II and III. Exclusion criteria were age < 18 years, ongoing disorder or medication affecting the immune system, active substance abuse, acute illness during the past four weeks, and pregnancy. A comprehensive set of immune-related samples were collected from patient blood, namely different subsets of B, T and NK cells (***Table 5***). However, for the overall purposes of study V, only CD19+ B cells were included in the final publication. The healthy reference population associated with the clinical laboratory at Karolinska University Hospital (Stockholm, Sweden) was chosen as reference data.

4.4 STATISTICAL METHODS

Statistical analyses were performed using GraphPad Prism version 7 for Windows (GraphPad Software Inc., San Diego, CA, USA), SPSS version 24 for Mac (SPSS, IBM, Armonk, NY USA), SAS version 9.3 (SAS Institute, Cary, NC, USA) and Excel 2016 for Windows/Excel 365 for Mac (Microsoft, Redmond, WA, USA).

4.4.1 Descriptive statistics

In study I, II, III and V, descriptive statistical analyses were performed to show means, median, proportions in % of the total number of observations, upper and lower limits of value ranges, and standard deviations (SDs).

4.4.2 Basic inferential statistics

Student's unpaired t-test with two-tailed *p*-values was used for significance testing for the differences in numerical values between two independent groups, i.e., cases and controls, in study I. Mann-Whitney U test was used for significance testing for the differences in categorical, ordinal, independent values, e.g., age, between cases and controls in study II and III. Fisher's exact test was used for significance testing for differences in categorial, nominal, independent variables, e.g., yes/no answers, between two independent groups such as cases and controls in study II and III.

In all instances, p-values < 0.05 were considered statistically significant.

4.4.3 Corrections for multiple comparisons

If multiple *p*-values are analyzed in conjunction, there is the risk of type 1 statistical error. This means the erroneous rejection of the null hypothesis, also referred to as "false positive". In order to reduce the risk of type 1 error, a correction for multiple comparisons approach was used. The Bonferroni correction was performed in study II together with two-way ANOVA (see next section) and the Benjamini-Hochberg correction was used in study I and III together with Student's unpaired t-test and Mann-Whitney U test, respectively.

4.4.4 Two-way ANOVA

Two-way ANOVA (analysis of variance) was used in study II for significance testing for the differences in numerical values in more than two independent groups, namely DD individuals sub-grouped for pathogenic *vs.* benign mutation and control subjects, as well as DD individuals

sub-grouped for acitretin *vs.* no acitretin treatment and control subjects. Two-way ANOVA was used in conjunction with the Bonferroni correction for multiple comparisons.

4.4.5 Conditional logistic regression

Conditional logistic regression is a statistical model used to assess the association between predictor variables (sometimes also called covariates) and a categorical outcome variable, e.g., a binary yes/no answer. Predictor variables can be continuous, categorical or dichotomous [309]. For the purposes of the population-based analyses in study II and IV, a conditional logistic regression model was used since the comparison individuals were matched on the index individuals with DD. Conversely, standard logistic regression is used for unmatched data. In study II and IV, matching was performed for birth year, gender and county of residence at the time of their first DD diagnosis. The main outcome of the conditional logistic regressions is expressed as odds ratios (ORs) for the association between DD and heart disease in study II, and the association between DD and diabetes in study IV. However, in this design, ORs can be regarded as risk ratios (RRs, or relative risk) due to several reasons (see next section).

4.4.5.1 Odds ratios and risk ratios

In the study design in study II and IV, ORs can be regarded as RRs due to a procedure called "incidence density sampling" [310]. The index persons, i.e., individuals with DD, must be so-called "incident cases", meaning they can change from a status of non-disease to disease over the specified study period. The comparison subjects must be selected from the at-risk population at the same time as the cases occur and maintain their eligibility of developing the disease until the end of the study period. Further, each individual with DD was matched with up to 100 comparison individuals, meaning the outcome is expressed as a proportion, i.e., risk. Thus, we can calculate the relative risk of DD patients having or developing heart disease or diabetes. In general, if the outcome is rare (< 10%), ORs can be directly regarded as RRs [311].

4.4.5.2 Crude and adjusted risk ratios

In study II, three different levels of RR were calculated. The crude risk ratio, called RR^1, did not adjust for any possible confounding factor. RR^2 adjusted for a lifetime diagnosis of alcohol misuse, and RR^3 adjusted for a lifetime diagnosis of heart disease risk factors (***Table 4***).

4.4.5.3 Confidence interval

A confidence interval (CI) is a given range in which there is a certain probability of finding the "true" value, where the true value is essentially unknown. It can also be described as the % of

the time the value arrived at will correspond with the (unknown) value in the population. If the interval crosses 1, it is deemed not significant, even if the corresponding *p*-value might be within the chosen significance threshold. In the conditional logistic regression analysis in study II and IV, a 95% CI was used for RRs, meaning there was a 95% probability of the true value residing within the interval.

4.5 ETHICAL CONSIDERATIONS

All studies in this thesis have been approved by the Swedish Ethical Review Authority, original reference numbers (diarienummer [dnr]) 2015/1798-31 (study I, II, III and V) and 2009/939-31/5 (study II and IV). No further applications for ethical permits are planned or required within the scope of either of the doctoral projects.

All patient and control subject participation were performed after signed informed consent. Consent forms are kept in a locked storage to which only the doctoral student and supervisor has the keys. All electronic data that is derived from or can be related to patient material is kept in coded form. All code-keys are kept in a password-guarded folder that is inaccessible from outside the Karolinska Institutet Center for Molecular Medicine network. Only the doctoral student and supervisor has access to this folder. All experiments were performed according to the Declaration of Helsinki. Patients were free to withdraw from the study at any time without further explanation, and without any impact on their medical care or other consequences. The blood sampling performed in study II, III and V is considered routine medical practice and patients provided signed informed consent beforehand, understanding the possible minor risks associated with the procedure. The same applied to the oral glucose tolerance test performed in study III, which might cause mild discomfort due to the overnight fast and possibly delayed intake of the first meal of the day. Electrocardiography performed in study II is considered essentially risk-free.

Register-based data includes potentially sensitive data for the individual. Thus, only the data needed for the research in question is extracted from the registers. The data is kept in password-protected servers inaccessible by anyone outside the scope of the immediate research group for each project. Only one person at the responsible institution has access to the link between research data and identity number, i.e., code key. In the scientific communication relating to patient data from The National Patient Register, it was presented in aggregates without the possibility of identification of any one individual patient. The same held true for randomly selected control subjects from The Total Population Register. The Swedish Ethical Review Authority does not need any informed consent from individual participants for the approval of a register-based study. The potential harm of inclusion in the register-based studies was considered to be minor. Conversely, the potential benefit from the outcomes of register-based studies is deemed to greatly exceed the potential harm for the included individuals.

