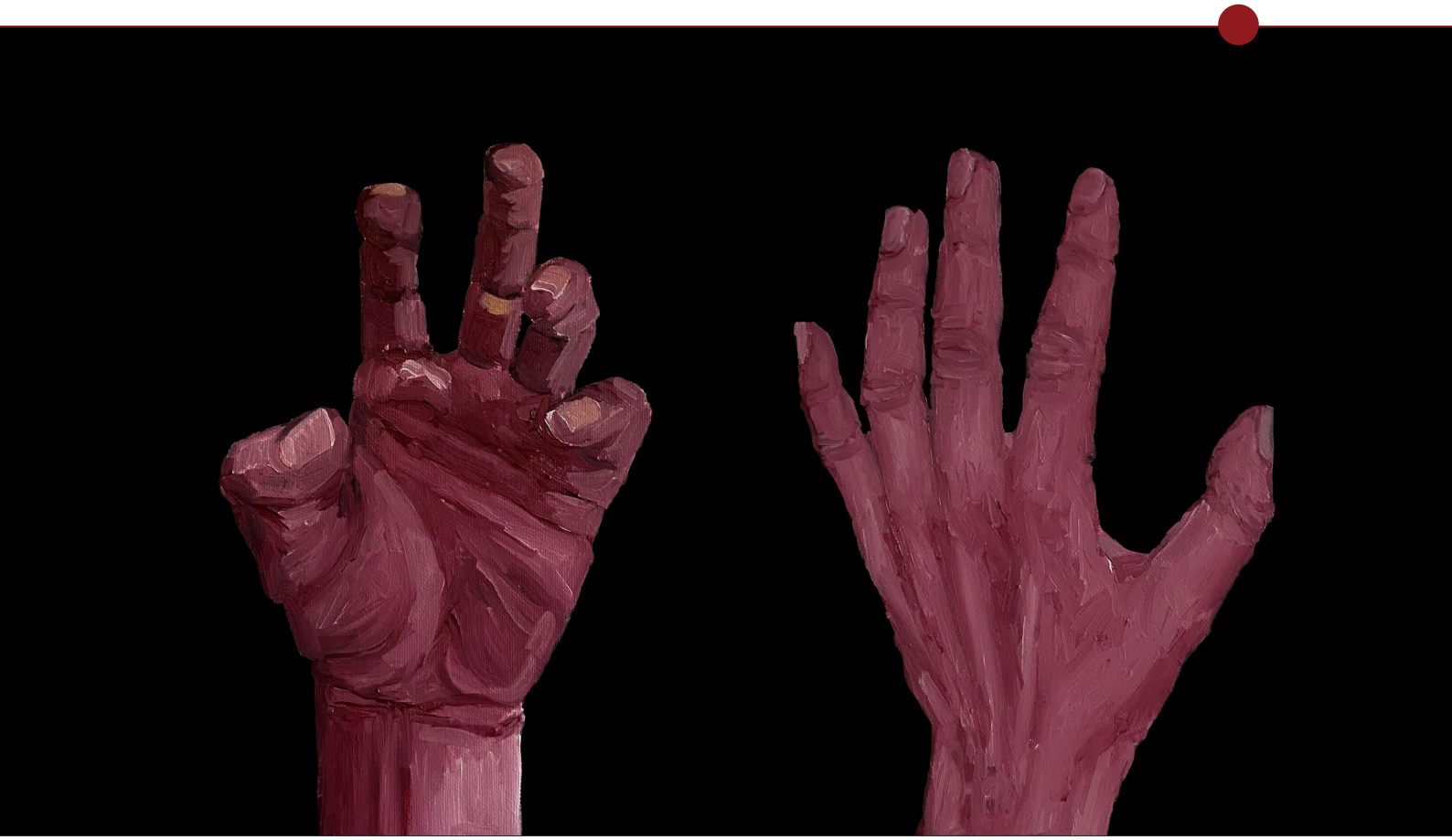
UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES



Hand eczema:

From molecular fingerprints to population-wide perspectives

PhD Thesis

Anna Sophie Quaade

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Preface

The main focus of this thesis is hand eczema, which is explored from two perspectives: a population-wide questionnaire study (*Manuscript I*), and a clinical study including molecular investigations of the disease (*Manuscripts II-IV*).

The thesis is based on scientific work conducted from April 2019 to February 2024 at the National Allergy Research Centre, Department of Dermatology and Allergy, Copenhagen University Hospital - Herlev and Gentofte, Denmark. The plasma proteomic and skin transcriptomic investigations of *Manuscripts II-IV* are based on a collaboration with the Department of Medicine, Division of Clinical Immunology, and the Department of Genomics and Genomic Sciences, Ichan School of Medicine at Mount Sinai, New York, USA. In addition, the skin transcriptomic data analyses in manuscript IV are further based on a collaboration with The Skin Immunology Research Centre, Department of Immunology and Microbiology, University of Copenhagen, Denmark.

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Anna Sophie Quaade

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Manuscripts

This PhD thesis is based on the following four manuscripts:

I. **Quaade AS**, Alinaghi F, Dietz JBN, Erichsen CY, Johansen JD. Chronic hand eczema: A prevalent disease in the general population associated with reduced quality of life and poor overall health measures. Contact Dermatitis. 2023 Dec 27;89(6):453–63.

II. **Quaade AS**, Wang X, Sølberg JBK, Ulrich NH, McCauley BD, Thyssen JP, Becker C, Johansen JD. Circulating biomarkers are associated with disease severity of chronic hand eczema and atopic dermatitis. Br J Dermatol. 2023 Jul 7;189(1):114–24.

III. **Quaade AS**, Wang X, Sølberg JBK, Mccauley BD, Thyssen JP, Becker C, Johansen JD. Inflammatory plasma signature of chronic hand eczema: Associations with aetiological and clinical subtypes. J Eur Acad Dermatology Venereol. 2023 Dec;(November):1–11.

IV. **Quaade AS**, Litman T, Wang X, Becker C, McCauley BD, Sølberg JBK, Thyssen JP, Johansen JD. Transcriptomic profiling of chronic hand eczema skin reveals shared immune pathways and molecular drivers across subtypes. Unpublished manuscript.

Additional Publications

Publications during doctoral studies not included in this thesis:

Quaade AS, Simonsen AB, Halling A, Thyssen JP, Johansen JD. Prevalence, incidence, and severity of hand eczema in the general population – A systematic review and meta-analysis. Contact Dermatitis. 2021 Jun 23;84(6):361–74.

Sølberg JBK, **Quaade AS**, Jacobsen SB, Andersen JD, Kampmann M, Morling N, Litman T, Thyssen JP, Johansen JD. The transcriptome of hand eczema assessed by tape stripping. Contact Dermatitis. 2022 Feb 8;86(2):71–9.

Sølberg JBK, **Quaade AS**, Drici L, Sulek K, Ulrich NH, Løvendorf MB, Thyssen JP, Mann M, Dyring-Andersen B, Johansen JD. The Proteome of Hand Eczema Assessed by Tape Stripping. J Invest Dermatol. 2023 Aug;143(8):1559-1568.e5.

Simonsen AB, Ruge IF, **Quaade AS**, Johansen JD, Thyssen JP, Zachariae C. Increased occurrence of hand eczema in young children following the Danish hand hygiene recommendations during the COVID-19 pandemic. Contact Dermatitis. 2021 Mar 5;84(3):144–52.

Simonsen AB, Ruge IF, **Quaade AS**, Johansen JD, Thyssen JP, Zachariae C. High incidence of hand eczema in Danish schoolchildren following intensive hand hygiene during the COVID-19 pandemic: a nationwide questionnaire study. Br J Dermatol. 2020 Nov 10;183(5):975–6.

Abbreviations

ACD	Allergic contact dermatitis
AD	Atopic dermatitis
CHE	Chronic hand eczema
CCL	Chemokine (C-C motif) ligand
CXCL	Chemokine (C-X-C motif) ligand
DEG	Differentially expressed gene
DEP	Differentially expressed protein
EASI	Eczema area and severity index
FLG	Filaggrin
HE	Hand eczema
HECSI	Hand eczema severity index
HRQoL	Health related quality of life
ICD	Irritant contact dermatitis
IL	Interleukin
QOLHEQ	Quality of life in hand eczema questionnaire
Th	T helper
TNF	Tumor necrosis factor alpha
VAS	Visual analogue scale

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Summary

Hand eczema (HE) is a very common, often chronic, skin disease with a multifactorial pathogenesis and diverse clinical presentations. The most common aetiologies are irritant contact dermatitis (ICD), allergic contact dermatitis (ACD), and atopic dermatitis (AD) manifesting on the hands.

Many studies have investigated the epidemiology of HE in general population settings, focusing mainly on disease prevalence. However, knowledge on other important epidemiological measures such as the prevalence of chronic and severe HE and the quality of life among those with HE at the population level is scarce.

The underlying immunological mechanisms and molecular profiles of HE are not fully understood, particularly across its various subtypes. Research into the molecular aspects of HE has highlighted three primary areas: compromised skin barrier function, skin microbiome imbalance, and a dysregulated local immune system. The details of the dysregulated immune system, including the dominating immune pathways and key molecular drivers of the disease, remain largely uncharacterized. Additionally, it remains to be investigated if HE is also associated with a systemic immune response, beyond the local skin inflammation.

The main focus of this thesis is HE. The above-described gabs in knowledge are explored through a nationwide questionnaire study (*Manuscript I*) as well as a clinical study, that includes molecular investigations of the disease (*Manuscript II-IV*).

1. In *Manuscript I*, we explored a wide range of epidemiological measures associated with HE within the adult, Danish general population. This was achieved by analysing responses from more than 40,000 participants in an electronic questionnaire study. We found that HE was very common with 13.3% of Danish adults reporting HE on any occasion during the past 12 months. The majority of those with HE reported chronic disease and one third moderate to severe disease. When comparing individuals with- and without HE, we found that those with HE reported poorer overall health and more sick leave for any reason. One third of those with current HE reported moderate to very strong impairment of their quality of life because of HE. We found that having moderate to severe, chronic, and work related HE were strongly associated with quality-of-life impairment.

Manuscripts II-IV are based on examinations of different subsamples of a clinical study population consisting of 110 patients with chronic HE (CHE) and/or AD and 40 healthy controls.

2. In *Manuscript II* we aimed to investigate if CHE was associated with systemic inflammation. We included patients with isolated CHE, patients with active AD, and healthy controls. We characterized the plasma inflammatory signature (266 inflammatory plasma proteins) across the cohort. We found that very severe CHE with no history of AD was associated with systemic immune activation characterized by increased circulating levels of T helper (Th) 2, Th1, and other inflammatory biomarkers. For patients with active AD, we found that moderate and severe disease was associated with systemic immune activation. Th2 associated systemic inflammation was shared between patients with very severe CHE without AD and patients with severe active AD, and three biomarkers correlated positively with clinical severity in both groups.
3. In *Manuscript III* we aimed to characterize the inflammatory plasma signature of different CHE subtypes. We included patients with CHE without concomitant AD and healthy controls. Patients were stratified according to both aetiological subtype (ACD, ICD, ACD/ICD), and unique clinical subtype (vesicular, hyperkeratotic, and chronic fissured). We did not find any biomarkers that could discriminate between ACD and ICD. Very severe ACD was associated with a mixed Th1/Th2 type systemic inflammation and several biomarkers correlated positively with clinical severity in this group. Hyperkeratotic CHE was associated with a rather psoriasiform systemic footprint and could be discriminated from both the vesicular and chronic fissured subtypes by increased levels of two biomarkers.
4. In *Manuscript IV* we aimed to characterize the transcriptome of CHE across different subtypes. We profiled the skin transcriptome from 220 full-thickness skin biopsies collected from hands (dorsal and palmar aspects) and arms from patients with CHE and/or AD and healthy controls. We first found evidence of regional transcriptomic variations, with palm skin showing a distinct molecular pattern. The molecular profile of CHE palm skin was primarily shared across subtypes categorized both by AD status and by unique aetiology (ACD, ICD, AD). This profile included a heterogenous dysregulated immune response with prominent activations from Th1 and Th2 pathways. We identified key upstream regulators that were shared across subtypes. These might serve as potential therapeutic targets. Although no genes were found to discriminate ACD from ICD, some differences in pathway and upstream regulator activity were noted between the subtypes.

Conclusion

In conclusion, HE is associated with a considerable burden, both for society and for the many affected by the disease. The high prevalence of HE, including the large proportion of individuals suffering chronic- and moderate to severe disease indicate unmet needs for treatment and prevention. In particular, individuals with chronic, severe, and occupational HE experience profound quality of life impairments, suggesting that targeted interventions could be aimed at these groups.

The molecular investigations of CHE included in this thesis provide novel insights into both local and systemic molecular fingerprints of the disease – overall and across its various subtypes. Collectively, a dysregulated Th1/Th2 skewed immune response was characterized, both in skin as well as in the systemic circulation of CHE patients with very severe disease. Our results indicate a shared lesional molecular endotype across different subtypes and highlight key molecular drivers that could serve as potential therapeutic targets.

Dansk Resumé

Håndeksem er en meget hyppig, ofte kronisk, hudsygdom med en multifaktoriel patogenese og forskellige kliniske præsentationer. De mest almindelige ætiologier, eller årsager, er irritativt kontakteksem, allergisk kontakteksem og atopisk eksem lokaliseret til hænderne.

Mange studier har undersøgt epidemiologien af håndeksem i forskellige generelle befolkninger. Disse studier fokuserer hovedsageligt på sygdommens forekomst (prævalens). Viden om andre vigtige faktorer såsom prævalensen af kronisk og svært håndeksem, samt livskvaliteten blandt individer med håndeksem på befolkningsniveau er meget sparsom.

De underliggende immunologiske mekanismer og molekylære mønstre ved håndeksem er ikke fuldt klarlagt. Specielt ikke på tværs af de forskellige ætiologiske og kliniske subtyper af håndeksem. Den nuværende forskning inden for feltet fremhæver tre primære områder: en kompromitteret hudbarriere, ubalance i hudmikrobiomet samt et lokalt dysreguleret immunsystem. Det dysfunktionelle immunsystem er ikke velkarakteriseret, herunder ved man ikke meget om, hvilke immunakser der er dominerende, samt om der er specifikke molekyler der driver inflammationen i håndeksem. Derudover er det uvist, om håndeksem, udover den lokale hudinflammation, også er associeret med en mere generaliseret aktivering af kroppens immunsystem, kaldet systemisk inflammation.

Fokus for denne afhandling er håndeksem. De ovenfor beskrevne vidensgab udforskes igennem et landsdækkende spørgeskemastudie (*Manuskript I*) samt et klinisk studie, der inkluderer molekylære undersøgelser af sygdommen (*Manuskript II-IV*).

1. I *Manuskript I* udforskede vi et bredt spektrum af epidemiologiske mål forbundet med håndeksem i den voksne danske befolkning. Dette blev opnået ved at analysere svar fra mere end 40,000 deltagere i en elektronisk spørgeskemaundersøgelse. Vi fandt, at håndeksem var en meget hyppig sygdom med en et års prævalens på 13.3% i den danske befolkning. Størstedelen med håndeksem rapporterede kronisk-, og en tredjedel moderat til svært eksem. Personer med håndeksem rapporterede dårligere generelt helbred og mere sygefravær end personer uden håndeksem. En tredjedel med håndeksem rapporterede moderat til meget stærkt forringet livskvalitet forårsaget af håndeksem. Vi fandt, at moderat

til svært-, kronisk- samt erhvervsbetinget håndeksem var stærkt associeret med nedsat livskvalitet.

Manuskript II-IV er baseret på undersøgelser af forskellige undergrupper fra en klinisk studiepopulation bestående af 110 patienter med kronisk håndeksem og/eller atopisk eksem samt 40 raske kontroller.

2. I *Manuskript II* undersøgte vi, om kronisk håndeksem var associeret med systemisk inflammation. Vi inkluderede patienter med isoleret kronisk håndeksem, patienter med aktivt atopisk eksem og raske kontroller. Vi karakteriserede den plasma-inflammatoriske signatur (266 inflammatoriske plasma proteiner) på tværs af kohorten. Vi fandt, at meget svært isoleret kronisk håndeksem, hos patienter uden tidligere atopisk eksem, var associeret med systemisk immunaktivering. Denne systemiske immunaktivering var karakteriseret ved forhøjede cirkulerende niveauer af T hjælper (Th)2, Th1 og andre inflammatoriske biomarkører. For patienter med aktivt atopisk eksem fandt vi, at moderat og svær sygdom var associeret med systemisk immunaktivering. Th2 associeret systemisk inflammation var delt imellem patienter med meget svært kronisk håndeksem uden tidligere atopisk eksem og patienter med aktivt atopisk eksem. Niveauer af tre cirkulerende biomarkører korrelerede positivt med sygdomssværhedsgraden i begge patientgrupper.