Special ethical consideration went into the planning, execution and evaluation of study I, regarding impaired cognitive function. The finding of impaired cognition can be considered a sensitive subject, but we deemed it important to study since patients may greatly benefit from possible future systemic treatments targeting the brain and other affected organs. Since cognitive function is in the center of countless facets of life, possible supporting measures in school or the workplace could be of great value for the individual. The data is presented in aggregate form without the possibility of identification of the individual patient.

5 RESULTS

5.1 DARIER DISEASE AND COGNITIVE IMPAIRMENT (STUDY I)

Study group characteristics of individuals with Darier disease (DD) and Hailey-Hailey disease (HHD), and their respective controls are shown in ***Table 7***. Successful matching was performed for both patient groups and controls in a 1:1 ratio. Height and weight were included but not matched for. There were no significant differences between cases and controls in either group.

Variable	DD patients	DD controls	HHD patients	HHD controls
n	29	29	25	25
Age (years)	54.0 ± 13.0	55.3 ± 9.9	51.5 ± 12.4	51.3 ± 12.5
Female gender (*n*)	20	20	17	17
Level of education (stratified value)	3.0 ± 0.7	3.7 ± 0.9	3.6 ± 1.1	3.8 ± 1.0
Height (cm)	169.0 ± 7.2	171.2 ± 7.3	170.9 ± 7.1	171.1 ± 9.4
Weight (kg)	82 ± 14	72 ± 10	75 ± 12	72 ± 14

Table 7: Characteristics of the study population in study I. Values are expressed as means ± standard deviation. DD: Darier disease; HHD: Hailey-Hailey disease.

The results of the cognitive assessment for individuals with DD *vs.* controls are found in ***Table 8***. A significant impairment for individuals with DD was seen for five of the 10 key measurements, all within the reaction time and rapid visual information processing tests. A trend towards impaired performance was also observed for the remaining five measurements, albeit not statistically significant.

Test	DD patients Mean ± SD	DD controls Mean ± SD	*p*-value
Motor screening test	1153.0 ± 505.6	964.3 ± 277.7	0.09
Paired associates learning 1	9.4 ± 4.4	10.6 ± 4.8	0.37
Paired associates learning 2	26.2 ± 17.6	23.9 ± 18.2	0.63
Reaction time 1	319.3 ± 97.1	265.8 ± 78.9	0.027*
Reaction time 2	454.1 ± 84.6	384.6 ± 37.1	0.0002*
Spatial working memory 1	19.5 ± 9.0	15.3 ± 8.5	0.08
Spatial working memory 2	9.4 ± 2.0	8.8 ± 2.3	0.24
Rapid visual information processing 1	0.86 ± 0.07	0.90 ± 0.05	0.016*
Rapid visual information processing 2	616.0 ± 165.1	508.6 ± 88.0	0.004*
Rapid visual information processing 3	0.07 ± 0.12	0.02 ± 0.03	0.024*

Table 8: Results of the cognitive assessment for Darier disease (DD) patients compared with control subjects. *Statistically significant difference after Benjamini-Hochberg correction within each set of tests.

The results of the cognitive assessment for individuals with HHD *vs.* controls are found in ***Table 9***. No significant differences between the groups were found after correcting for multiple comparisons.

Test	HHD patients Mean ± SD	HHD controls Mean ± SD	*p*-value
Motor screening test	947.6 ± 765.6	927.4 ± 273.6	0.90
Paired associates learning 1	10.3 ± 4.1	11.5 ± 4.6	0.33
Paired associates learning 2	23.4 ± 14.7	21.4 ± 17.9	0.67
Reaction time 1	267.3 ± 83.2	258.6 ± 82.7	0.71
Reaction time 2	416.1 ± 48.6	389.0 ± 34.3	0.027#
Spatial working memory 1	14.9 ± 8.9	13.8 ± 9.4	0.68
Spatial working memory 2	8.8 ± 2.3	8.4 ± 2.7	0.54
Rapid visual information processing 1	0.88 ± 0.06	0.91 ± 0.05	0.08
Rapid visual information processing 2	496.4 ± 92.8	502.9 ± 87.4	0.80
Rapid visual information processing 3	0.09 ± 0.17	0.02 ± 0.03	0.029#

Table 9: Results of the cognitive assessment for Hailey-Hailey disease (HHD) patients compared with control subjects. #Statistically insignificant difference after Benjamini-Hochberg correction within each set of tests.

5.2 DARIER DISEASE AND HEART DISEASE

5.2.1 Clinical study (study II)

Study group characteristics of individuals with DD control subjects are shown in ***Table 10***. Successful matching was performed for cases and controls in a 1:1 ratio. The only significant difference between the group was acitretin treatment, however a seven-day washout period predated the study visit (see section 4.3.2.2).

Variable	DD patients	Control subjects	*p*-value
n	25	25	
Age (years)	52 ± 13 (27–78)	51 ± 13 (27–76)	0.80#
Female gender (*n*)	15	15	1†
BMI (kg/m^2)	28.2 ± 5.3 (18.9–42.3)	27.0 ± 5.0 (19.8–38.7)	0.45#
Height (cm)	169.6 ± 9.9 (152–193)	170.3 ± 11.5 (48.5–193)	0.83#
Weight (kg)	81.4 ± 17.8 (54–119)	78.8 ± 18.2 (49.3–117)	0.73#
Current smoker (*n*)	5	2	0.417†
Diabetes family history (*n*)	13	11	0.778†
Acitretin treatment (*n*)	14	0	0†
Hypertension treatment (*n*)	3	4	1†
Dyslipidemia treatment (*n*)	3	2	1†

Table 10: Characteristics of the study population in study II and III. Continuous values are expressed as means ± standard deviation (min-max). Categorical values are expressed as number. #Mann-Whitney U test; †Fisher's exact test. BMI: body mass index; DD: Darier disease.

The results of heart biomarkers, blood lipids and ECG values for control subjects and DD patients sub-grouped for mutation status are found in ***Table 11***. A significant difference was found in LDL/HDL ratio in DD patients sub-grouped for pathogenic *vs.* benign mutation, and in QT interval in DD patients with pathogenic mutation *vs.* controls.

Test parameter	Control subjects (*n* = 25)	DD benign mut (*n* = 9)	DD pathogenic mut (*n* = 14)
NT-proBNP	89 (11-954) ± 187	64 (18-187) ± 55	126 (17-535) ± 144
ST2	28.3 (12.7-48.2) ± 9.7	28.4 (19.5-37.8) ± 6.2	32.3 (20.4-47.3) ± 6.8
Galectin-3	14.7 (7.5-34.7) ± 5.6	13.2 (9.5-20.5) ± 3.3	14.6 (9.2-18.3) ± 2.7
Troponin T	24.0 (5.0-8.0) ± 1.1	7.0 (6.0-9.0) ± 1.1	9.0 (5.0-17.0) ± 4.1
Triglycerides	1.1 (0.5-3.9) ± 0.7	1.4 (0.6-3.1) ± 0.8	1.5 (0.8-2.8) ± 0.6
Cholesterol	5.3 (3.6-7.0) ± 0.9	5.4 (3.3-7.9) ± 1.4	5.6 (4.2-7.7) ± 1.0
HDL	1.7 (0.8-2.6) ± 0.5	1.5 (1.0-2.2) ± 0.4	1.4 (0.6-2.0) ± 0.4
LDL	3.1 (2.0-5.1) ± 0.8	3.2 (1.6-5.4) ± 1.2	3.6 (2.6-5.7) ± 1.0
LDL/HDL	2.1 (0.9-4.3) ± 1.0	2.4 (1.1-5.20) ± 1.4	3.1 (1.4-9.5) ± 1.9*
HR (bpm)	62 (46–84) ± 10	61 (50–78) ± 10	55 (47–80) ± 7.7
PQ interval (ms)	159 (108–208) ± 24	150 (114–180) ± 22	146 (114–192) ± 20
QRS-duration (ms)	94 (74–114) ± 9.7	96 (80–116) ± 13	103 (88–118) ± 7.7
QT interval (ms)	415 (354–464) ± 26	434 (372–552) ± 55	453 (406–518) ± 34#
QTc interval (ms)	419 (368–455) ± 23	433 (402–560) ± 49	432 (379–498) ± 34

Table 11: Heart biomarkers, blood lipids and ECG values for control subjects and DD patients sub-grouped for pathogenic *vs.* benign mutation status. Values are reported as means ± standard deviation (min-max). Two-way ANOVA with Bonferroni correction was used as statistical analysis. Units for laboratory parameter are found in ***Table 5***. **p* < 0.001 pathogenic *vs.* benign mutation. #*p* < 0.001 pathogenic mutation *vs.* control. bpm: beats per minute; HDL: high-density lipoprotein; HR: heart rate; LDL: low-density lipoprotein; ms: milliseconds; mut: mutation; NT-proBNP: N-terminal-pro brain natriuretic peptide; ST2: suppression of tumorigenicity 2.

The results of heart biomarkers, blood lipids and ECG values for control subjects and DD patients sub-grouped for acitretin treatment are found in ***Table 12***. Significant differences were found in LDL/HDL ratio in DD patients with acitretin treatment *vs.* both controls and DD patients with no acitretin treatment, and in QT and QTc interval between DD patients with acitretin treatment and controls.

Test parameter	Control subjects (*n* = 25)	DD acitretin (*n* = 14)	DD no acitretin (*n* = 11)
NT-proBNP	88 (11-954) ± 187	120 (17-535) ± 152	81 (18-187) ± 52
ST2	28.3 (12.7-48.2) ± 9.7	30.4 (20.4-37.8) ± 6.1	31.3 (19.5-47.3) ± 7.5
Galectin-3	14.7 (7.5-34.7) ± 5.6	15 (11-20.5) ± 2.4	12.7 (9.2-18.3) ± 3.1
Troponin T	6 (5-8) ± 1.1	7 (5-17) ± 8.7	8 (6-10) ± 7.8
Triglycerides	1.1 (0.5-3.9) ± 0.7	1.6 (0.8-3.1) ± 0.7	1.5 (0.56-2.9) ± 0.8
Cholesterol	5.3 (3.6-7) ± 0.9	5.8 (4.6-7.9) ± 1.1	5.2 (3.3-7.1) ± 1.2
HDL	1.7 (0.8-2.6) ± 0.5	1.3 (0.6-2) ± 0.4	1.5 (1-2.2) ±0.4
LDL	3.1 (2-5.1) ± 0.8	3.8 (2.6-5.7) ± 1.1	3.1 (1.6-4.6) ± 0.9
LDL/HDL	2.1 (0.9-4.3) ± 1.0	3.2 (1.6-9.5) ± 2.1*	2.3 (1.1-3.8) ± 0.9#
HR (bpm)	62 (46-84) ± 10	57 (50-80) ± 9.3	58 (47-78) ± 9.1
PQ interval (ms)	159 (108-208) ± 24	151 (114-192) ± 21	146 (114-180) ± 22
QRS-duration (ms)	94 (74-114) ± 9.7	101 (82-118) ± 9.3	99 (80-118) ± 12
QT interval (ms)	415 (354-464) ± 26	455 (378-552) ± 46§	432 (372-504) ± 37
QTc interval (ms)	419 (368-455) ± 23	442 (379-560) ± 48§	421 (404-446) ± 15

Table 12: Heart biomarkers, blood lipids and ECG values for control subjects and DD patients sub-grouped for acitretin *vs.* no acitretin treatment. Values are reported as means ± standard deviation (min-max). Two-way ANOVA with Bonferroni correction was used as statistical analysis. Units for laboratory parameter are found in ***Table 5***. **p* < 0.001 DD acitretin *vs.* control. #*p* < 0.001 DD acitretin *vs.* no acitretin. §*p* < 0.001 DD acitretin *vs.* control. bpm: beats per minute; HDL: high-density lipoprotein; HR: heart rate; LDL: low-density lipoprotein; ms: milliseconds; NT-proBNP: N-terminal-pro brain natriuretic peptide; ST2: suppression of tumorigenicity 2.

5.2.2 Register-based study (study II)

The results of register-based data on DD and the risk of heart disease are found in ***Table 13***. There was a significant risk increase for heart failure among individuals with DD of 59% (RR^1 1.59, CI 1.16-2.19), an increased risk of any heart diagnosis among all DD individuals of 32% (RR^1 1.32, CI 1.02-1.70) and especially female DD individuals of 59% (RR^1 1.59, CI 1.13-2.25). The mean age at first heart diagnosis occurred almost seven years earlier in individuals with DD (70.0 *vs.* 76.9 years).

Diagnosis	Individuals with DD (*n* = 935) *n* (%)	Comparison subjects (*n* = 93,487) *n* (%)	RR^1 (CI)	RR^2 (CI)	RR^3 (CI)
Heart failure	52 (5,56)	3,616 (3,87)	1.59 (1.16-2.19)	1.58 (1.15-2.18)	1.53 (1.11-2.11)
Myocardial infarction	29 (3,10)	2,828 (3,03)	1.03 (0.70-1.52)	1.03 (0.70-1.52)	0.95 (0.64-1.41)
Arrhythmia	15 (1,60)	1,330 (1,42)	1.13 (0.68-1.90)	1.12 (0.67-1.89)	1.10 (0.65-1.85)
Any heart diagnosis:					
Males & females	80 (8,56)	6,504 (6,96)	1.32 (1.02-1.70)	1.31 (1.01-1.70)	1.26 (0.97-1.65)
Males	36 (9,63)	3,431 (9,17)	1.07 (0.73-1.57)	1.06 (0.72-1.56)	1.10 (0.74-1.63)
Females	44 (7,84)	3,073 (5,48)	1.59 (1.13-2.25)	1.59 (1.13-2.25)	1.46 (1.02-2.08)

	Mean age at first diagnosis (years)	
Heart failure	70.0	76.9
Myocardial infarction	65.9	71.9
Arrhythmia	67.9	61.4

Table 13: Darier disease (DD) and the risk of common heart diseases, register-based data. Risk ratios (RR) and corresponding 95% confidence intervals (CI) are expressed for each heart disease diagnosis. Also included is the mean age at first heart disease diagnosis for individuals with DD *vs.* control subjects. RR^1: crude risk ratio; RR^2: risk ratio adjusted for a lifetime diagnosis of alcohol misuse; RR^3: risk ratio adjusted for a lifetime diagnosis of heart disease risk factors (see ***Table 4***). Green signifies $p < 0.05$ (for actual *p*-values, see study II, Table 5, in appendix).