3. I *Manuskript III* undersøgte vi den plasma-inflammatoriske signatur for forskellige ætiologiske og kliniske subtyper af kronisk håndeksem. Vi inkluderede patienter med kronisk håndeksem uden samtidig atopisk eksem og raske kontroller. Patienter blev stratificeret efter både ætiologisk subtype (allergisk kontakteksem, irritativt kontakteksem samt allergisk- og irritativt kontakteksem) og klinisk subtype (vesikuløst, hyperkeratotisk og kronisk fissureret håndeksem). Vi fandt ikke nogen biomarkører der kunne diskriminere imellem allergisk- og irritativt kontakteksem. Meget svært allergisk kontakteksem var associeret med et blandet Th1/Th2 type systemisk immunrespons og flere biomarkører korrelerede positivt med den kliniske eksemsværhedsgrad blandt disse patienter. Hyperkeratotisk håndeksem var associeret med et mere psoriasiformt blodbillede og denne subtype kunne diskrimeres fra både de vesikuløse og de kronisk fissurerede subtyper ved højere niveauer af to cirkulerende biomarkører.

4. I *Manuskript IV* undersøgte vi transkriptomet ved kronisk håndeksem på tværs af forskellige subtyper. Hudtranskriptomet blev profileret fra 220 hudbiopsier der var taget fra hænder (håndflader og håndrygge) og arme fra patienter med kronisk håndeksem og/eller atopisk

eksem samt raske kontroller. Vi fandt tegn på regionale variationer i hudtranskriptomet, hvor hud fra håndfladerne viste et specifikt molekylært mønster. Vi fandt, at den læsionelle transkriptomprofil primært var delt imellem håndeksemsubtyper, der var kategoriseret både efter atopisk eksem status og unik ætiologisk subtype (atopisk eksem, allergisk- og irritativt kontakteksem). Denne molekylære endotype var karakteriseret ved et heterogent, dysreguleret immunrespons med prominente aktiveringer af Th1 og Th2 immunakser. Vi identificerede molekyler der drev inflammationen (upstream regulators) på tværs af subtyperne. Disse molekyler kan repræsentere potentielle *targets* for nyere mere målrettede behandlinger. Vi fandt ikke nogen gener, der kunne diskriminere imellem allergisk- og irritativt kontakteksem, men vi observerede visse forskelle i aktiveringen af immunakser og upstream regulators imellem disse subtyper.

Konklusion

Håndeksem er forbundet med en betydelig byrde, både for samfundet og for de mange, der er berørte af sygdommen. Den høje forekomst af håndeksem, herunder den høje proportion af individer der lider af kronisk samt moderat til svær sygdom indikerer et behov for bedre forebyggelse og behandling af håndeksem. Personer med kronisk, svært og erhvervsbetinget håndeksem oplever betydelig negativ påvirkning af deres livskvalitet, hvorfor målrettede interventioner kan fokusere på disse grupper.

Denne afhandlings molekylære undersøgelser af håndeksem afdækker ny viden om sygdommens lokale og systemiske molekylære fingeraftryk – både overordnet og på tværs af forskellige subtyper. Samlet set blev der identificeret et dysreguleret, Th1/Th2 domineret immunrespons både i huden og i den systemiske cirkulation hos patienter med meget svær sygdom. Resultaterne viser en primært delt læsionel molekylær endotype på tværs af subtyper og fremhæver centrale molekyler der potentielt kunne repræsentere terapeutiske mål.

1. Introduction

Our hands provide a unique interface for interaction with the world around us. They are incredibly versatile and play a crucial role in numerous daily activities at home, at work and in our free time. We meet and greet through a handshake and communicate non-verbally with others through gestures. The sensory capabilities of the hands contribute to tactile exploration and perception, and we use touch to display emotional affection. We primarily keep our hands unclothed, visible to people around us, and highly exposed to many physical, and environmental factors. We wash and disinfect our hands to keep them clean and avoid catching or spreading infectious diseases. Several times a day.

Eczema located on the hands, hand eczema (HE), occurs when the integrity of the skin barrier becomes compromised. Frequent causes are irritant damage, contact allergy and atopic dermatitis (AD) (also known as childhood eczema). The skin becomes dry, red, often with blisters and cracks which causes itch, pain, and discomfort for many affected. It is a multifactorial and heterogenous disease that can be categorized into several different subtypes. As such, HE is used as an umbrella term that encompasses all these subtypes.[1,2]

HE is one of the most common skin diseases in Scandinavia, affecting around one out of ten adults each year and it is also the most common occupational disease in many western countries, including Denmark.[3,4] HE can cause decreased quality of life, sick-leave, and for some even change of career or unemployment.[5–7] Socioeconomically, the consequences of HE are considerable.[8] Nevertheless, research into the personal and societal impacts of HE mainly focusses on selected groups, such as certain occupational groups and hospital patients, which limits the generalizability of the results to the general population.[9]

Research into the molecular profile and the pathogenesis of HE is scarce. Studies mainly point to an important role of skin barrier impairment, a dysregulated local immune system, and microbiome dysbiosis.[10–18] The extent to which HE may be associated with inflammation beyond the skin, affecting the body's overall immune system, is uncertain. Moreover, the dominating immune pathways and molecules driving the disease remain largely uncharacterized. It is also unclear whether specific molecular markers can distinguish different types of HE. Uncovering these aspects could enhance our understanding of the disease pathogenesis of HE and potentially lead to improved managing options for those affected.

In this thesis HE is investigated from two different perspectives. The first, a nationwide cross-sectional questionnaire study with the overall aims to examine the quality of life of unselected individuals with HE and to contrast health measures such as sick leave and health perception between individuals with and without HE (*Manuscript I*). The second, a clinical study with the overall aim to characterize the molecular characteristics of HE through profiling of biomarkers from blood (*Manuscripts II-III*) and from skin (*Manuscript IV*).

2. Background

2.1 Hand eczema

2.1.1 Classifications and definitions

HE is heterogenous and can be classified into subtypes based on factors such as temporality, morphology, anatomical localization, and aetiology of HE.[19] An overview over such factors is presented in Figure 1. Subclassification of HE can facilitate targeted patient management, including enhanced diagnosis, customized treatment, and patient education. Frequently, multiple sub-diagnoses are required for a comprehensive characterization of HE.[2]

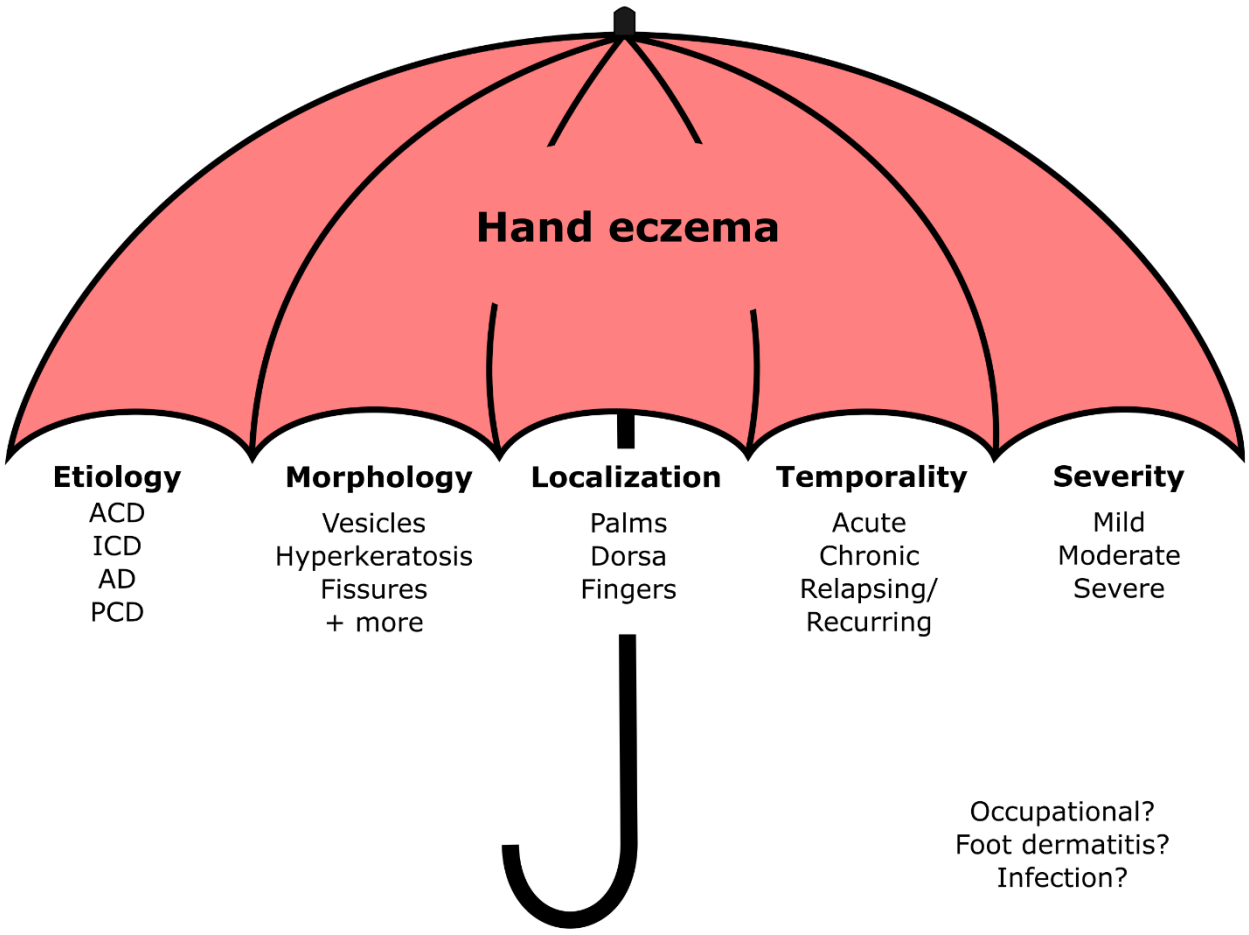

Figure 1. *Hand eczema can be classified according to different factors. Allergic contact dermatitis, ACD; atopic dermatitis, AD; irritant contact dermatitis, ICD; protein contact dermatitis, PCD.*

Although uniform disease classifications are important for standardizing studies and trials, different classification approaches using differing terminologies have historically been used for HE with no clear international consensus. The newest HE classification guidelines were published in 2022 from the Guideline Development Group established by the European Society of Contact Dermatitis (ESCD).[1]

2.1.1.1 Anatomical localizations

HE is defined as eczema located on the wrists, palms, dorsa of hands, and fingers. HE can primarily be localized to one specific area, i.e., presenting as pulpitis, interdigital, palmar, or dorsal eczema.[19,20]

2.1.1.2 Temporal and morphological classifications

Acute HE can be defined as HE with a duration of less than three months and with under two eruptions per year. Chronic HE (CHE) can be defined as eczema with a duration of three months or more or with two or more eruptions per year.[21] Other terms such as persistent, recurrent, intermittent, and cyclic HE are also used to describe the temporal course of HE.[20] Often, acute eczema is morphologically characterized by erythema, oedema, papules, oozing, and vesicles whereas chronic eczema is characterized by xerosis, lichenification, fissures, and scaly skin. However, the morphology of HE can vary over time and mixed, polymorphous presentations are often seen.[19,20]

In 2011 the Danish Contact Dermatitis Group (DCDG) characterized six clinical HE subtypes.[20] This classification was based on the clinical examination of HE and incorporated both the morphology, temporality, and anatomical localization of HE. Figure 2 shows photos of the different clinical subtypes. This classification was used in the clinical study in this thesis and thus described in brief here:

Chronic fissured HE presents with dryness, scaling, possible hyperkeratotic areas, and fissures but only a limited number of vesicles, Figure 2a.

Recurrent vesicular HE presents with recurrent vesicular eruptions typically on the sides and palmar aspects of palms and fingers. The intervals between eruptions varies and can be so frequent that the eczema presents as chronic, Figure 2b.

Nummular HE refers to well-circumscribed, coin-like, lesions characterized by erythema, keratosis, and vesicles, Figure 2c.

Hyperkeratotic palmar eczema refers to hyperkeratosis, with possible fissures but no vesicles. Lesions are well demarcated and located on the palms possibly extending to the fingers, Figure 2d.

Pulpitis refers to hyperkeratosis with possible fissures and occasional vesicles, located on the fingertips, Figure 2e.

Interdigital eczema refers to erythema and scaling, but rarely vesicles, located in the proximal areas of the interdigital spaces, Figure 2f.

The subtypes chronic fissured HE, and interdigital eczema have not been included in the newest consensus based recommendation on the classification of clinical subtypes from 2022, whereas the remainder four subtypes have.[1]

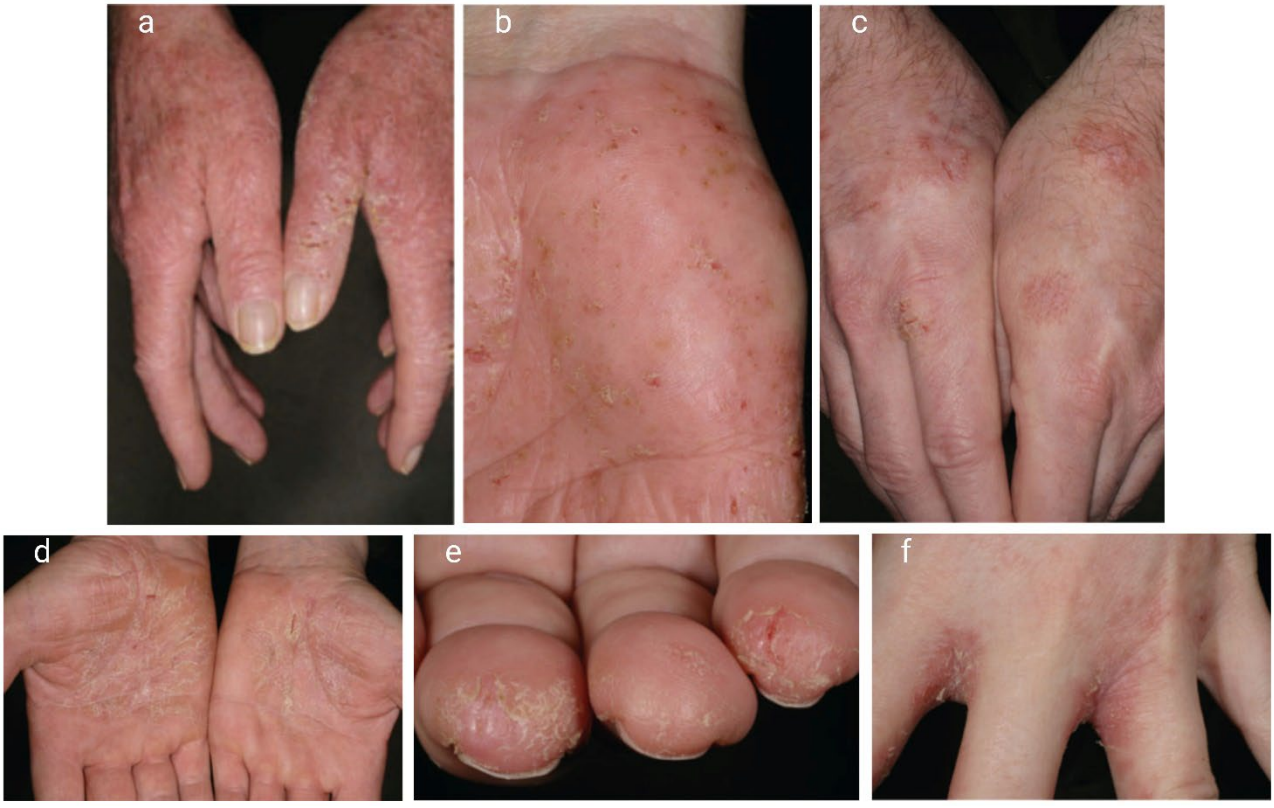

Figure 2. *Clinical hand eczema subtypes. Photos (a-f) are reprinted from Menné et al., 2011[20] with permission from John Wiley & Sons A/S.*

2.1.1.3 Aetiological classifications

Aetiological HE subtypes refer to the identifiable causes of HE. These include ICD, ACD, atopic HE, and, less commonly, protein contact dermatitis/contact urticaria. Mixed or unknown aetiologies are frequently encountered.[1,22] Aetiological HE subtypes cannot be distinguished from one another based on the clinical presentations of HE. The diagnostic work-up include clinical examination, and taking a thorough medical history, including information on family and personal history of HE, AD, asthma, allergic rhinitis, and psoriasis. Further, a thorough assessment and quantification of exposures to irritants, allergens, and proteins is performed, primarily from patient history and through review of product labels and safety data sheets. Based on these findings, further testing is planned, most commonly by patch tests and skin prick tests. The diagnostic criteria for the aetiological HE subtypes are summarized in **Table 1**, based on DCDG recommendations and ESCD HE guidelines.[1,20]

Etiological Subtypes	**Diagnostic Criteria**	**Additional Notes**
Irritant Contact Dermatitis	Diagnosed based on significant exposure to irritants correlating with onset or exacerbation of HE.	Common irritants are wet work, glove use, detergents, and mechanical friction. ICD is an exclusion diagnosis, ACD in particular should be ruled out.
Allergic Contact Dermatitis	Diagnosed with a positive patch test reaction and ascertained or qualified exposure to the contact allergen on the hands.	Patch testing is the gold standard for diagnosing ACD and should be performed in accordance with ESCD patch test guidelines.[23]
Atopic Hand Eczema	Diagnosed mainly based on personal history of atopic dermatitis.	Often dorsal eczema and often complicated by other aetiologies, especially ICD.
Protein Contact Dermatitis	Diagnosed based on a history of immediate skin reaction to a source of protein and a positive skin prick-test.	Less common; not discussed in detail in this thesis.