5.3 DARIER DISEASE AND DIABETES

5.3.1 Clinical study (study III)

The study group characteristics are presented in section 5.2.1 and ***Table 10***.

The results of metabolic parameters in individuals with DD patients sub-grouped for both mutation status and acitretin treatment, and DD patients *vs.* control subjects are found in ***Table 14***. There was a significant difference in HOMA2-%B between DD patients and controls,

signifying pancreatic β-cell dysfunction. Individually significant differences in DD sub-groups were found, however none persisted after correcting for multiple comparisons.

Test parameter	DD patients (*n* = 25)	Control subjects (*n* = 25)	*p*-value
Fasting plasma glucose	5.3 ± 0.4	5.6 ± 0.5	0.07
2-h plasma glucose	5.6 ± 1.7	5.7 ± 1.3	0.68
HbA1c	36 ± 4	36 ± 4	0.36
Pro-insulin/insulin (ratio)	0.85 ± 0.32	0.80 ± 0.49	0.19
C-peptide	0.8 ± 0.2	0.7 ± 0.2	0.24
HOMA2-%S	79.6 ± 32.7	94.9 ± 52.4	0.53
HOMA2-%B	122.7 ± 27.1	103.5 ± 22.1	0.01*
	DD pathogenic mut (*n* = 15)	**DD benign mut (*n* = 9)**	***p*-value**
Fasting plasma glucose	5.2 ± 0.3	5.5 ± 0.4	0.04#
2-h plasma glucose	5.6 ± 2.1	5.8 ± 1.0	0.77
HbA1c	37 ± 4	34 ± 2	0.02#
Pro-insulin/insulin (ratio)	0.87 ± 0.30	0.83 ± 0.35	0.62
C-peptide	0.8 ± 0.2	0.8 ± 0.2	0.55
HOMA2-%B	128.1 ± 30.0	115.4 ± 21.0	0.40
	DD acitretin (*n* = 14)	**DD no acitretin (*n* = 11)**	***p*-value**
Fasting plasma glucose	5.3 ± 0.3	5.4 ± 0.4	0.12
2-h plasma glucose	5.6 ± 2.2	5.6 ± 0.6	0.76
HbA1c	35 ± 3	37 ± 4	0.62
Pro-insulin/insulin (ratio)	0.85 ± 0.28	0.94 ± 0.41	0.63
C-peptide	0.9 ± 0.2	0.7 ± 0.2	0.05#
HOMA2-%B	134.6 ± 29.2	107.6 ± 14.1	<0.01#

Table 14: Glucose metabolism and pancreatic β-cell function in Darier disease (DD) compared with controls, and sub-groups of DD patients: pathogenic *vs.* benign mutation status and acitretin *vs.* no acitretin treatment. Values are reported as means ± standard deviation (min-max). Mann-Whitney U test with Benjamini-Hochberg correction was used as statistical analysis. Laboratory parameter units are found in ***Table 5***. *$p < 0.01$, significant after Benjamini-Hochberg correction. #$p < 0.05$ in individual values, however insignificant after Benjamini-Hochberg correction.

5.3.2 Register-based study (study IV)

The results of register-based data on DD and the risk of diabetes are found in ***Table 15***. There was a significant risk increase for type 1 diabetes (T1D) among individuals with DD of 74% (RR 1.74, CI 1.13-2.69).

Diagnosis	Individuals with DD (*n* = 770)		Comparison subjects (*n* = 76,987)		RR (CI)
	n (%)	Male/female	*n* (%)	Male/female	
Diabetes type 1	22 (2.86)	8/14	1,288 (1.67)	643/645	1.74 (1.13-2.69)
Diabetes type 2	22 (2.86)	8/14	2,471 (3.21)	1,228/1,247	0.88 (0.57-1.36)

Table 15: Darier disease and the risk of diabetes type 1 (ICD-10: E10) and type 2 (ICD-10: E11), register-based data. Risk ratios (RR) and corresponding 95% confidence intervals (CI) are expressed. Green signifies $p < 0.05$.

5.4 DARIER DISEASE AND THE IMMUNE SYSTEM (STUDY V)

The results are shown in ***Figure 7***. Of all individuals with DD included ($n = 16$), six were found to have CD19+ B cell values below the lower limit of the normal laboratory reference range of 9-24%. The mean value was 11.4%, the median value 13.5%, and no patient displayed a value above or near the upper limit of the normal range. Note that several other immune-related samples were collected, while only CD19+ B cells were included in the final publication due to the overall purposes of the investigation (see section 4.3.3 and ***Table 5***).

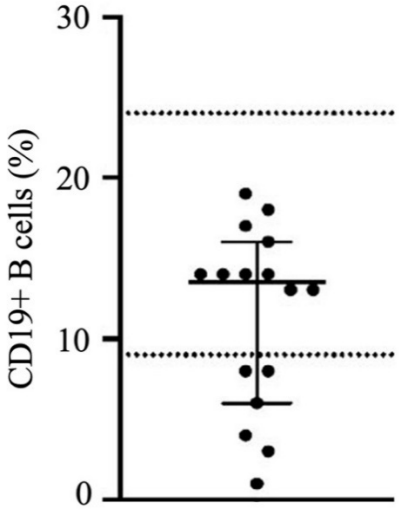

Figure 7: CD19+ B cell percentage in individuals with Darier disease (DD, $n = 16$). Values are expressed as median (13.5), with 95% confidence interval. Dotted lines represent the reference laboratory range of 9-24%. DD mean value is 11.4%. Dots represent individual values.

6 DISCUSSION

6.1 DARIER DISEASE IS ASSOCIATED WITH SEVERAL COMORBIDITIES

Prior to the investigations performed in this thesis, the only well-established comorbidities associated with Darier disease (DD) were psychiatric disorders. In literature from the late 1950's there are accounts of DD patients as "mentally subnormal/retarded", descriptions that would be seen as unethical today, and that provide little or no scientific value as they are only anecdotal or observational [75, 78, 312]. More recent evidence links DD to bipolar disorder, depression including suicidality, schizophrenia, psychosis, intellectual/learning disabilities and epilepsy [76, 174, 175, 313-324].

The overarching rationale for the existence of comorbidities in DD is the fact that SERCA2 is a ubiquitously expressed "housekeeping" protein, and that the role and importance of SERCA2 and ER stress in numerous organs have been reported. In the studies included in this thesis, the spectrum of known comorbidities associated with DD has been widely broadened.