Table 1. *Diagnostic criteria for aetiological hand eczema subtypes. Allergic contact dermatitis, ACD; European society of contact dermatitis, ESCD; hand eczema, HE; irritant contact dermatitis, ICD. The table is based on HE guidelines from the DCDG and the ESCD[1,20]*

All aetiologies should be considered uniquely or combined. To obtain a unique aetiological sub diagnosis of HE, other contributing aetiologies should be excluded. For example, to obtain a unique ICD diagnosis, ACD should be excluded through patch testing. If the HE cannot be categorized within the above-described groups, it is categorized as 'aetiologically unclassifiable HE'.[1,20]

HE can also be classified into major subgroups based on AD status (e.g., HE with or without concomitant AD at other body sites, or with or without a history of AD).[17] This method allows for easier and more uniform patient stratification across studies but overlooks any variability inherent in aetiological subtypes of HE. Some distinguish between exogenous (ICD, ACD, protein contact dermatitis) and endogenous HE (atopic, hyperkeratotic, and recurrent vesicular HE).[2] This distinction was not employed in the studies included in this thesis.

2.1.1.4 Severity

The severity of HE can be assessed through clinical examination and self-assessments using different instruments. An instrument often used to assess the clinical severity of HE is the Hand Eczema Severity Index (HECSI).[24] In the HECSI, clinical signs such as erythema, infiltration, and vesicles, and the extent/area of eczema is graded separately for five different anatomical locations: fingertips, fingers, palms, dorsa, and wrists. The sub scores from each location is added up to obtain the total HECSI score which varies from a minimum of 0 to a maximum of 360 points.[24] Severity strata based on these scores are: mild (almost clear), 1-16; moderate, 17-37; severe, 38-116; very severe ≥117.[25]

A photographic grading system including 16 photographs in the above described four severity groups, the photographic guide, can be used for self-assessment of HE severity.[26,27] HE severity can also be subjectively assessed on a visual analogue scale (VAS). In a validation study, Hald et al. found good agreement between the dermatologist graded clinical severity and patient reported severity on the photographic guide but only a moderate correlation between dermatologist rated severity and selfreported VAS scores.[27]

2.1.1.5 Other classifications

HE can be classified as work related (or occupational) or non-work related.[2] Danish physicians are obliged to report suspicion of occupational HE to the Danish Labour Marked Insurance. Occupations at high risk for occupational HE include hairdressers, beauticians, bakers, and

dentists.[4,7] Finally, HE can be present with- or without concomitant foot dermatitis or with or without bacterial infection, most commonly caused by *Staphylococcus (S.) aureus*.[2]

2.1.2 Epidemiology

To accurately estimate the prevalence and other epidemiological endpoints associated with HE, factors such as the chosen population (general population, hospital patients, specific occupational groups), country, geographical region, and the sex and age of the individuals involved should be considered.[19] Moreover, the methods used to assess the various endpoints should also be taken into consideration. Results from general population studies are considered more generalizable to the wider society than those from selected populations.[9]

2.1.2.1 General population questionnaire studies

Numerous studies have investigated the epidemiology of HE in the general population. Most of these studies originate from Nordic countries and use questionnaire data obtained from a representative population sample to assess epidemiological HE outcomes, mainly prevalence.[3,9] The nationwide registries in the Nordic countries, which include personal identity numbers and demographic details, facilitate the acquisition of these random or representative samples from the general population.[28] In Denmark, such information can be obtained from the central person register (CPR).[29]

Although termed general population studies, many studies report on samples from specific geographical areas, typically in or around larger cities. Only a limited number of studies/surveys have reported results from nationwide samples.[30–32] Historically, most studies have used paper-based questionnaires and more recent studies employ web-based questionnaires, or mixed methods.[30,31,33] The response rates of general population HE studies seem to have been decreasing over the years, although this issue has not been systematically approached in the research. Where response rates over 50% could be expected 10-15 years ago[33,34], most recent web-based epidemiological studies in Denmark and neighbouring countries report response rates ranging from 35-45%.[30,31] Similarly, a recent questionnaire study from the Netherlands reported a response rate of 42.8%.[35]

Many studies use HE related questions from the Nordic Occupational Skin Questionnaire 2002 (NOSQ-2002)[36] and validated questions to assess the prevalence of HE.[3,9]

Meding and Barregård examined the validity of self-reports of a 1-year prevalence of HE in a population of Swedish dentists, office workers, and mechanics and found a low sensitivity (53-59%) and a high specificity (96-99%). The question used was "Have you had HE on any occasion during the past 12 months?".[37] Bregnhøj et al. examined the validity of self-reports of a point prevalence of HE in a cohort of young hairdressing apprentices using questions adapted from the NOSQ-2002 and found a moderate sensitivity (70.3%) and high specificity (96.3%).[38] Similarly, Yngveson et al. reported comparable sensitivity (73%) and specificity (99%) estimates for a self-reported point prevalence of any hand dermatosis among Swedish school children.[39] The selfreported lifetime prevalence of HE has not been previously validated. The prevalence of selfreported HE assessed through slightly different questions has been reported to be comparable to the validated questions.[3,9] By contrast, the selfreported prevalence of a physician based diagnosis of HE is much lower than the prevalence assessed through validated questions, suggesting an even lower sensitivity.[3,37]

2.1.2.2 Prevalence

Evidence of the prevalence and incidence of HE, alongside other epidemiological endpoints in the general population, was compiled in a review in 2010[9], and this review was updated through a systemic review and meta-analysis in 2021.[3]

In the meta-analysis, 66 studies including over 500,000 unselected individuals were included in analyses, and the pooled estimates of total self-reported point-, 1-year and lifetime prevalences among adults were 3.4%, 9.7% and 15.6%, respectively.[3] The total pooled adult incidence rate was 7.5 cases/1000 person years based on data from 8 studies. All prevalence and incidence estimates were 1.5-2 times higher in females than males, and the self-reported pooled point prevalence was similar to the clinically determined pooled point prevalence (3.5% vs 4.0%), suggesting good validity selfreported current HE prevalence. No clear time trends in HE prevalence was found but the incidence of HE appeared to have increased in more recent years (1964-2007 vs 2008-2019).[3] Only a few studies reported prevalence and incidence estimates of children and adolescents and the pooled estimates for adults were not further stratified by age nor by geography due to lack of data.[3] Importantly, the incidence of HE has been shown to peak among young women and decrease with age.[40] Evidence of HE prevalence outside of the Nordic countries and in particular outside of Europe is sparse.

2.1.2.3 Severe and chronic hand eczema

Only a few studies have assessed the severity of HE in a general population setting, some through clinical scoring of current HE using different tools,[41–44] and some using self-reports of severity at worst, at current and/or averagely the past year.[30,33,35,44] Although the estimates vary some between studies, the majority of cases with current HE are found to be mild and roughly one third are moderate-very severe.[3] Prevalence estimates of severe and very severe current HE remain uncertain due to small case populations in many studies.[3] A larger Dutch study performed in 2020 found that most adults with HE in the past year self-reported mild HE (75.1%), followed by moderate (22.3%), severe (2.3%), and very severe HE (0.4%).[35] A Danish study on adolescents performed in 2021 reported higher severity levels: 58.4% mild, 34.5% moderate, 6% severe, and 1.1% very severe.[30] Both studies reported on the average HE severity the past year as assessed by the photographic guide. The same two studies are the only to have reported the prevalence of CHE defined as HE that lasts for more than three months or recurs more than once during the past year. In the Dutch study, 63.8% with HE in the past year had CHE and in the Danish study a similar estimate of 62.6% was found.[30,35]

2.1.2.4 Health-related quality of life

It is well-known from studies examining selected patient and occupational populations that HE has a profound impact on health related quality of life (HRQoL), particularly in chronic and severe cases.[45–48] However, the large proportion of individuals who do not seek medical attention for their HE and who are not employed in particular occupations are not included in these studies, making it difficult to generalize the results to the population level.[33,49] Three Swedish studies performed in 1990[50], 2009[34], and 2011[51] have examined the HRQoL of individuals with HE in general population settings. One study found that 80% of those with HE had experienced negative emotional and social consequences related to their HE.[50] The two latter studies employed the generic HRQoL instruments EuroQol-5D and Short Form 36 and both studies found that the HRQoL was lower in individuals with HE than in those without.[34,51]

The Quality of Life in Hand Eczema Questionnaire (QOLHEQ) is a validated instrument used to assess disease specific HRQoL in individuals with HE.[52,53] This means that the instrument specifically assesses impairments caused by HE. The Danish version of the QOLHEQ has recently been validated.[54] This version consists of 30 questions/items that are grouped in the following four

domains or subscales: symptoms, treatment and prevention, emotions, and functioning, as shown in Figure 3.[55] Individuals are asked how often they have been bothered by their HE in various situations in the past week. Scores from each domain are combined in a total score which ranges from 0-117, and high scores indicate strong HRQoL impairment.[54] The interpretability of international QOLHEQ scores have been proposed by Osterhaven et al.[56] No Danish interpretability study has been performed.

The QOLHEQ has been employed in selected populations[57–59], but so far not in a general population setting.

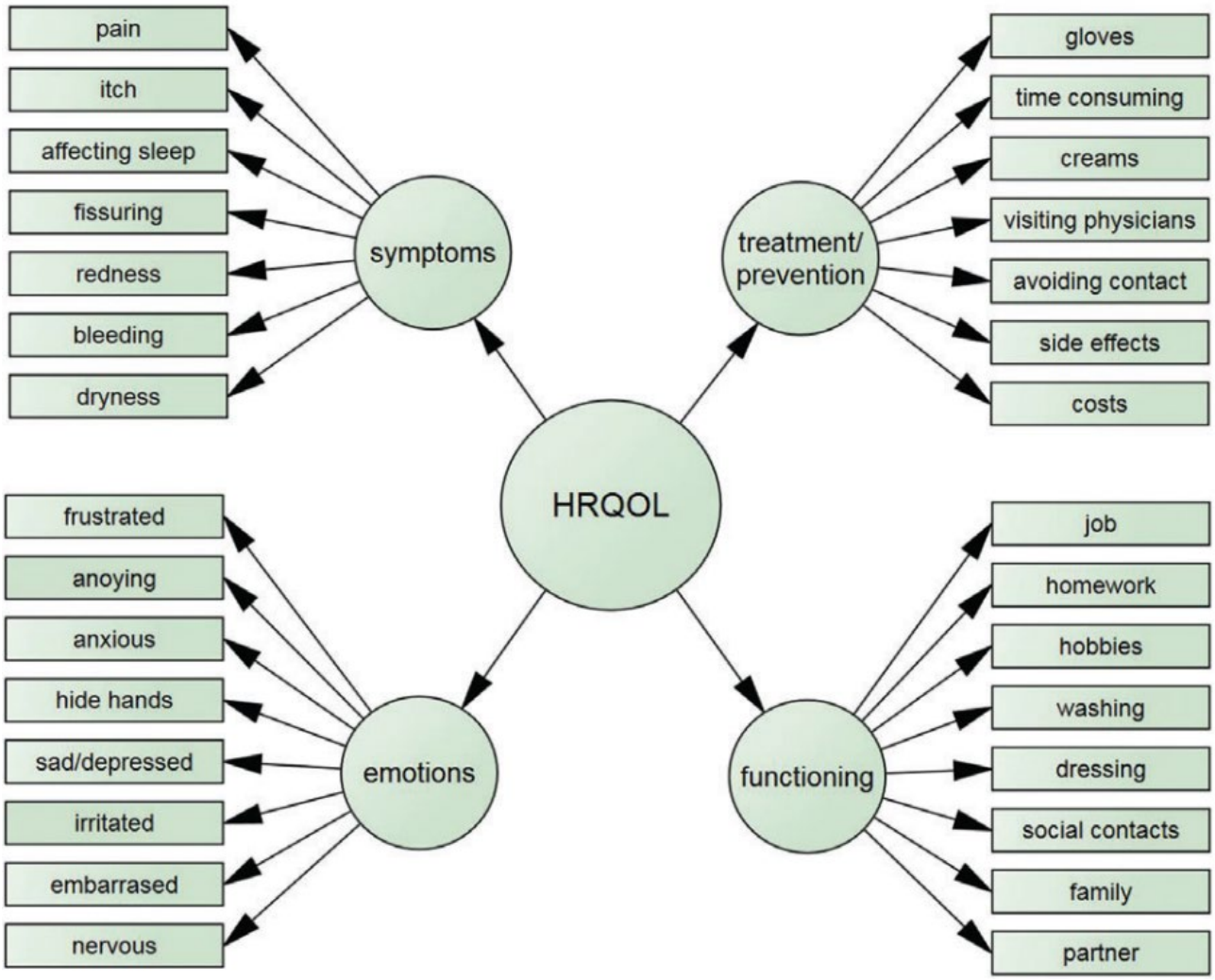

Figure 3. *Overview of the four domains in the Quality Of Life in Hand Eczema Questionnaire (QOLHEQ). Figure published by Oosterhaven et al, 2017 in Contact Dermatitis[55]. Used with permission from John Wiley & Sons A/S. Health Related Quality of Life, HRQoL.*

2.1.2.5 Socioeconomic burden

The socioeconomic burden of HE is considered to be substantial mainly due to direct and indirect costs related to sick leave, presenteeism, job loss, job change, and treatment as summarized in a recent review.[8] The general population studies included in this review reported high sick leave prevalences because of HE, but none of these studies compared sick leave (of any cause) between individuals with and without HE.[8] Hald et al. examined medical attention seeking behaviours in unselected Danish individuals with HE and found that 67% had consulted a general practitioner and 44% a dermatologist because of their HE. Having consulted a medical doctor was associated with having severe HE. Many individuals, including those with severe HE, were not referred to a dermatologist, which might negatively affect the prognosis of HE.[33] The study by Hald et al. was performed in 2006 and updated evidence on medical attention seeking behaviour among unselected individuals with HE in Denmark and other countries is lacking.

The reasons why individuals with HE do not seek medical attention and continue working while affected by HE (presenteeism) are not fully understood.[8,33] Further, the personal consequences and indirect societal costs associated with this are also unclear. It has been suggested that HE is often seen as a minor issue.[60] Therefore, increasing awareness about its substantial personal and occupational effects, along with more comprehensive education on managing the disease for the general public and healthcare professionals, is essential.[60] To this end, identifying high-risk groups at the population level could aid in targeting such measures.

2.1.3 Risk factors

Previous or current AD is a strong individual risk factor for the development of HE.[61,62] Filaggrin (FLG) gene mutations in individuals with AD are associated persistent and severe HE whereas no clear association between FLG mutations and non-AD HE have been found.[19,63,64] Results from a Danish population-based twin cohort including both monozygotic and dizygotic twins suggested that unrecognized genetic risk factors, independent of AD, could explain the development of HE.[65] However, Genome-Wide Association Studies (GWAS) on HE are lacking. Environmental risk factors include contact with irritants and allergens.[1,19] The strong association between female sex and HE has generally been attributed to sex differences in exposure to irritants and allergens, both in domestic and occupational settings.[9,66]

2.1.4 Treatment

For years, the management toolbox for HE has been somewhat static with limited active treatment options. The existing standard therapy for HE includes emollients, patient education on protective and preventive measures such as avoidance of triggers and use of gloves.[1] Recommended active treatments include topical corticosteroids, calcineurin inhibitors, and phototherapy. Conventional systemic agents such as alitretinoin and off label use of methotrexate, cyclosporine, and azathioprine are considered in severe and topical treatment resistant cases.[1]

Recent advances in AD treatment, particularly with biologics and small molecules, have revolutionized its management, and new AD targets are continually emerging.[66] Although the pathogenesis of HE remain elusive, as described below, some of these novel AD treatments have demonstrated a successful cross-over in treating HE.[67]

Evidence from two recent phase II trials showed that systemic targeting of interleukin (IL)-4/IL-13 with dupilumab, and broader pathway inhibition with the oral Janus Kinase (JAK)/spleen tyrosine kinase inhibitor, gusacitinib, were efficacious in treating severe CHE without concomitant AD.[57,68] Another recent phase II trial showed the topical JAK inhibitor delgocitinib to be effective in treating CHE.[69] Further, studies have also shown IL-4/IL-13 inhibitors and JAK inhibitors to be effective in treating CHE in patients with concomitant AD.[58,70] Investigations of several other novel HE therapies are ongoing, and new targets are likely to emerge based on findings from future molecular HE research and the understanding gained from studies on AD.[67]

None of these newer treatments have been approved for the treatment of HE without concomitant AD and evidence on efficacy as well as on safety in larger phase III trials are yet to be published. Nevertheless, it appears likely that these or other novel treatments will be added to the HE management toolbox in the foreseeable future.

2.2 Atopic dermatitis

AD is an inflammatory skin condition characterized by intense pruritus and chronic or recurrent eczematous lesions.[71] AD most often begins in early childhood and affects around 15-20% of children in western countries.[71] Most individuals outgrow their AD, yet for others the disease persists into adulthood. Additionally, it is also possible to have first onset of AD in adulthood. The point prevalence of AD among adults in Western countries is estimated to be around 5%.[72] This thesis encompasses research on adults; therefore, the subsequent sections will specifically address adult AD. The pathophysiology of AD is complex and further described in a subsequent section of this thesis. Well established risk factors for AD development include parental history of atopy, and FLG gene mutations.[71] However, many individuals with FLG gene mutations do not develop AD, and as such the prevalence of a FLG gene mutation is not diagnostic for AD.[73]

AD is associated with other atopic comorbidities including allergic rhinitis, asthma, and food allergies.[74–76] Individuals with AD experience decreased quality of life and AD has also been associated with non-atopic comorbidities such as psychiatric, ocular and infectious diseases, particularly in moderate-severe disease.[77,78] In accordance, the personal and socioeconomic burdens of the disease are vast.[79] In adults, AD lesions are most often chronic and localized to the face, neck, flexures, or hands.[71] In a meta-analysis, patients with AD were found to have a 3-4 fold increased prevalence of HE as compared to controls.[80] In another meta-analysis, it was found that one third of unselected individuals with HE had a history of AD.[3]

AD is diagnosed through medical history and clinical examination; no valid diagnostic biomarkers exist. The Hanifin and Rajka criteria are often used as a diagnostic tool.[81] They consist of four major and 21 minor criteria of which 3 major and 3 minor criteria must be fulfilled to obtain a diagnosis of AD.[81] The major criteria are as follows: pruritus, typical morphology and distribution of eczema (flexural eczema lichenification in adults), chronic or relapsing eczema, and personal or family history of atopy.[81] The severity of AD can be clinically assessed by the Eczema Area and Severity Index (EASI), which grades percentage of affected skin areas, and the intensity of redness, thickness and lichenification across different body regions (head and neck, trunk, upper- and lower limbs). The total score ranges from 0-72 with higher scores indicating high severity.[82]

The Patient-Oriented Eczema Measure (POEM) represents a tool that is often used to assess the severity and impact of AD from a patient perspective.[83]

2.3 Immunology and biomarkers of eczema

The skin is the largest human organ, and our skin barrier functions through a dynamic and complex way to maintain the body's homeostasis and shield and protect the 'inside body' against outside agents such as microorganisms, chemicals, and physical factors.[84,85] Structurally, the skin barrier is divided into the outermost skin layer, the epidermis, and the inner skin layer, the dermis, and the epidermis is further subdivided into four strata, or layers as depicted in Figure 4.[85] Functionally, the skin barrier can be divided into a microbial, a chemical, a physical and an immunological barrier, Figure 4. These skin barrier types are interconnected and cross-talk in the skin's response to contact with exogenous stressors, i.e. irritants and allergens.[85]

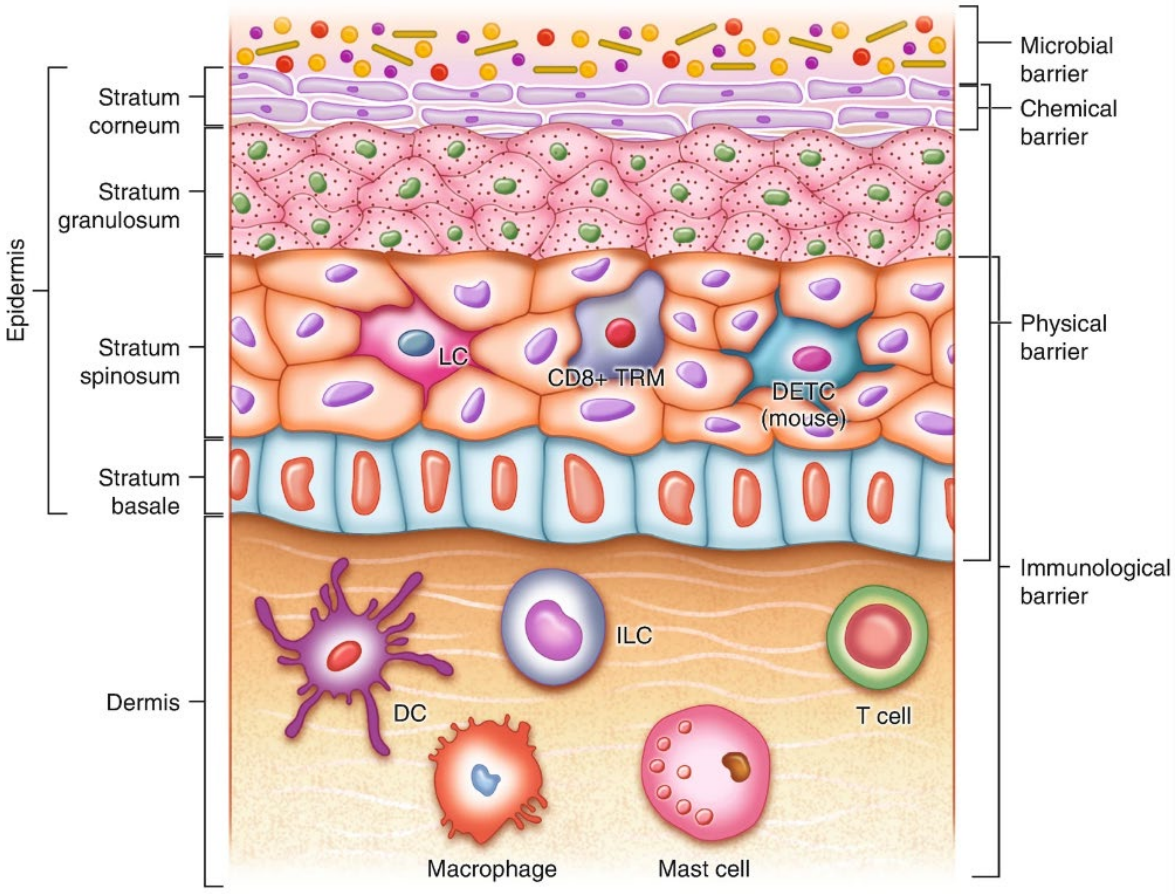

Figure 4. *The skin barrier. Reprinted from S. Martin and C. Bonefeld, published in Contact Dermatitis sixth edition vol. 1*[85]*, with permission from Springer Nature. Dendritic cells, DC, dendritic epidermal T cells, DETC (only in mice); innate lymphoid cells (ILC); tissue-resident memory CD8⁺ cells, CD8⁺ TRM.*

The skin inflammation seen in eczema can occur when the integrity of the skin barrier is compromised, and some of these defence mechanisms are (over) activated. The way the skin's defence mechanisms are activated and the immunological response that follows are complex and can be dependent on the trigger. For example, different mechanisms and immunological responses are seen for ICD and ACD.[85] Furthermore, some individuals have an inherently impaired skin barrier e.g., those with loss-of-function mutations in the FLG gene.[71]

2.3.1 Allergic- and irritant contact dermatitis

ACD and ICD are the two most frequent causes of contact dermatitis, and the most common clinical presentation of contact dermatitis is on the hands. Research into immunology and biomarkers of contact dermatitis primarily derive from mouse models and human studies examining patch test reactions to allergens and irritants.[85–87]

ACD, or contact allergy, is a delayed hypersensitivity reaction. It is characterized by an immunologic T-cell mediated reaction to a contact allergen that penetrates the skin. However, other cell types such as innate lymphoid cells, dendritic cells, neutrophils, and mast cells are also involved in ACD.[85,86] There are two phases of ACD: the sensitization phase and the elicitation phase. In the sensitization phase, the immune system encounters the contact allergen for the first time. No visible eczema is seen in this phase. In response to the allergen penetrating the skin, an innate immune response as well as stress responses are induced. This results in activation of keratinocytes, resident immune cells, fibroblasts, as well as recruitment of immune cells from the blood. Dendritic cells migrate to the skin draining lymph node and present antigens to naïve T-cells. This induces proliferation and differentiation of allergen-specific T-cells into effector and memory T-cells. These T-cells can migrate from the lymph node to the skin, where they can persist as tissue resident memory T-cells. In the elicitation phase, re-exposure to the same allergen can activate dendritic and Langerhans cells that in term may activate the now present tissue resident memory T-cells. The activated tissue resident memory T-cells secrete pro-inflammatory cytokines and recruit other inflammatory cells to the skin site. The elicitation phase occurs hours to days after re-exposure and the associated inflammatory response clinically manifests as eczema.[85,86]

ACD is considered a Th1-cell dominated disease. However, the specific immune polarization can depend on the allergen.[86] For example, fragrance, and rubber have been shown to induce a Th2-cell

dominated response as opposed to a more Th1/Th17 dominated immune response in nickel allergy.[88,89] Furthermore, continuous activation of the innate immune system is seen in ACD.[86]

ICD is induced by physical or chemical damage of the skin barrier and is primarily driven by innate immune responses and not as ACD adaptive T-cell mediated immune responses.[85] Upon skin damage, an inflammatory response is initiated that is mediated by skin cells such as keratinocytes as well as resident innate immune cells. Pro-inflammatory cytokines released by keratinocytes include IL-1α, IL-1β, and tumor necrosis factor α (TNF).[90] This inflammatory response clinically manifests at as eczema at the exposure site.[85]

There are currently no valid biomarkers to discriminate ACD from ICD and patch testing remains the gold standard for an ACD diagnosis.[86,87] Several human patch test studies have examined samples from irritant and allergic patch test reactions using tape strips[89,91] or biopsies.[92–94] One study using tape strips suggested IL-16[89] and another loricrin[91] as discriminating biomarkers between ACD and ICD. Lefevre et al. performed transcriptomic profiling of skin biopsies collected from irritant and allergic patch test reactions. Using machine learning, they found that a combination of skin biomarkers including chemokine (C-X-C motif) ligand (CXCL) 9-10 and ZBP1 could discriminate ACD from ICD.[93] Fortino et al. also investigated transcriptomic profiles of biopsies collected from irritant and allergic patch test reactions and suggested CD47, BATF, FASLG, SELE, and IL-37 as biomarkers with a diagnostic relevance to distinguish ACD from ICD.[94] The biopsies in the two studies were collected at different time points.[93,94] More research is needed into biomarkers of ACD and ICD, including validation of biomarkers across studies and in clinical contact dermatitis populations, e.g. in HE patients.[86]

Research into systemic, blood biomarkers of contact dermatitis is scarce, but ACD has previously been associated with systemic immune activation characterized by increased serum levels of different inflammatory proteins in clinical populations.[95,96] No study has yet examined systemic biomarkers of ACD or ICD on the hands.

2.3.2 Atopic dermatitis

The pathophysiology of AD is complex and still not fully understood. It involves genetic susceptibility, skin barrier dysfunction, environmental factors, microbiome abnormalities and a dysregulated immune system.[71]

Two different hypotheses on AD pathogenesis have been suggested, the 'outside-in' hypothesis, where skin barrier dysfunction triggers immune activation, and the 'inside-out' hypothesis, where immune dysregulation is primary and skin barrier dysfunction secondary. However, current research emphasizes the integration of both processes as equally important.[97,98]

The dysfunctional skin barrier in AD is characterized by low expression of epidermal barrier markers, for example deficiency of FLG, as well as lipid impairment, elevated skin PH, and trans epidermal water loss.[71,90] This makes AD skin more susceptible to external agents that can trigger an inflammatory response. Furthermore, physical skin damage by scratching can further impair the skin barrier and attenuate an inflammatory response, resulting in an itch-scratch cycle.[99]

The dysregulated AD immune system involves alterations of both adaptive and innate pathways. AD is primarily, but not exclusively, a Th2 driven disease.[71,100] Epidermal barrier disruption triggers the release of pro-inflammatory mediators from keratinocytes. This activates innate immune cells and initiates a Th2 inflammatory response. This response is characterized by upregulation of the Th2 cytokines IL-4, IL-5, and IL-13 that play a key role in driving the inflammatory response in AD.[98] Th2 cytokines can promote production of Immunoglobulin E (IgE) and induce or exacerbate pruritus through communication with cutaneous sensory neurons.[71] Additionally, the Th2 inflammatory response further diminishes skin barrier function and affects the skin microbiome composition.[101]

Besides Th2 activation, Th22, Th1, and Th17 pathways are also activated dependent on the AD endophenotype.[98,102] Namely, immune pathway activations in AD can be dependent on factors such as disease duration, age at onset, and ethnicity. Acute AD is mainly Th2/Th22 dominated, while chronic AD primarily involves Th1 activation. Moreover, elderly AD patients show increased Th1 and Th17 pathway activity and reduced Th2/Th22 activity compared to younger patients, and Asian patients show a more Th17 skewed immune profile in comparison to European American patients.[103]

Moderate to severe, but not mild, AD has been associated with systemic inflammation characterized by high circulating levels of inflammatory and cardiovascular disease (CVD) associated proteins.[104–107] The skin has been suggested as the likely source for these upregulated markers[106], but this has not been well-established.

Several studies have investigated biomarkers of AD as summarized in two recent reviews.[108,109] Chemokine (C-C motif) ligand (CCL) 17, a chemoattractant of Th2 cells, is established as the AD biomarker that shows the greatest correlation with clinical AD severity (in blood). Nevertheless, elevated CCL17 levels (skin and blood) are not AD specific and have also been associated with other diseases. In a review by the International Eczema Council, several other biomarkers showed to correlate with AD severity with overall high evidence for biomarker generalizability (e.g., CCL22, LDH, IL-18, E-selectin). Further, blood levels of CCL17 and skin levels of CCL13, IL-13 and CCL22 showed moderate to high evidence as biomarkers for clinical improvement in longitudinal systemic treatment studies.[109] With the introduction of a variety targeted therapeutics, current research focuses on the stratification of AD patients based on the key molecular drivers of their disease (endotype) to enable a more personalized approach.[108]

2.3.3 Hand eczema

A limited number of studies have examined the molecular profile of clinical HE. Some by use of skin biopsies and some by use of tape-strips or skin scrapings, as summarized in Table 2 and Table 3, respectively. Eight studies have employed broader molecular investigations of HE by proteomic or transcriptomic profiling methods or by incorporating a higher number of selected markers.[10–17] Collectively, these studies primarily point to a dysfunctional skin barrier in HE. However, newer studies also point towards a dysregulated immune system and a shared molecular profile between CHE and AD. Nevertheless, the study populations, sampling, and profiling methods between studies are heterogenous and no valid HE biomarkers exist that can distinguish between subtypes.