6.1.1 Darier disease and cognition

Before this investigation no closely matched experimental case-control study for general cognition had been carried out. A significant impairment in broad cognitive function was found to be associated with DD. This finding is consistent with a 2015 Swedish nationwide population-based study on individuals with DD (n = 770), that found the risk increase of a diagnosis of intellectual disability among individuals with DD to be 620% (RR 6.2, 95% CI 3.1-12.4) [314]. Further, in a subset of male individuals with DD *without* such a diagnosis of intellectual disability (n = 114), taken from conscription data, a significant reduction in IQ test scores was revealed when compared with 885 matched comparison subjects from the TPR [314].

The evidence for the importance of SERCA2 in the brain is plentiful. SERCA2 is critical in maintaining intracellular Ca^{2+} homeostasis in neurons. In turn, Ca^{2+} homeostasis is critical for proper nerve cell function, including synaptic activity, neurotransmitter release and density of dendritic spines. SERCA2b appears to be the main isoform in the brain and expressed ubiquitously, whereas SERCA2a and SERCA2c are expressed only in some nerve cells. SERCA2 and Ca^{2+} is implicated in essential brain functions such as memory formation and long-term memory, learning, cognition and even motor function [216, 217]. Pathophysiological links for SERCA2 has been found for dementia including Alzheimer's disease [325-327], Parkinson's disease [328, 329], and bipolar disorder and schizophrenia [330-332]. Despite these strong indications, much of the evidence behind the role of SERCA2 in the brain is only now unfolding. Our results further illustrate the importance of SERCA2 for proper cognitive function, adding to the available knowledge of SERCA2 and the brain.

The significant impairment in cognitive function in individuals with DD were found in the reaction time and rapid visual information processing test categories. Interestingly, deficits in the areas of cognition that these tests assess have been found to be correlated with an increased risk of all-cause mortality [333, 334]. Another study found that impairments in information processing speed is correlated to, and even a potential risk factor in the development of, psychological distress [335]. Moreover, a 2017 study found that reaction time is strongly correlated to intelligence/general mental ability, and that the strength of the correlation increased with age [336]. Of note, as previously mentioned in the results section, a trend was also seen for impaired results in the DD group for all other cognitive tests.

The implication of these results for the individual patient with DD are likely far-reaching. Impairments in cognitive function can influence everyday life in profound ways, affecting everything from school, work and social interactions to decision making, planning, remembering, problem-solving, and even thinking. The links to increased mortality, psychological distress and general mental ability further illustrates the importance this knowledge of impaired cognitive function in DD has for the individual patient. Possible supporting action in school might be motivated, as well as in the workplace. There is a potential benefit in evaluating the occurrence of psychological distress. For the individual patient with DD that has struggled in school and/or the workplace, there may be great comfort in being provided an explanation as to why this happened. Lastly, these findings highlight the need for treatments that also targets the brain.

In summary, the association between DD and impaired cognitive function yields important insights into the importance of SERCA2 in the brain, and provides meaningful clinical implications for individual patient with DD.

6.1.2 Darier disease and heart disease

On the population level, individuals with DD were shown to have a 59% increased crude risk for a heart failure (HF) diagnosis and 32% for any heart diagnosis, while female individuals with DD had a 59% crude risk increase for any heart diagnosis. Further, our results show that the first heart diagnosis in DD occurred almost seven years earlier as compared to controls.

In the clinical study, there was a significant difference in LDL/HDL ratio in DD sub-grouped for mutation status, potentially indicating a pathology in serum lipids in the pathogenic mutation sub-group. However, no difference was found when compared with controls. The pathogenic mutation sub-group showed a significant increase in QT interval compared with controls, but the difference disappeared when correcting for heart rate (i.e., QTc interval). In the DD acitretin sub-group, the LDL/HDL ratio was significantly higher than compared with both the no acitretin treatment sub-group and controls, despite the seven-day washout period. This is in line with previous findings showing an increase in serum lipids upon acitretin treatment [337, 338], and such an increase should persist even after a seven-day washout. The difference could also coincide with the increase seen in the pathogenic mutation sub-group,

making it hard to draw a conclusion. The acitretin treatment sub-group showed significantly increased QT and QTc intervals compared with controls. Prolonged QTc interval is a known risk factor for cardiac arrhythmias [339]. There was no increased risk for a diagnosis of arrhythmia among all individuals with DD in the population-based study, however, no sub-grouping for acitretin treatment could be performed due to the fact that no such data is collected in the register. To summarize, while some individual differences were found to be significant, no conclusive results were able to be drawn from the clinical study.

HF is a severe disorder that affects roughly 1% of the population, its prevalence and incidence increasing with age [340]. SERCA2a is the major SERCA isoform in the heart [341], responsible for pumping Ca^{2+} back into the sarcoplasmic reticulum (SR) and ER after it has been released in order to activate cardiac muscle contraction [215]. SERCA2 has been broadly implicated in the pathophysiology behind heart disease in general, including HF [191, 342-344]. SERCA2a expression has been found to be downregulated in HF, causing a significant impact on both diastolic and systolic function [345-348]. In a 2011 gene transfer trial, restoring SERCA2a expression was successful in improving both HF and the overall clinical course in patients [349], while a 2016 follow-up trial failed to repeat this improvement [350]. However, in an animal HF model, SERCA2a gene transfer improved cardiac function [351]. SERCA2 haploinsufficient mice, i.e., a model simulating DD patients on the cellular level, have also been shown to develop HF [190, 352, 353]. Furthermore, ER stress has been found to be implicated in several heart diseases including HF [354-358]. Thus, there is a robust scientific foundation underlying the association between DD and heart disease found in our population-based cohort.

A gender difference in any heart diagnosis was observed, with females displaying a 59% increased risk while males did not. Even though no sub-grouping for gender was done for HF in particular, this difference is most likely explained by a specific risk increase for HF among females. Why is this the case? There is an established 2:1 female:male ratio in HF [359, 360], but this does not account for the whole difference. Unfortunately, the register data does not contain information about the subtypes of HF, e.g., systolic/diastolic or reduced/preserved ejection fraction, in the cohort. The explanation may lie in SERCA2 function itself. Females are more likely to present with diastolic HF and HF with preserved ejection fraction [359, 360], and studies have shown that SERCA2 dysfunction and Ca^{2+} dyshomeostasis in HF leads to diastolic dysfunction and HF phenotypes with preserved ejection fraction, thus indicating a predominantly female type of HF [190, 352, 353]. Taken together, this may explain the gender difference in heart diagnoses seen among individuals with DD.

Knowledge of an increased risk of HF is important as this is a potentially serious disease that all the while can be easily screened for using a blood test for NT-proBNP, a widely used biomarker for HF [361]. The first heart diagnosis in DD also occurred almost seven years earlier than in the general population, further warranting considering screening for earlier detection. In the clinical study, we included NT-proBNP and troponin T, also a common biomarker which can increase in both myocardial infarction and HF [362]. ST2 [363, 364] and

galectin-3 [365] are fairly novel biomarkers for detecting heart disease including HF that are not commonly used in clinical practice, but were included in the clinical study. No significant differences were seen for either heart biomarker.

Like HF, deranged serum lipid levels are also easily accessible for screening. The difference in LDL/HDL ratio seen in the pathogenic mutation and acitretin treatment sub-groups might well be explained by acitretin treatment and not DD in itself [337, 338]. However, it still constitutes the most common systemic treatment for DD, and these patients will benefit from early detection of pathological serum lipid values either way.

In conclusion, the increased risk of heart disease in general, and heart failure in particular, and the signs of impaired serum lipid levels in DD warrants being taken into account in routine clinical care of these patients, including primary prevention and the possibility of screening for early diagnosis and treatment.

6.1.3 Darier disease and diabetes

On the population level, individuals with DD have a 74% crude risk increase for a diagnosis of type 1 diabetes (T1D). No change in risk was seen for a diagnosis of type 2 diabetes (T2D). In the clinical study, the main finding was a significant increase in HOMA2-%B in individuals with DD compared with control subjects, a sign of increased basal insulin secretion in pancreatic β-cells. HOMA2-%B was also increased in the DD acitretin treatment sub-group, albeit not significant when correcting for multiple comparisons.

Diabetes is a multi-facetted spectrum of quite differing disease sub-types, the primary subtypes being T1D and T2D. T1D is believed to be caused by an autoimmune attack on the insulin-producing β-cells of the pancreas, initially leading to a chronic inflammatory infiltrate and ultimately destroying the cells. The primary cause and target of the attack is still unknown [366]. Investigations have identified ER stress as one of several basic mechanisms of T1D, underlying the core pathophysiological changes of autoimmunity and inflammation [367]. A 2016 review illustrates how Ca^{2+} dyshomeostasis and ER stress, as seen in DD, may result in neoautoantigens due to faulty protein folding or posttranslational protein modifications, thus stimulating an endogenous autoimmune attack on β-cells [368]. Directly targeting these mechanisms *in vitro* reduces β-cell death [369]. β-cells from individuals with T1D also show increased levels of several ER stress markers [370]. Furthermore, SERCA plays a major role in T1D, both as a key Ca^{2+} regulator and through the ER stress mechanisms described [371]. The risk increase seen in DD further adds to the body of knowledge about the T1D pathophysiology.

ER stress has been implicated as a pathophysiological mechanism participating in T2D diabetes [372-374], and SERCA also seems to play a key role [371]. For example, SERCA2 haploinsufficient mice display pathologically low insulin secretion and are prone to develop diet-induced obesity and diabetes [375]. SERCA2b expression is also significantly reduced in

diabetic conditions [376, 377]. Altogether, the scientific grounds for an association between DD and both T1D and T2D is strong.

The significantly increased basal insulin secretion in individuals with DD suggests pancreatic β-cell dysfunction which is in turn indicative of T2D development. In nondiabetic individuals, increased basal insulin secretion is associated with a pre-diabetic phenotype and a steady decline in the ability to correctly regulate glucose metabolism [378]. Further, in a large longitudinal cohort study, HOMA2-%B values were shown to increase until around seven years prior to T2D diagnosis, whereafter they declined [379]. Similarly, increases in insulin secretion were seen in rat pancreatic β-cells upon drug-induced SERCA inhibition [380].

There is a discrepancy in the results between the clinical indications of a prediabetic state and the lack of an increased risk of T2D on the population-level. One explanation might be that T2D is never fully developed, and thus a diagnosis is never assigned, because the pancreatic β-cells can somehow compensate for the SERCA2 loss of function. At the moment, the cause for this discrepancy remains unknown.

Taken together, the association between DD and both T1D and T2D necessitates being considered in routine clinical care of DD patients. The diagnosis of T1D most likely precedes DD, as the incidence peak for T1D is around 5-9 years of age [381] and around 11-15, at the earliest, for DD [76]. It might therefore be of interest to screen for diabetes among children in DD families, since this is done by a simple blood test. The same holds true for T2D among older DD patients, where screening is easily performed and probably justified. Information on other risk factors for T2D development, such as obesity, physical activity and diet, might also be of benefit for the individual patient, in order reduce the risk of developing a prediabetic state and improve prognosis.

6.1.4 Darier disease and the immune system

Out of 16 individuals with DD, six exhibited values of CD19+ B cells below the normal reference range. CD19 is expressed in B cell lineages [382], both during the development phase when undergoing V(D)J recombination, and in mature plasma cells [383]. Numerous other cells in the immune system were also analyzed, however not included in the final publication since the overall investigation focused on V(D)J recombination specifically. Due to the possibility of a future publication including results of the other markers, the results cannot be disclosed in this thesis.

The rationale behind examining a link between DD and the immune system is that individuals with DD are prone to secondary infection [76, 384-386], and that there might exist an underlying immunological deficiency. Two early investigative case series yielded conflicting results, with indications of a depression in cell-mediated immunity [387, 388]. Further case series investigated several immune cells/samples and other immune-related markers and found no significant differences compared with the normal reference values [389, 390]. A 2010

investigation found a significant reduction of CD1a+ Langerhans cells and CD123+ dendritic cells in individuals with DD. The authors postulate that the genetic damage of DD might cause certain subsets of dendritic cells in the skin to be lost, leading to a worsening of secondary infections that frequently occur [391]. Interestingly, pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, that are activate during infections, have been shown to both downregulate *ATP2A2* gene expression, and accentuate herpes simplex virus (HSV) growth, leading to susceptibility to, and worsening of symptoms of, secondary HSV infections in DD [392]. Further, an *ATP2A2* gene silencing 3D skin barrier dysfunction model resulted in more severe HSV infection [393].

The role of SERCA in the immune system has not been as thoroughly studied as in the brain, heart and pancreas. SERCA2 and SERCA3 are expressed in immune cells, and often co-expressed [187, 230, 394, 395]. One theory as to why 10 out of 16 DD patients display normal values of CD19+ B cells is that their SERCA2 deficiency is sufficiently low to cause skin symptoms, while SERCA3 upregulation can compensate for the loss of function of SERCA2 in B cells. This mechanism of compensatory upregulation has been seen in other tissues [341], and was also observed in B cells in the experimental part of study V. However, to the author's best knowledge, no evidence for such a mechanism exists for keratinocytes. It has even been theorized that the loss of SERCA3 co-expression with SERCA2 in skin partly explains the skin phenotype in DD [283].

In summary, there may well be an association between DD and the immune system, but a rescue effect in immune cells by compensatory upregulation of SERCA3 might mitigate the impact of SERCA2 loss of function. However, as few studies on the subject exists, the characteristics of such an association, and not least the possible clinical implications for DD patients, remain to be explored.

6.2 METHODOLOGICAL CONSIDERATIONS

The main study designs of this thesis are population-based cohort studies (register-based) and experimental, matched case-control studies (clinical). Like all study designs, they have advantages and disadvantages, strengths and limitations. The studies included in this thesis are subject to the potential effect of several types of biases.