In 2015 Molin et al. employed mass spectrometry to profile the proteome of palmar skin biopsies obtained from six CHE patients and six healthy controls.[10] The authors found 185 barrier proteins to be differentially expressed in CHE versus controls. The results indicated an important role of skin barrier dysfunction in CHE. In 2016, Kumari et al. investigated the gene expression of various selected barrier genes as well as the cytokine TSLP in biopsies from 15 CHE patients before and after treatment with oral alitretinoin. The authors found a dysregulation of barrier markers, and

upregulation of TSLP before treatment. The findings were normalized with decreased clinical severity (post treatment).[11] These results supported the role of skin barrier dysfunction in CHE but also showed that this could be reversed through treatment. In 2021, Voorberg et al. profiled the transcriptome of lesional, non lesional and healthy palmar epidermal biopsies collected from 10 patients with vesicular HE and 10 healthy controls.[12] The authors found increased expression of markers involved in keratinocyte host defence, as well as immune signalling genes, although with low expression levels, in lesional HE skin. Further, several markers involved in epidermal differentiation were dysregulated. The authors compared their results with earlier reported AD transcriptomic profiles and noted a large overlap.[12] These results indicated a common pathophysiology between vesicular HE and AD. Samples from the same participants were recently reanalysed in a study that also incorporated transcriptional data from non-palmar full-thickness biopsies from AD patients.[16] The authors found primarily shared transcriptional profiles between vesicular HE and AD but also noted differences, such as more prominent interferon signalling in vesicular HE than in AD.[16]

Reference (year)	Population and samples	Profiling methods	Findings
Molin et al. (2015)[10]	6 CHE patients, 6 healthy controls, palmar skin biopsies. Validation by immunohistochemistry in biopsies from 7 different patients and 7 different controls.	Mass spectrometry, immunohistochemistry of selected markers	CHE vs healthy: 185 skin barrier proteins differentially expressed Skin barrier dysfunction in CHE.
Kumari et al. (2016)[11]	15 CHE patients, biopsies before and after oral alitretinoin treatment	Real-time quantitative polymerase chain reaction (qPCR) and immunohistochemistry Profiling of selected barrier markers and TSLP	Lesional CHE: Dysregulation of barrier markers, upregulation of TSLP before treatment. Normalization of findings post treatment with decreased clinical severity.
Voorberg et al. (2021)[12]	10 vesicular HE patients, 10 healthy controls, palmar epidermal biopsies	Transcriptome profiling (RNA-seq), real time qPCR of selected markers	Lesional CHE vs healthy: Increased expression of keratinocyte host defence, immune signalling genes (at low expression levels), dysregulation of epidermal differentiation markers. No non-lesional abnormalities. Overlap with AD transcriptomic profiles.
Politiek et al. (2016)[110]	7 hyperkeratotic HE patients, 2 healthy controls. Palmar skin biopsies	Immunohistochemistry of selected barrier proteins Genomic profiling (blood) of mutations in 135 palmoplantar keratoderma genes	Lesional hyperkeratotic HE vs healthy: Downregulation of K9, K14. Upregulation of K5, K6, K16, K17. Filaggrin expression similar in lesional, perilesional and control skin. No monogenetic mutations found.
Wang et al. (2018)[111]	30 patients with moderate-severe CHE, 30 healthy controls. Biopsies from 6 patients and 5 controls.	Measurements of pH, and TEWL in all. Immunohistochemical profiling of caspase-14 in 6 patients and 5 controls.	Lesional CHE vs healthy: increased pH value, decreased water content, impaired integrity of the stratum corneum. Decreased expression of caspase-14.
Rosenberg et al. (2023)[16]	Same participants as.[12] Data from non-palmar full thickness biopsies from AD patients and healthy controls from other studies were reanalysed.	Transcriptome profiling (RNA-seq)	Primarily shared lesional transcriptomic profiles between vesicular HE and AD. Interferon signalling and necroptosis were more prominent in vesicular HE than AD.

***Table 2**. Molecular hand eczema profiling studies (skin biopsies). Atopic dermatitis, AD; Chronic hand eczema, CHE; hand eczema, HE; trans epidermal water loss, TEWL.*

Sølberg et al. examined the transcriptome and proteome of HE by use of tape strips.[14,15] The participants included in these studies were sub-samples from the clinical study population characterized in this thesis. These studies showed that both the transcriptome and the proteome of HE could be assessed through tape-stripping.[14,15] The transcriptomic investigations showed upregulation of several immune signalling markers in lesional CHE skin, with no correlation between these markers and clinical severity.[14] Comparisons between subtypes categorized by AD status, unique aetiology, and clinical subtype revealed some differences. No differences were noted between palmar and dorsal samples. The proteomic investigations also showed upregulation of several immune signalling as well as skin infection proteins and downregulation of epidermal barrier proteins. Further, differences between palmar and dorsal samples were noted.[15] Tauber et al. profiled various selected markers from both blood and skin (through skin scrapings of dorsal CHE) in 2020. Through unsupervised latent class analyses, CHE patients were categorized into two groups with one characterized by increased skin barrier impairment, severity, and bacterial colonization and the other with opposite characteristics.[13] Most recently, Bar et al. presented findings (unpublished) on the transcriptome of CHE profiled from tape strips collected from CHE patients with and without AD. They characterized upregulation of genes associated with several immune pathways: Th1, Th2, Th17/22. Further, they found that the lesional molecular CHE profile was shared between patients with and without concomitant AD.[17]

Other studies have found skin microbiome alterations in HE with increased colonization rates and bacterial density of S. *aureus* being associated with increased clinical severity of HE.[18,112–114]

Some studies have profiled selected epidermal barrier markers in hyperkeratotic HE with the results indicating a larger resemblance to psoriasis than other HE subtypes.[115–117]

It is unclear if HE is associated with systemic immune activation, as circulating HE biomarkers have not been investigated.

Reference (year)	Population and samples	Molecular evaluations	Findings
Tauber et al. (2020)[13]	71 CHE patients Skin scrapings from dorsal HE skin by use of a micro abrasive tool Skin swabs Blood samples	Blood: total IgE, FLG gene mutations. Skin scrapings, RT-qPCR: selected skin barrier and immune signalling genes. Skin swabs: IL-8, S. Aureus, S. epidermidis TEWL	Latent class analysis categorized patients into a group with high severity, high barrier impairment, high s. aureus colonization, high IL-8 and a second group with opposite characteristics.
Sølberg et al. (2021)[14]	30 CHE patients, 16 healthy controls Tape-strips, palmar and dorsal	RNA-seq	Lesional CHE vs healthy: upregulated immune signalling markers. No correlations with clinical severity. No difference between dorsal and palmar CHE. Comparisons between CHE with and without AD: largest difference in NL skin areas, downregulation of CXCL8 in CHE without AD. Difference between ACD and ICD CHE: 6 markers
Sølberg et al. (2023)[15]	34 CHE patients, 16 controls Tape strips palmar and dorsal	Mass spectrometry	Identification of 2919 stratum corneum proteins from tape strips Differences between dorsal and palmar samples Lesional CHE skin vs healthy: upregulated immune signalling and skin infection proteins, downregulated barrier proteins.
Bar et al. (2023)[17], conference abstract	95 CHE patients, 45 of these with concomitant AD, 20 controls. Tape strips (L, NL, N)	RNA-seq	Lesional CHE vs healthy: Upregulation of genes associated with Th1, Th2, Th17/22. No difference between CHE with or without AD. Positive correlations with immune markers and severity.

Table 3. *Molecular hand eczema profiling studies (tape strip and skin scrapings). Atopic dermatitis, AD; Chronic hand eczema, CHE; hand eczema, HE; trans epidermal water loss, TEWL.*

2.4 Molecular profiling of eczema

The molecular profiling of eczema involves examining various biomolecules in different tissues, with skin and blood being the primary focus. This profiling aims to understand the molecular mechanisms underlying eczema at different biological levels such as: DNA, RNA, and proteins. Knowledge on genetic mutations associated with eczema can be gained from DNA investigations. Profiling of RNA provides information gene-expression and activity, and protein analyses investigates the functional proteins, providing a direct measure of the biological processes affected.

1. **Tissue Selection for Profiling**:
    - **Skin**: Provides insights into local molecular changes and immune responses.
    - **Blood**: Reflects systemic changes and immune responses, offering a broader view of the body's reaction to eczema.
2. **Molecular Profiling Methods**:
    - **'Omics' Approaches**: These methods provide a comprehensive view of the molecular components within a tissue. An example is whole transcriptome sequencing, which is used to analyse the full range of RNA transcripts in a sample.[14]
    - **Targeted Profiling**: Focuses on specific, selected markers within a tissue. The selection of these markers can be more or less explorative. An example is targeted proteomics, where a wide range of proteins thought to be relevant to eczema is investigated.[107] Another example is targeted genomic profiling of FLG gene mutations that are known to be associated with AD.[13]

3. Thesis Objectives

The overall aims of this thesis were 1. To investigate various epidemiological, HE associated endpoints in the adult, Danish general population, and 2. To investigate and characterize biomarkers and molecular patterns of HE and AD.

The specific aims of the manuscripts included in this thesis were:

- To examine the prevalence of HE in the Danish general population including the prevalence of severe and CHE. Furthermore, to examine and compare sick-leave prevalence and overall health perception between individuals with and without HE. Lastly, to examine the HRQoL as assessed by the disease specific QOLHEQ in unselected individuals with current HE (*Manuscript I*).

- To characterize the inflammatory plasma signature of patients with CHE without concomitant AD and of patients with more generalized AD and examine if these diseases were associated systemic inflammation. Further, to investigate if levels of circulating biomarkers were associated with the clinical disease severity of CHE without AD as well as in more generalized AD (*Manuscript II*).

- To characterize the inflammatory blood signature of different aetiological of clinical CHE subtypes. Further, to investigate potentially discriminating circulating biomarkers between subtypes (*Manuscript III*).

- To investigate and characterize the biopsied skin transcriptome of CHE and further investigate associated immune pathways and upstream regulators of the disease. Further, to investigate molecular differences between CHE subtypes categorized by AD status and unique aetiology (*Manuscript IV*).

4. Materials and Methods

This thesis is based on examinations of two separate study populations, and a short summary of the materials and methods employed is provided in this section, separately for the epidemiological questionnaire study (*Manuscript I*) and the clinical study (*Manuscripts II-IV*). Detailed descriptions of materials and methods are found in *Manuscripts I-IV.*

4.1 Manuscript I

4.1.1 Ethical approvals

The study received approvals from the Danish Data Protection Agency and the Danish Health Data Authority prior to start.[49] The study did not require approvals from the local ethics committee. It was highlighted to participants that any participation was voluntary.

4.1.2 Study population

This was a cross-sectional questionnaire study. A random sample of 100,000 individuals living throughout all of Denmark was drawn from the CPR. The sample was drawn at random but was restricted to adult individuals between 18-75 years with Danish citizenship and birthplace. Data obtained from the CPR encompassed civil registration numbers, sex, age, and municipality codes for all individuals. Municipality codes were used to group both responders and non-responders into the five Danish Municipality Groups: capital, metropolitan, provincial, commuter and rural municipalities.[49] In spring 2021, participants received an invitation to complete the electronic questionnaire via their personal electronic e-mail that were linked to civil registration number (eBoks), with a reminder sent to non-responders after two weeks.

4.1.3 Questionnaire

The questionnaire was constructed in Research Electronic Data Capture (REDCap) and consisted of questions on HE, AD, contact allergy, overall health measures, income and more. Participants who reported HE answered the specific QOLHEQ, self-reported their HE severity by the photographic guide and answered other HE specific questions. Validated questions were used to assess the prevalence of HE and AD. Other questions used were primarily taken from the NOSQ-2002 and some questions were added or modified.

4.1.4 Statistical analysis

A sample size of 33,670 participants was calculated prior to study start. A response rate of 35% was estimated and thus 100,000 individuals were invited to participate. IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Endpoints were computed excluding any missing answers. Differences between groups for categorical variables were assessed using Chi square tests and differences between groups for continuous variables were estimated both through parametric and non-parametric methods. QOLHEQ scores were calculated using a publicly available SPSS syntax created for this purpose and interpretation of QOLHEQ scores were based on international severity bands.[53,56] Binary logistic regression models were used to examine factors associated with HRQoL impairment, sick leave, low self-reported health rating and low household income.

4.2 Manuscripts II-IV

4.2.1 Ethical approvals

The study received approvals from the local ethics committee, and the Danish Data Protection Agency. The study adhered to the Helsinki declaration, and informed consent was obtained from all participants prior to inclusion in the study.[118]

4.2.2 Study population

Recruitment of study participants took place at the Department of Dermatology and Allergy, at Gentofte Hospital, Denmark, during the period from March 2019 to September 2020. A total of 110 patients and 40 age and sex matched healthy controls were recruited. Patients with physician diagnosed CHE and/or AD were eligible for inclusion meaning that both patients with CHE without concomitant AD, patients with AD without concomitant CHE, and patients with both AD and CHE were recruited. The sample size of 110 patients and 40 controls was determined prior to study start, and the aim was to recruit minimum 30 patients with CHE with no history of AD, and minimum 50 patients with active AD (with or without CHE). An overview of patient recruitment is shown in

Figure 5. Patients were first diagnosed by the treating physician and inclusion and exclusion criteria were confirmed by another physician (ASQ) at inclusion, Figure 6.

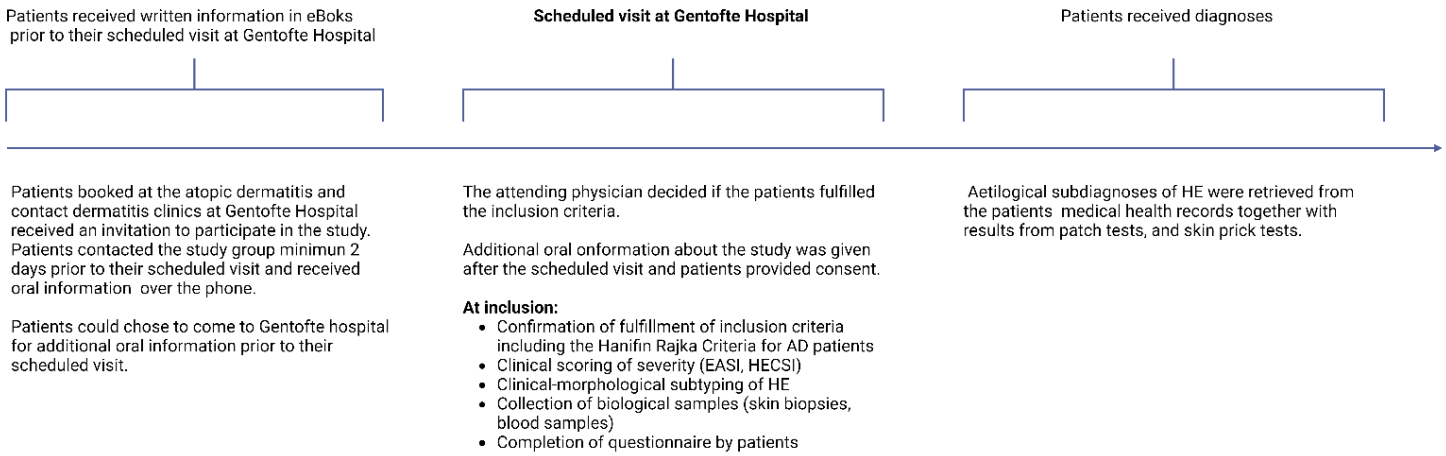

Figure 5. *Overview of patient recruitment.*

To be included, participants had to fulfil the following criteria:

- Over 18 years, Scandinavian skin type, able to read and speak Danish
- No ongoing infections and no treatment with antibiotics within the past four weeks
- No use of self-tanners or phototherapy within the past four weeks
- No pregnancy or lactation
- No history of other dominant skin disease, such as psoriasis (patients)
- No history of inflammatory skin disease or autoimmune disease including no history of AD or HE. No immune-modulating medications (controls)

Patients were encouraged not to apply active topical treatments 2 days prior to visit. The use of systemic eczema treatment was not an exclusion criterion, but any use of such treatments was registered. At inclusion, AD was diagnosed by the Hanifin and Rajka criteria, HE severity was assessed by HECSI and AD severity by EASI. Patients with concomitant AD and HE were scored using both tools. The clinical subtype of HE was also noted at inclusion and patients answered a questionnaire including questions on comorbidities, medications, and self-reported severity of HE on a VAS and by the photographic guide. Selfreported measures of AD included the POEM and self-reported itch on a VAS. The aetiological HE subtype was determined based on information

retrieved from patients' medical health records post inclusion, where patch test and skin prick test results, as well as information on clinical relevance and final diagnosis were available. All included patients with HE had been patch tested. The majority of HE patients were newly referred and thus included in the study at their first scheduled visit at the department and patch tested after inclusion. Some HE patients (mostly those with concomitant AD) were included at other scheduled visits at the department.