Selection bias might have occurred in the clinical studies since patients with very mild DD can be undiagnosed and thus not included. Perhaps these patients have weaker associations with comorbidities, leading to a misrepresentation of the intended target population, i.e., all individuals with DD. However, since there is no clear genotype-phenotype correlation in DD, a solid underlying pathophysiological mechanism for the comorbidities exist, and the fact that the inheritance pattern in DD promotes patients with mild symptoms to also seek a diagnosis, the potential effect of selection bias was most likely minimal. Likewise, in the register-based studies, potentially undiagnosed individuals with DD are not included, but here too the effect is considered marginal. It might also be the case that individuals with DD, solely due to their

more frequent interactions with healthcare, are more likely to be diagnosed with certain comorbidities. This can be seen as an example of *detection bias*, where one group (individuals with DD) is more closely monitored then the other (comparison subjects). As T1D is usually diagnosed before DD, detection bias is unlikely, but it may influence HF diagnosis as this diagnosis is assigned much later in life.

Misclassification of DD may potentially exist, but since it is a very specific diagnosis, the extent of such a bias is thought to be minimal. Misclassification of comorbidity diagnoses are likely more prevalent as these diagnoses are far more common, but since the validity of the NPR is very high the potential impact is expected to be small [296].

Confounding is a possible bias that can never be fully accounted for. Close matching in both the register-based and clinical studies likely abolish the major, obvious confounders. In the register-based data, crude RR does not adjust for potential confounding diagnoses, and it is considered by many to be the best measure as it mimics the real world, which the data are trying to reflect. In the heart disease register-based data, adjusted RRs were also included, taking several confounders into account. However, one can never be entirely certain that a potentially important confounder remains unaccounted for. For example, the register data lacks information on potentially important lifestyle factors. Thus, crude RR is likely a more appropriate end-point measurement. Another potential confounding factor might be the chronic skin disease per se. For example, the presence of a severe chronic skin disease has in itself been shown to be associated with increased risk of heart disease mortality [396]. The same might be true for cognitive function and diabetes. This could have been accounted for in the matching process, albeit making it markedly more difficult to find suitable control subjects.

6.2.1 Register-based studies

The main strengths in research carried out with data from the Swedish national registers are their almost complete coverage, the high validity of diagnoses, and the incorporation of the personal identity number which enables linkage between different registers [294-296]. The validity of a diagnosis of DD is believed to be very high since it is highly specific and most likely assigned by a dermatologist. The cohort of individuals with DD within the NPR is presumably the largest in the world. Only around 200 individuals with DD are expected to be alive in Sweden, but in the studies up to 935 individuals were able to be included, which is a uniquely high number in all research pertaining to DD. Due to the matching of each individual to ~100 comparison subjects, a strong analysis of associations and risks on the population level are made possible.

As previously mentioned, the register-based studies are subject to the potential influence of a loss of undiagnosed cases of mild DD, detection bias, misclassifications and confounding. In study IV, no stratification for gender was performed, which might have yielded further valuable insights. Furthermore, mean age at first diagnosis was not included. However, this parameter has limitations of its own, since patients might have received their diagnosis of DD in ICD-8,

and thus the first ICD-9 code of DD leads to an incorrect mean age at diagnosis. Thus, in study II, this parameter must be interpreted with caution. In study IV, no adjustment for diabetes risk factors was done for the RR, but as was previously discussed, adjusted RRs are not without their own limitations, and thus crude RR might be considered more suitable.

In general, a potential limitation of the national registers is incomplete coverage, which might occur in the case of migration or the unreported death of a Swedish citizen abroad. However, the error resulting from these potential inaccuracies are minor [295].

6.2.2 Clinical studies

DD is rare, impacting the number of individuals available to enroll in research, and in turn affecting the statistical power of the clinical studies. Cohorts of up to 29 patients were included, estimated to constitute around 15% of the cases in Sweden. Thus, in relative terms, a high proportion of Swedish DD patients are included in the studies. Nevertheless, in absolute numbers it is still a small study population. Statistical power can be enhanced by increasing the number of control subjects. Therefore, the 1:1 matching ratio can be seen as a limiting factor, leading to the risk of type 2 statistical error (i.e., "false negative"). It might be that the lacking power explains why some of the results from the register-based studies were not replicated in the clinical studies. In general, however, tight matching, an experimental setup with prospective data collection, and thorough inclusion and exclusion criteria reduces the potential bias in the clinical study design, improving the overall validity. Further, correcting for multiple comparisons additionally strengthens the end-point analysis, making the significant *p*-values even more trustworthy.

There might be important confounders unaccounted for in the matching and inclusion/exclusion criteria. Acitretin is such a potential confounder, where a seven-day washout period aimed to account for some of the potential influence of the treatment. However, effects of the drug likely persist. In the heart clinical study, sub-group analyses were made to evaluate potential differences, and some were seen in individual parameters. However, the groups were very small. No sub-group analyses were performed in the diabetes clinical study, leaving us without knowledge of potential differences. However, it is unlikely that acitretin treatment would influence the main result of impaired pancreatic β-cell function, since acitretin has been shown to probably even have the opposite effect [397, 398]. No sub-grouping was performed in the cognition clinical study either. It would have been interesting to be able to evaluate a possible genotype-phenotype correlation in cognitive function in patients with pathogenic *vs.* benign mutation variants.

To the best of our knowledge, the experimental evaluations of cognition, heart disease and metabolic phenotype/diabetes were adequate and accurate. All the while, there might be better ways to evaluate the intended outcomes. For example, a 2001 study used echocardiography in a group of 10 DD patients to evaluate cardiac function [399]. Even though no difference was

observed when comparing values with established standard ranges, such an analysis might have provided interesting insights in our patient cohort.

In study V there are obvious limitations pertaining to the fact that only a single cell marker was evaluated, no matched control group was used for comparison, the group was relatively small (n = 16), and no confounder was taken into account except thorough exclusion criteria. A possible strength lies in that pathogenic mutation status was a prerequisite for inclusion, and that the result of the analysis resonates with the overall results of the investigation.

7 CONCLUSIONS

Darier disease (DD) has been found to be associated with comorbidities in several organs, including the brain, heart, pancreas, and possibly the immune system. The underlying biological mechanisms for these associations have been thoroughly reviewed, and there is strong evidence behind each. Individuals with DD display impaired cognitive function, have increased risks of heart disease in general and heart failure in particular, an increased risk of a type 1 diabetes diagnosis, a metabolic phenotype indicative of pancreatic β-cell dysfunction and a prediabetic state, as well as a possible defect relating to the immune system. Through the use of experimental case-control studies and register-based cohort studies, the investigations in this thesis have widened the range of known comorbidities for DD to include multiple organs.

In summary, it can safely and accurately be concluded that DD is a systemic condition and not just a disorder confined to the skin. Future treatment attempts for DD should take this in consideration.