4.2.3 Biological samples

Three mm skin punch biopsies were collected from all participants under local anaesthesia. Two mm biopsies were chosen for a few lesional palmar samples. Two lesional biopsies were collected from patients with concomitant CHE and AD at other body sites, and one lesional biopsy was collected from patients with isolated CHE. Lesional biopsies were collected from the most affected area while also trying to avoid particular sensitive areas such as palmar creases. One non-lesional biopsy was collected from the arm of all patients. One healthy (or normal) biopsy was collected from each healthy control, the localization of this biopsy was chosen with the aim to match with the overall localizations of lesional patient biopsies. Biopsies were stored in a stabilization solution and kept overnight at 4°C, and then at -80°C until they were analysed. Blood samples were collected from all participants. Both whole blood and plasma (obtained after centrifuging blood samples) were stored at -80°C until analysis. Biological samples were shipped to collaboration partners at Ichan School of Medicine, Department of Genomics and Genomic Sciences, NY, USA, where they were analysed.

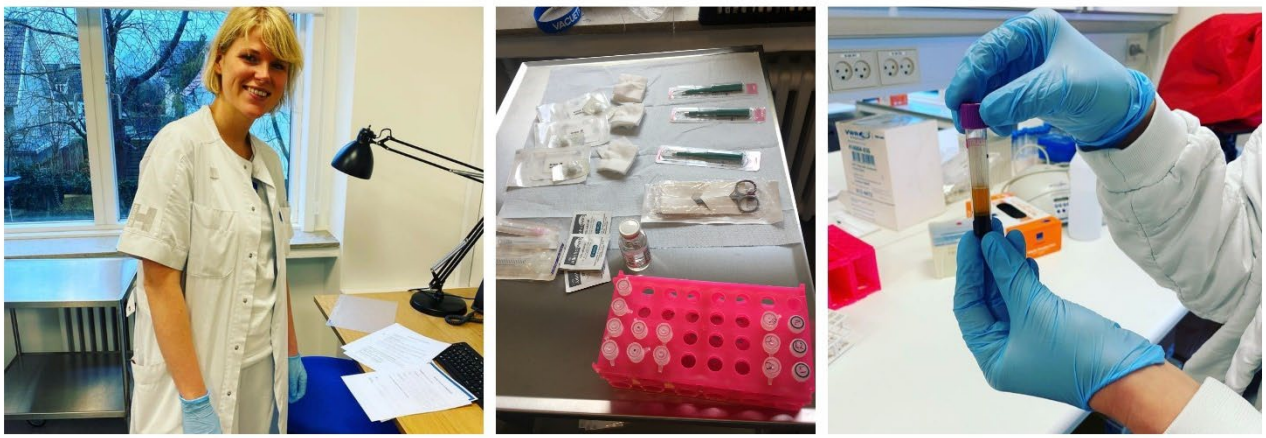

Figure 6. *Photos from study participant recruitment. ASQ (left photo) recruited all participants together with research nurse Anne Marie Topp. Tools to collect the skin biopsies are showed in the middle. A centrifuged blood sample is shown to the right.*

4.2.4 Profiling of plasma proteins

Plasma from all 150 participants was analysed using Olink Proseek® Multiplex Assay (Olink Bioscience, Uppsala Sweden). The panels CVDII, CVDIII, and inflammation were used. Each panel measured 92 inflammatory and CVD risk proteins.

4.2.5 Genotyping of filaggrin gene mutations

DNA was collected from whole blood from all 150 participants. Using Taqman® allelic discrimination assay, the DNA was analysed for four common mutations in the FLG gene (R501X, 2282del4, R2447X, and S3247).

4.2.6 Transcriptomic profiling of skin biopsies

RNA was extracted from 295 skin biopsies collected from the 150 participants. Transcriptomic profiling was performed on 241 biopsies (from 140 participants) as 54 samples showed degradation of RNA.

In short, RNA quantification was performed by use of the Bioanalyzer RNA 6000 kit from Agilent (Agilent, Santa Clara, CA, USA). Library build was performed with the Truseq Stranded Total RNA Gold (Illumina, California USA). Illumina HiSeq 4000 platform (Illumina, California USA) was used to perform 100 base pair paired end sequencing, resulting in an average sequencing depth of 43 M reads per sample. Quality control was performed through different steps, the reads were aligned to the human genome, and the raw counts were quantified. Extracted counts from each sample were compiled into a counting table.

A total of 60,458 genes were profiled, but only the 25,441 genes with entrez gene ID's were included in analyses. This was chosen to focus on well-annotated genes. After quality control and exclusion of samples collected from body sites other than palms, dorsa, and arms, 220 samples from 128 participants were included in analyses (*Manuscript IV*).

4.2.7 Study populations in Manuscripts II-IV

Of the 150 included participants, different sub-samples were included in the different manuscripts as shown in Table 4. Further, different sub-categorizations of patients were employed in the different manuscripts.

	Manuscript II	**Manuscript III**	**Manuscript IV**
Participants	108 patients with CHE and/or AD and 40 healthy controls	51 patients with CHE without concomitant AD and 40 healthy controls	96 patients with CHE and/or AD and 32 healthy controls
Sub-categorization	*AD status:* AD with active lesions, n=57 (current CHE, n=47; no CHE, n=10) CHE with no history of AD, n=40 CHE with previous AD, n=11	*Aetiological subtypes*[1]*:* ACD, n=14 ICD, n=5 ACD and ICD, n=6 Unknown, n=15 *Clinical subtypes*[2]*:* Chronic fissured, n=8 Vesicular, n=13 Hyperkeratotic, n=4	54 lesional CHE palm samples. *AD status:* Current AD, n=16 Previous AD, n=9 No history of AD, n=29 *Aetiological subtypes (only unique):* ACD, n=12 ICD, n=5 AD, n=8
Molecular profiling	Targeted plasma proteomics, four FLG gene mutations	Targeted plasma proteomics, four FLG gene mutations	Skin transcriptomics, four FLG gene mutations

Table 4*. Participants, sub-categorizations, and molecular profilings in Manuscripts II-IV.*
[1] Only patients with no history of AD.
[2] Only unique clinical subtypes with ≥ 4 patients. Allergic contact dermatitis, ACD; atopic dermatitis, AD; chronic hand eczema, CHE; filaggrin, FLG; irritant contact dermatitis, ICD.

4.2.8 Statistical analysis

Descriptive statistics of study population characteristics were performed using IBM SPSS Statistics for Windows, version 25.0 (*Manuscript II*) and version 28.0 (*Manuscripts III-IV*) (IBM Corp., Armonk, NY, USA).

Analyses on plasma protein expression (*Manuscript II and III*) were performed using R 3.6.1.[119] The plasma proteins included in analyses are shown in Figure 7. Differentially expressed proteins (DEPs) between contrasts were defined as Benjamini-Hochberg false discovery rate (FDR) adjusted p-values <0.05. Some analyses were adjusted by age (*Manuscript III*). Volcano plots, boxplots, scatter plots and correlation heatmaps were created by use of different R packages.

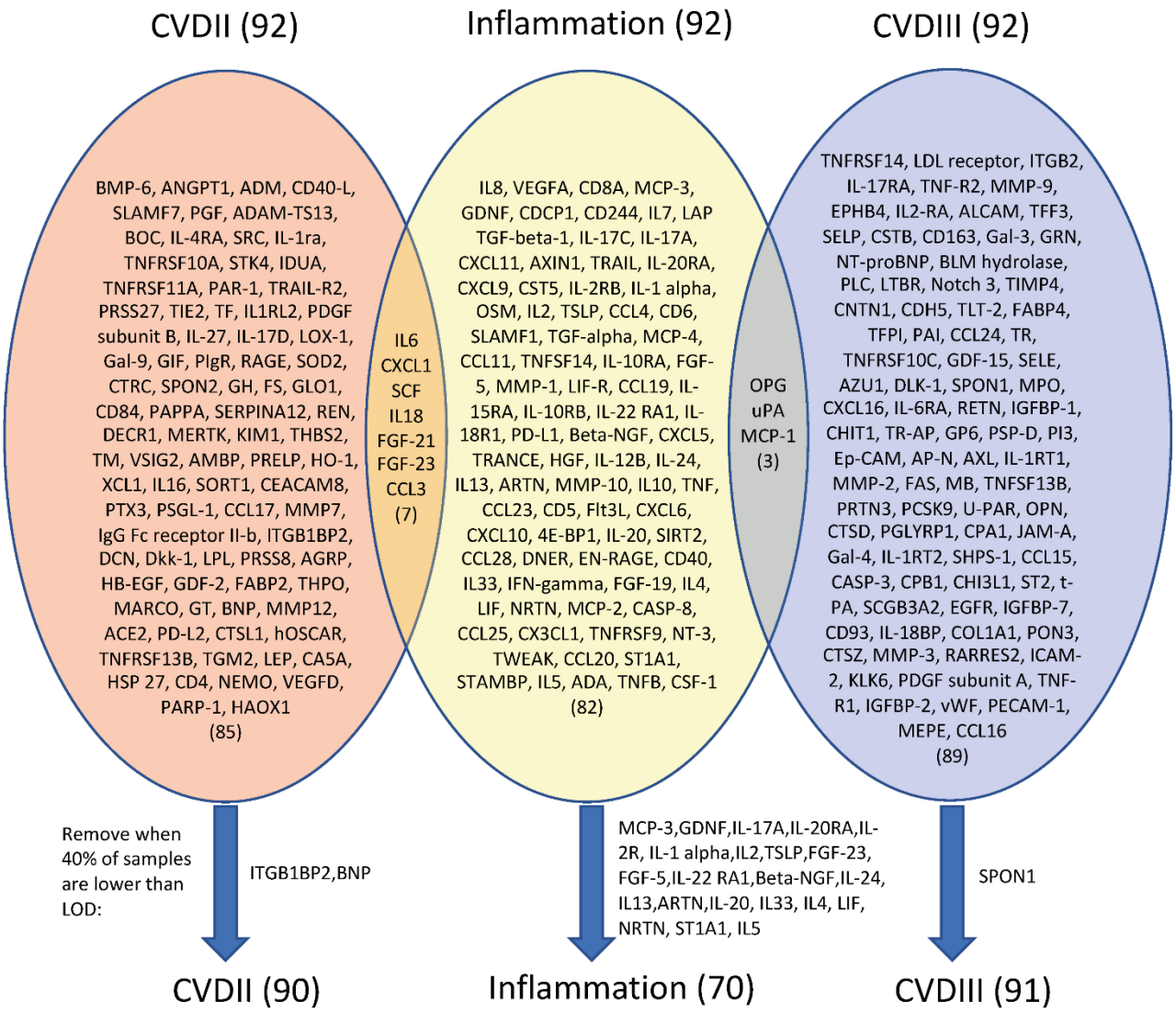

Figure 7. *Plasma proteins in the three Olink panels, 241 proteins were included in analyses in Manuscript II and III. Published in supplementary material in Manuscript I[118], used under CC-BY license. Cardiovascular disease, (CVD).*

Transcriptomic analyses and data visualizations in *Manuscript IV* were performed by use of Qlucore Omics explorer 3.9 (Qlucore AB, Lund, Sweden). Normalization of the raw counts to

counts per million (cpm) was performed. The data were subsequently floored at 0.01 and log2 transformed in Qlucore Omics explorer. Differentially expressed genes (DEGs) between contrasts were defined as Benjamini-Hochberg FDR adjusted p-values < 0.05 and absolute fold changes (FCH) ≥ 2. All comparisons were unpaired and adjusted by age. Principal component analyses (PCA) plots and unsupervised hierarchical clustering heatmaps were used to visualize sample similarities/dissimilarities. Variance filtering was used to focus on hypervariable genes. Analyses and data visualizations of canonical pathways and upstream regulators were performed using ingenuity Pathway Analysis (IPA) (Qiagen Inc. https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis). The upstream regulator analyses focused on the molecule types: cytokines and transcription regulators.

A detailed description of the statistical methods employed can be found in the individual manuscripts.

5. Main Results

This section provides a summary of the key findings from each manuscript. The complete manuscripts are included as appendices at the end of this thesis

5.1 Manuscript I: Chronic hand eczema: *A prevalent disease in the general population associated with reduced quality of life and poor overall health measures*

Out of 100,000 invited individuals, 42.7% responded to the questionnaire and 40.9% responded to the question on the lifetime prevalence of HE and were included in analyses. Non-responders were younger than responders and females were more likely to respond than males. The main findings of the study are summarized in Figure 8.

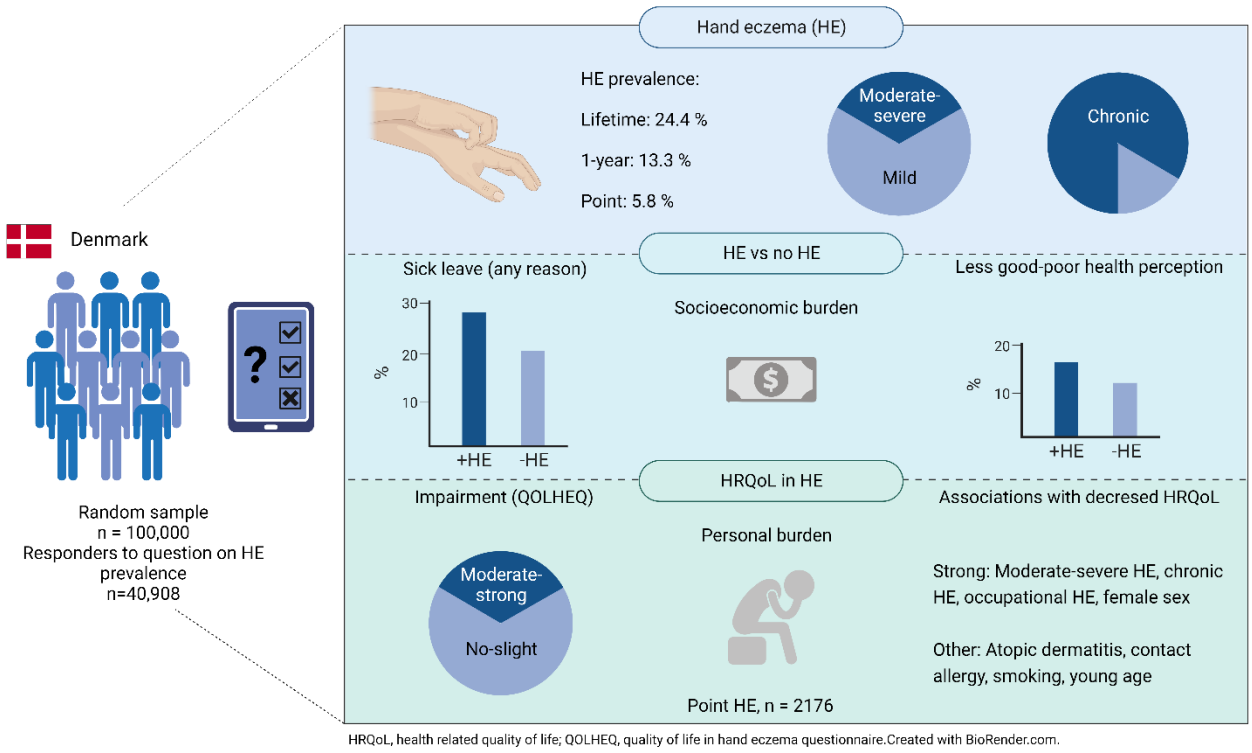

***Figure 8**. Overview of Manuscript I. Adapted from graphical abstract published with Manuscript I[49], used under CC-BY license.*

HE was highly prevalent in the adult, Danish general population, with a total 1-year prevalence of 13.3%. The prevalence of HE was higher in females than males, around one third reported moderate to severe disease and the vast majority (over 80%) reported CHE. The high CHE prevalence was

mainly caused by a high proportion of individuals reporting multiple eruptions of HE. The severity of HE as assessed by the photographic guide, was similar between females and males whereas females reported higher HE severity on a VAS. More individuals living in rural municipalities reported moderate to severe disease than those living in capital and metropolitan municipalities.

Individuals with HE within the last year reported lower overall health ratings and more sick leave (for any reason and for more than seven days the past year) than those without HE. The associations between HE and less good to poor health perception and sick leave prevalence were confirmed in logistic regression analyses that were adjusted for potential confounders such as sex, age, and AD.

The QOLHEQ questionnaire was completed by 2176 (92.5%) of those with current HE. In line with the results for self-reported HE severity, around one third reported moderate to very strong impairment of their HRQoL, as measured by the QOLHEQ. The QOLHEQ scores for all individuals with current HE indicated a slight overall HRQoL impairment and a moderate impairment of the two domains: symptoms and treatment and prevention. Females and younger age groups reported stronger HRQoL impairment both overall and across most domains, and rural residents reported stronger HRQoL impairment of the symptoms domain as compared to capital residents.

Through logistic regression analyses it was found that moderate to severe HE, CHE, occupational HE, and female sex were strongly associated with moderate to very strong HRQoL impairment in individuals with current HE. Other factors that were associated with moderate to very strong HRQoL impairment included AD, a positive patch test result (of patch tested individuals), young age, and smoking. Annual household income and municipality of residence were not found to be associated with HRQoL impairment among individuals with current HE.

5.2 Manuscript II: *Circulating biomarkers are associated with disease severity of chronic hand eczema and atopic dermatitis*

A total of 108 patients with CHE and/or AD and 40 healthy controls were included in this study. The patients were stratified into three groups: 57 patients with AD with active lesions (47 with concurrent CHE, and 10 without), 40 patients with CHE with no history of AD ($CHE^{NO\text{-}AD}$), and 11 patients with CHE and previous AD ($CHE^{PREVIOUS\_AD}$).[118]

The prevalence of FLG gene mutations was higher in AD ($p<0.01$), and $CHE^{PREVIOUS\_AD}$ patients (at borderline significance) as compared with controls, but the prevalence of FLG gene mutations in $CHE^{NO\text{-}AD}$ patients and controls was comparable.

Plasma protein expression of 241 inflammatory and CVD risk proteins was examined across the cohort, and the main findings are summarized in Figure 9.

Very severe, and to a lesser degree severe $CHE^{NO\text{-}AD}$ was associated with systemic immune activation when compared with healthy controls. Moderate $CHE^{NO\text{-}AD}$ was not associated with systemic immune activation as compared to controls. The top two DEPs between very severe $CHE^{NO\text{-}AD}$ and controls were the Th2 associated inflammatory chemokines CCL17 and CCL13, and other DEPs included proteins associated with Th1 (CXCL9-11), general inflammation (MMP12), activation of eosinophiles and monocytes (CCL2), as well as pleiotropic cytokine IL-6, Figure 9a.

Levels of five circulating biomarkers correlated positively with clinical severity (HECSI score) among the 40 $CHE^{NO\text{-}AD}$ patients, Figure 9b.[118] We also found significant positive correlations between age and 10 proteins, but age did not correlate significantly with clinical severity.

No DEPs were found between $CHE^{PREVIOUS\_AD}$ and controls, and this group was too small to stratify by clinical severity.

For the AD group, we found severe and moderate AD to be associated with systemic immune activation whereas no DEPs were found between patients with mild AD and controls. Further, significant positive correlations were found between EASI score and seven proteins, Figure 9b.

Systemic type 2 inflammation was shared between very severe CHE without AD and severe AD, with CCL17 and CCL13 being the top two DEPs in both groups. Very severe $CHE^{NO\text{-}AD}$ was associated with increased expression of Th1 associated CXCL9-11, whereas severe AD was not, Figure 9a. In general, the FCHs and significance level of DEPs were higher in the AD group than in

the CHE^{NO_AD}. Three circulating proteins correlated positively with clinical severity of both CHE^{NO_AD} and AD: CCL17, CCL13, and MMP12, Figure 9b.

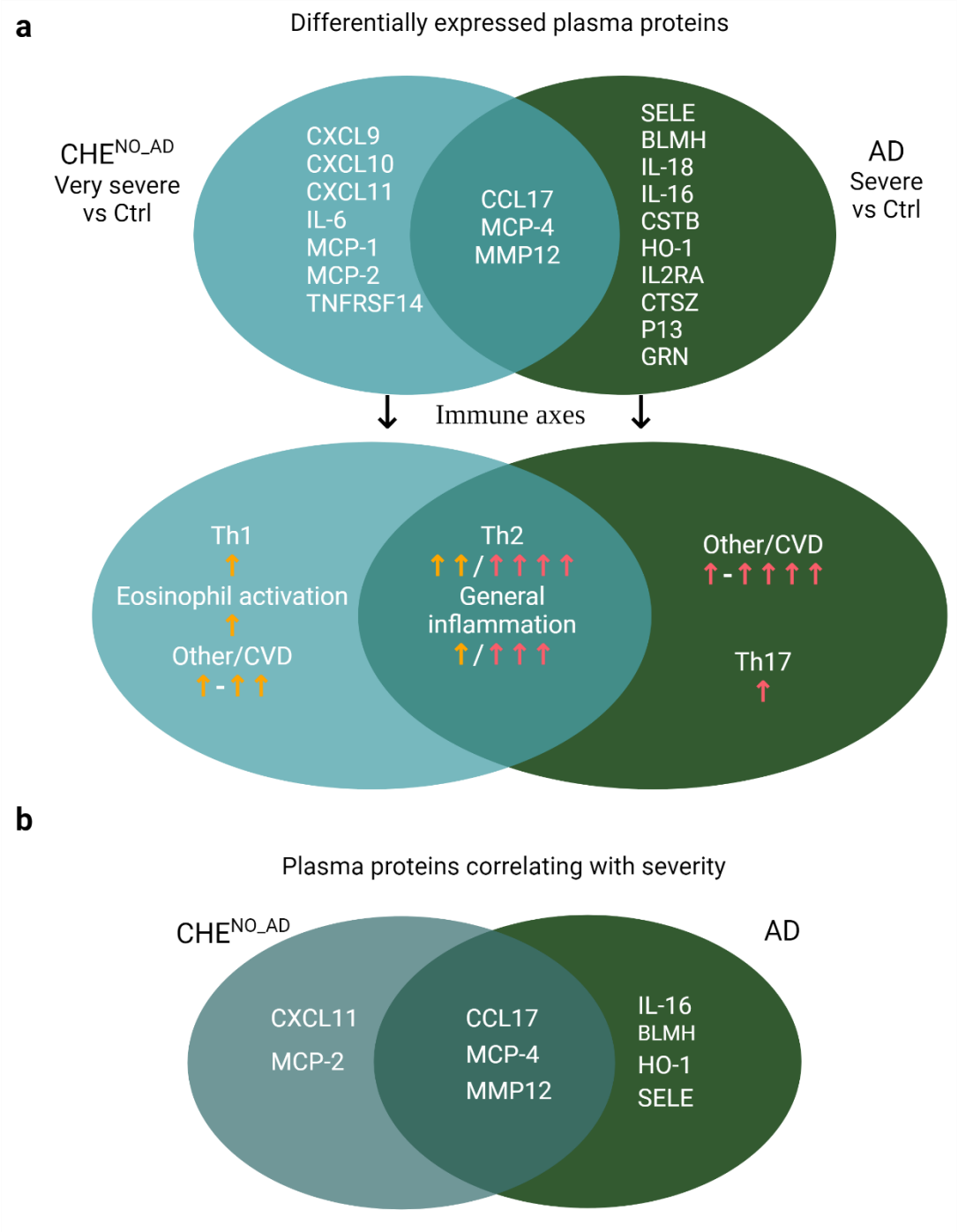

***Figure 9**. Overlapping differentially expressed proteins (**a**), and their associated pathways (**b**) between patients with very severe CHE^{NO_AD} versus controls and patients with severe AD versus controls. (**c**) shows overlapping circulating biomarkers that correlated with clinically assessed eczema severity in patients with CHE^{No_AD} and patients with AD. Created with Biorender.com. Adapted from figure published in Manuscript II[118]. Used under CC-BY license.*

5.3 Manuscript III: *Inflammatory plasma signature of chronic hand eczema: associations with etiological and clinical subtypes*

In this study, 51 CHE patients without concomitant AD and 40 healthy controls were included. The patients were categorized according to CHE aetiology and clinical subtype as shown in Figure 10[120] and plasma protein expression was assessed and compared across the cohort.

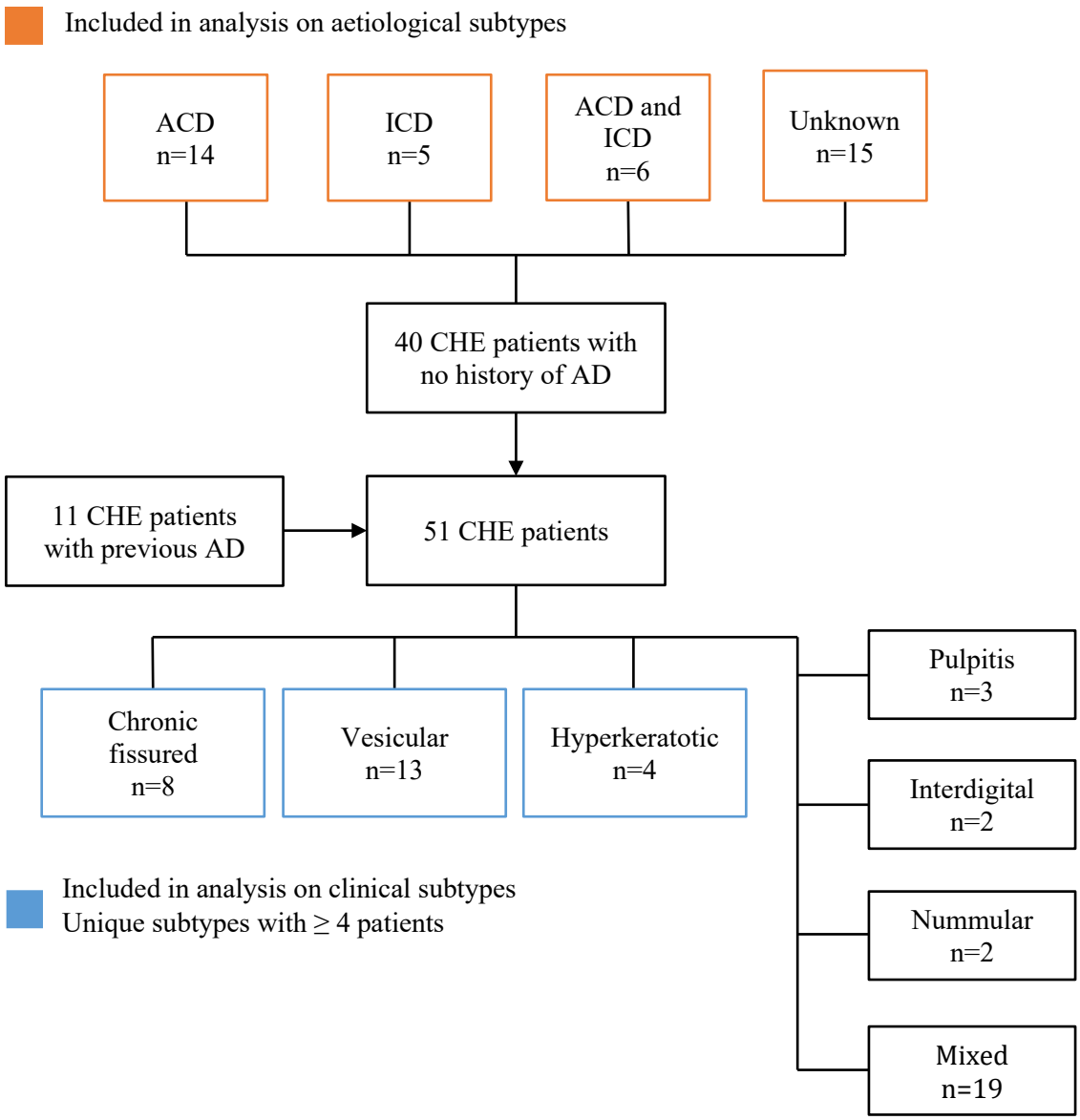

***Figure 10**. Overview of patients included in the different subgroups in Manuscript III. Adapted from figure published in Manuscript III. [120] Used under CC-BY-NC license.*

No circulating biomarkers were found that could discriminate between the different aetiological CHE subgroups. When comparing the subgroups separately to controls, one DEP was found for combined ACD/ICD, whereas no difference was found between ACD or ICD and controls. The ACD subgroup was the only subgroup that was large enough to stratify by clinical severity.

We found that very severe ACD was associated with systemic immune activation as compared with controls, whereas only one DEP was found in severe and none in moderate ACD when compared with controls. The DEPs in very severe ACD included markers associated with Th2 (CCL17, CCL13), Th1(CXCL9-11) alongside other proinflammatory proteins. In the 14 ACD patients, positive and significant correlations were found between the clinical severity and circulating levels of six proteins, as shown in Figure 11. These proteins included markers of both Th1 and Th2 associated inflammation. When examining plasma protein expression according to unique clinical subtypes, we found that hyperkeratotic HE was associated with increased expression of several inflammatory proteins as compared to controls, whereas the vesicular and chronic fissured subtypes were not. The inflammatory proteins associated with hyperkeratotic CHE included Th1 and TNF associated markers, but not Th2 associated markers. Hyperkeratotic CHE could be discriminated from both vesicular and chronic fissured CHE by increased levels of CCL19 and CXCL9-10.

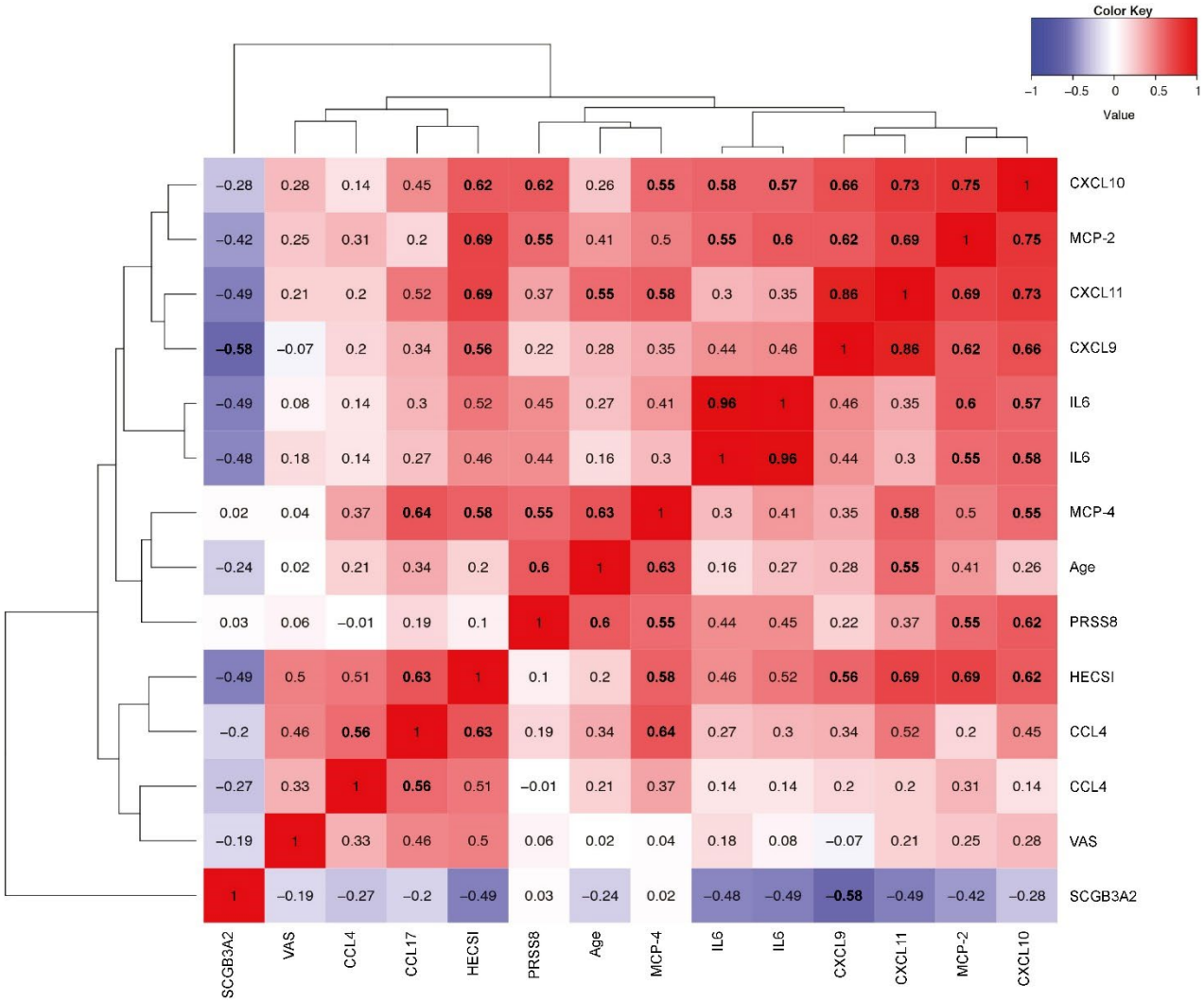

Figure 11. *Correlations between plasma proteins, age, and clinical and selfreported severity in 14 patients with chronic allergic contact dermatitis of the hands. Correlation coefficients are displayed, and bold values denote significance at the p<0.05 level. Published in Manuscript III.* [120] *Used under CC-BY-NC license.*

5.4 Manuscript IV: *Transcriptomic profiling of chronic hand eczema skin reveals shared immune pathways and molecular drivers across subtypes*

In this study we investigated the transcriptome of CHE as profiled from full-thickness skin biopsies. In our preliminary analyses, we looked at the transcriptomic profile of 220 biopsies collected from palms, dorsa (of hands), and arms from 96 patients with CHE and/or AD and 32 healthy controls. We observed that healthy and lesional CHE palm skin exhibited distinct transcriptional signatures.