8 POINTS OF PERSPECTIVE

1. Why is knowledge about comorbidities associated with DD important?

Viewing DD as a multi-organ condition and not simply a dermatological disorder yields valuable insights about the way DD should be managed in routine care. It is important to characterize the entire disease spectrum in order to best care for and treat this group of patients. Furthermore, it provides knowledge about the role of SERCA, and by extension ER stress and Ca^{2+} homeostasis, in organ biology and pathophysiology.

2. What are the ramifications for individuals with DD?

As mentioned in the discussion, the impact for the individual patient stemming from knowledge about these comorbidities potentially carries great weight. For example, heart disease and diabetes are easy targets for screening, leading to early diagnosis, appropriate treatment and better prognosis. Even so, the question of screening is often the subject of debate and controversy, and one might argue against the cost-effectiveness aspect of a screening approach, or that the risks are too low to motivate screening. In the case of DD, the patients will come for follow-up visits anyway, and one should therefore keep these risks in mind.

Overall, patients might benefit from special medical, societal and other appropriate attention. Looking forward, there might be the need for specific, targeted treatments that take comorbidities into account.

3. Are there additional comorbidities associated with DD?

To potentially further characterize and increase the spectrum of DD comorbidities, it would be interesting to follow up on indications linking DD, or SERCA, to conditions such as basal cell carcinoma [400-402], internal malignancies [403-405], ophthalmic pathologies [406], immune defects [407], and dementia (mainly Alzheimer's and Parkinson's disease) [217]. Like with the impact of the other comorbidities, linking DD to any of these conditions could motivate specific treatments or other healthcare interventions.

4. How should we treat DD?

Currently, no treatment targeted to the disease mechanism exist. We are limited to mostly symptomatic treatments and medications used to handle complications of the disease. My view is that the potentially severe consequences of the comorbidities associated with DD call for targeted treatments, as we can no longer confidently say that all that DD patients suffer from is merely a skin rash. It may well be that the reason to treat DD in a particular fashion is dependent primarily on the comorbidities and not the skin.

5. Does a genotype-phenotype correlation pertaining to DD comorbidities exist?

Many researchers have tried to find some type of genotype-phenotype correlation in DD regarding several symptoms such as skin rash severity, quality of life, psychiatric symptoms etc. To the best of my knowledge, only one study has found any type of correlation, namely a specific mutation locus leading to a more severe neuropsychiatric phenotype in a small subset of patients in a larger DD cohort (n = 75) [318]. A large cohort is needed in order to be able to draw conclusions on genotype-phenotype correlations, limiting the feasibility of such studies due to the rarity of the disease. Unfortunately, data on mutation status is not recorded in the NPR, so a register-based approach is unviable. Nevertheless, in order to further characterize the disease spectrum of DD and gain insights into the role of SERCA in several organs, the genotype-phenotype correlation regarding comorbidities would be interesting to investigate.

6. Which are the outlooks for future research concerning DD?

Apart from further exploring the comorbidities of DD and genotype-phenotype correlations, it would be interesting to perform whole genome sequencing on mutation-negative individuals with DD and search for explanations to their disease phenotype. Such research might yield many interesting insights. In addition, research is ongoing concerning a possible novel treatment for DD that directly targets the disease mechanism. Initially planned to be part of this thesis, due to the project growing beyond the scope of what is possible to include in a PhD thesis, the focus shifted to comorbidities. The laboratory work on the novel treatment and a clinical trial for which a large research grant has been received constitute the immediate future research on DD.

9 ACKNOWLEDGEMENTS

I am deeply grateful for the participation of all patients and control subjects, without whom there would have been nothing to work with in this thesis.

There are several persons I want to direct my sincere gratitude towards:

Jakob Wikström, my main supervisor. With unequaled drive, tempo, knowledge and sense of direction, during our journey together you have enabled for me to develop exponentially from the unsuspecting novice researcher you took under your wing. For this I am and will be forever grateful.

Martin Cederlöf and **Enikö Sonkoly**, co-supervisors. Always available to assist in my recurring inquiries, your support has been essential in making much of the work in this thesis possible. Martin, your knowledge of statistics and registers is all-encompassing and invaluable. Enikö, by having you as a co-supervisor I have never felt out of place when soliciting advice from colleagues in the lab (which happened absolutely all the time).

All the colleagues in the lab who have helped and worked with me, for your patience, persistence, knowledge and skill: **Etty Bachar-Wikström**, **Naricha Pupinyo**, **Mansi Manchanda**, **Ritu Bansal**, **Ankit Srivastava**, **Lorenzo Pasquali**, **Kunal Das Mahapatra**, **Warangkana Lohcharoenkal** and **Pernilla Nikamo**. Even though the lab work didn't make it into this thesis (don't worry, it's coming later), it has provided unrivaled learning opportunities and helped me grow as a researcher, much thanks to you.

Hanna Brauner and **Anders Näsman**, with whom I wrote a completely unrelated review article, originally intended as a case report. You have aided me in the process of reviewing and trying to make sense of a completely different dermatological disease and its field of research, an experience which helped me further develop my skills and abilities.

Barry Sleckman and your research group, for initiating contact with us and establishing a transatlantic collaboration through which I have learnt a lot.

Johanna Bern, **Linnea Sand** and **Tara Ahanian**, medical students at the time. The dedication and hard work you put forth in your respective master's theses could be harnessed and included in our publications together.

My co-authors, if not aforementioned: **Ivone Leong**, **Henrik Larsson**, **Kerstin Brismar**, **Jan Lapins**, **Niki Radros**, **Britta Krynitz**, **Mireia Jasans-Barcélo**, **Lena Hagströmer** and **Darius Sairafi**, for being willing to participate in our ventures and providing valuable input in many ways.

Erik Sundström, supervisor, the excellent lecturers, and especially all the great colleagues who attended the Research School in Molecular Medicine for Clinicians together with me. You helped lay the robust scientific foundation on which I am today standing and made sure we had a lot of fun in the meantime.

All the fantastic colleagues at the Dermatology Clinic at Karolinska University Hospital, for creating the wonderful clinic I am lucky to be working at every single day, and in particular my bosses **Maria Sund Böhme** and **Lena Lundeberg**, enabling for me to take so much precious research time during my residency.

All my friends and extended family, for all the crucial support, joy and energy you have given me.

My father **Michael**, for instilling in me the core values and perspectives that permeates everything I do in life and making me never doubt what I am capable of accomplishing.

My mother **Catharina**, without whom not even a fraction of this work would have been possible (not least thanks to all the babysitting). Always looking out for me, always taking care of me, always available for me to unload my emotions, always there as a cornerstone in my life. You are the best mother I could ever imagine being blessed with.

Finally, **Clara**, my wife, the love of my life, my best friend and kindred spirit, and **Hedda** and **Adam**, my beloved children, the lights of my heart and the subjects of my limitless love. You make me want to be the best possible version of myself. At times when I have struggled you have been there and reminded me of what is truly important in life.

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