We next concentrated our primary analysis on 54 lesional and 16 healthy palm samples. In unsupervised analyses, we found that the lesional samples showed high overall molecular similarity, with no apparent separation according to factors such as AD, FLG mutation or clinical CHE severity. We did not find any correlations between gene expression levels and clinical severity.

In differential expression analysis, we found a large difference (2333 DEGs) between CHE and healthy palm skin, Figure 12.

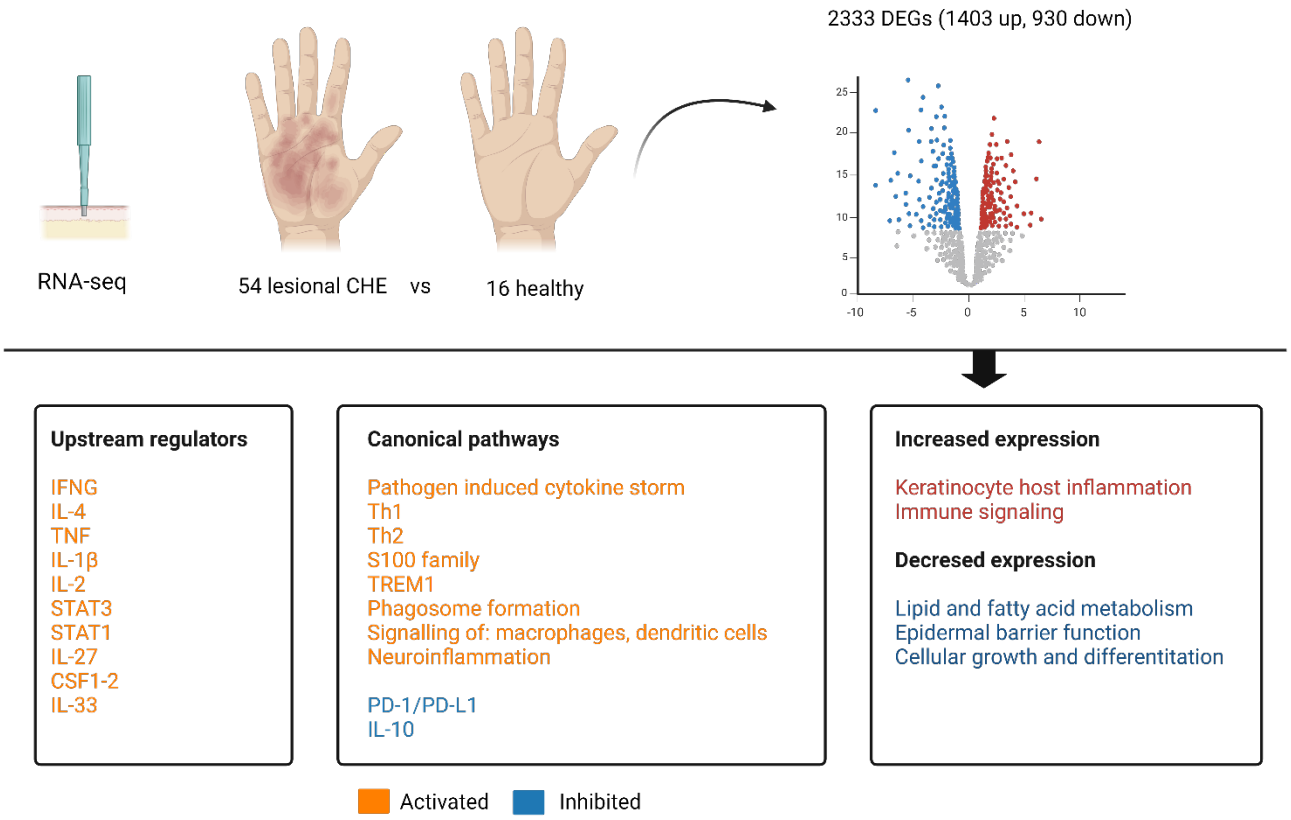

***Figure 12**. Overview of main findings from the lesional transcriptomic signature of chronic hand eczema as compared to healthy palm skin. Created with Biorender.com.*

Top DEGs that showed increased expression were associated with keratinocyte host inflammation and immune signalling and top DEGs showing decreased expression were associated with epidermal barrier function, metabolism of lipids, as well as cellular growth and differentiation. Pathway analysis of these 2333 DEGs showed a heterogenous inflammatory response in lesional CHE palm skin with significant activations of multiple pro-inflammatory pathways including prominent activations of Th1 and Th2 immune pathways, Figure 12. Accordingly, analyses on upstream regulators also showed most significant activations of Th1 and Th2 associated molecules as well is IL-1 family members, in particular IL-1β, Figure 12.

When looking at lesional CHE palm samples stratified by AD status (current AD, previous AD, no history of AD), we found no differences between the three subgroups when they were compared separately to each other. When we compared the subgroups separately to healthy palm skin, we found that the lesional molecular signatures mostly overlapped between the three subtypes.

We found overall similar pathway enrichments and upstream regulator predictions across the subtypes. The shared pathways and upstream regulators aligned with those characterized for all 54 lesional CHE palm samples as compared to healthy palm skin.

However, some Th1 associated molecules showed the greatest activity and significance in CHE without AD. Th2 associated molecules such as IL-4 showed a similar activation pattern in the three subtypes.

We found that the unique aetiological CHE subtypes (ACD, ICD, and AD) also showed overall similar molecular signatures, and no DEGs were found to discriminate ACD from ICD in differential expression analysis. Two non-coding DEGs with unknown biological relevance to dermatitis were found between AD and ICD. When we compared gene expression separately between each subtype to healthy palm samples, we noted an overall shared lesional molecular signature. However, ACD showed the highest number of DEGs, and the highest foldchanges of several markers. As such, this subtype was found to be the most disrupted as compared to controls. When comparing enriched pathways among the three subtypes, we again noted that these were primarily shared between the aetiological subtypes. However, some differences were noted in the activity patterns between subtypes. ACD showed the greatest activity of adaptive immune pathways such as Th1 and Th2, whereas ICD showed the greatest activity of pathways associated with innate immunity and skin barrier restoration. In comparison analyses of upstream regulators, we also noted

a more pronounced Th1 skewing in ACD than in ICD and AD, with highest significance and activity of interferon alpha and STAT1 being found in ACD.

6. Methodological Considerations

This section elaborates on methodological considerations including strengths and weaknesses of the four manuscripts.

6.1 Manuscript I

6.1.1 Study design

The epidemiology of HE can be studied through different methods. Studies that include clinical examination by medical professionals of unselected participants to determine outcomes such as prevalence and severity of HE are considered to have higher validity than studies using self-reported measures. Given the high estimated sample size (>30,000 unselected individuals), and the nationwide nature of this study, we chose to a investigate the desired outcomes through a web-based questionnaire study. This method is cost-effective and more feasible when examining larger cohorts, but there are several limitations associated with questionnaire studies. We chose a web-based questionnaire as opposed to a paper-based questionnaire as a more cost-effective and environmentally friendly approach. This approach further enabled adaptive questioning using branching logic, allowed for automated data entry into REDCap, eased subsequent data-analysis, and the questionnaire was easily accessible on all electronic devices. A Danish study comparing paper- and web-based questionnaires (to eBoks) found 10 times higher costs associated with paper invitations per respondent, higher response rates for the paper versus the digital version (45.9% vs 36.2%), a lower number of missing values in the digital version, and no difference between non-responders in the two groups.[121] This could imply that the level of selection bias is not increased by using digital questionnaires.

6.1.2 Response rate, selection-, and recall bias

The response rate obtained in our cohort was 42.7% with 40.9% answering the question on the lifetime prevalence of HE. This response rate was higher than our estimation in the sample size calculations (35%). Although this is a response rate that can be considered as realistic and comparable to similar recent studies, it would still be preferable with a response rate over 50%. We obtained demographic characteristics for both non-responders and responders for the non-responder analysis, which is a study strength, but we did not have any means of collecting information on HE

prevalence in non-responders, which is a limitation. Selection bias cannot be ruled out, meaning that more people with HE might have answered the questionnaire than people without. We sought to minimize this type of selection bias by highlighting in the invitation letter, that all answers were needed, regardless of individuals having a history of eczema (or rash) or not. Another important limitation of the study is potential recall bias. We sought to minimize this bias by mainly employing validated questions/instruments.

6.1.3 Missing answers

The questionnaire was rather lengthy, and we did observe some signs of 'questionnaire fatigue' with more missing answers in the last questions in the questionnaire. In retrospect, a shorter questionnaire could potentially have reduced the proportion of missing answers. Further, the questionnaire commenced with overall questions on eczema at different body sites (these questions were not included in *Manuscript I*). Beginning the questionnaire with the most important question on the lifetime prevalence of HE, could potentially have resulted in more respondents on this question. Responders were not required to have answered a question to proceed to the next. This was chosen in fear of participants skipping the entire questionnaire if encountering a question they felt strongly against answering, but this might also have led to more missing answers for some questions. We sought to mitigate this through adding 'I don't know' and 'I do not wish to answer this question' options. Indeed, apart from the response rate, a limitation of this study is the missing answers. Missing answers can be handled through differing methods (e.g., by data imputation). We chose to exclude missing answers when computing the endpoints and provide comprehensive data on missing answers in supplementary material as an easy interpretable and transparent method which also allow the use of our data by others (e.g., in future meta-analyses). We saw that the missing data frequencies in individuals with and without HE were comparable for outcomes compared between these groups, which is a strength. For the HE related outcomes which were only answered by individuals with HE (e.g., the QOLHEQ), a low missing data frequency was observed. This increases the validity of the results specific for the HE population, e.g., the QOLHEQ scores, and the results on severity.

6.1.4 Other considerations

Another limitation of the study is the lack of incorporation of socio-economic variables such as education and occupational status. We used household income as a proxy for socio-economic status.

This variable was dichotomized into over or under 600,000 DKK per year. In retrospect, a stratification into income percentiles might have provided a more nuanced understanding of the impact of income on the study outcomes. We interpreted QOLHEQ scores based on international recommendations in lack of a Danish interpretability study. It is possible that the interpretability of the Danish QOLHEQ scores differ from the international context due to cultural differences or variations in healthcare systems, which may affect how quality of life is perceived and reported in Denmark. The study was performed during May-June 2021 after the third COVID-19 wave. The Danish society had almost completely reopened at this time point following longer periods with lock downs. It is possible that increased hand wash and use of hand sanitizers among the public might have resulted in higher HE prevalence estimates in the study. It is also possible that the circumstances under the COVID-19 pandemic might have affected other reported outcomes measured, such as the selfreported HRQoL.

6.2 Manuscripts II-IV

The clinical study was explorative with the overall aim of investigating biomarkers of AD and CHE. Recruitment was based on AD and/or CHE diagnosis. The predetermined sample size included 110 patients and 40 controls, aiming at minimum 30 patients exclusively with CHE and no history of AD and 50 with active AD. This was chosen to ensure a robust comparative analysis and enable subgroup investigations. No further sample size calculations were made to ensure certain numbers of patients with specific HE subtypes. This is a limitation as the groups compared in *Manuscripts II-IV* are not of similar size, with some groups having small sample sizes. Nevertheless, previous studies performing broader molecular profiling from HE biopsies are few and include between 6-15 HE patients, with no stratification according to subtype.[10–12] Accordingly, *Manuscript IV* is the largest study to date investigating the molecular HE profile from biopsies.

Prior to study start, recruiting CHE patients was anticipated to be challenging, given the expectation that few would consent to hand skin punch biopsies. Further, we aimed to study CHE and AD in a real-life clinical setting. Therefore, the exclusion criteria did not include systemic eczema therapy, and no longer wash out period of topical corticosteroids was required. As the study commenced, and we experienced an unexpected willingness from CHE patients to participate and donate skin punch biopsies, the recruitment primarily focused on patients who did not receive systemic eczema treatments. The short washout period of topical cortico-steroids and the inclusion of patients in

systemic treatment might have caused a different molecular eczema profile. Most patients abstained from use of active topical treatments for two days, but this was generally considered as an obstacle for the willingness of patients to participate. Most included CHE patients had severe to very severe disease and did not wish for their eczema to worsen. Any longer wash out periods would inevitably have resulted in increased patient discomfort and a smaller sample size.

AD was diagnosed through the Hanifin Rajka criteria. This represents a well-known and widely used method for this purpose. AD patients were not further subcategorized, e.g., into intrinsic and extrinsic AD. We aimed to make this categorization by profiling total blood IgE levels from all participants, but these analyses unfortunately failed. Total IgE levels measured in the clinic were only available for a subset of patients and were thus left out. All patients were adults with a Scandinavian skin type, and long lasting, chronic AD, thus representing a rather homogenous sub population.

CHE was aetiologically and clinically subcategorized based on classifications from the DCDG. These classifications are widely similar to the newest HE guidelines from 2022. One limitation of this study is potential misclassification of HE (in particular in *Manuscript III and IV*). The clinical subtyping, as well as clinical scoring of severity, was performed by ASQ in all cases which minimizes interobserver variability. However, the diagnostic work-up and the following aetiological sub-diagnosis of HE was made by different physicians, with this information being extracted from the patient files post inclusion. It is possible that an allergen has been missed in some cases, and more likely, that an irritant aggravating component has been missed in some ACD and AD CHE cases. Additionally, any clinically relevant contact allergy resulted in an ACD categorization, and this subgroup was not further sub-stratified based on the specific contact allergy, for example fragrance allergy. This would have been preferable but was not possible as few patients had the same contact allergy (*Manuscript III and IV*). The patient sample represented the clinical reality of a very heterogenous and multifactorial disease. With these limitations in mind, we sought to stratify the patients into as ‘clean’ aetiological and clinical groups as possible, and by mainly performing analyses on the unique subtypes (*Manuscript III and IV*).

Skin biopsies were collected from all participants (*Manuscript IV*). Non-lesional biopsies were collected from the arms of patients if possible. This was chosen to provide a uniform sampling strategy in the heterogenous patient population consisting of both AD and CHE patients. For patients with CHE, non-lesional biopsies from the hands would have been preferable and enabled

site-matched paired analyses. It is a limitation of *Manuscript IV* that non-lesional biopsies from hand skin were not collected. However, skin biopsies from the hands are particularly painful, and due to ethical considerations, we did not collect more than one biopsy from the hands. Furthermore, for many of the included CHE patients with palmar eczema, finding a non-eczema affected area on the palms that was suitable for collection of a biopsy would have been difficult. Lesional biopsies were collected from the hands from all patients with CHE and one additional lesional biopsy was collected if patients had active AD lesions at other body sites. The most affected area was chosen. However, this was not always possible for patients with palmar eczema, as sampling from sensitive structures such as palmar creases and fingers were generally avoided.

6.3 Manuscripts II-III

The profiling of inflammatory plasma proteins were the first analyses to be completed, and followingly to be reported. Three Olink panels were chosen (Inflammation, CVDII, and CVDIII). These panels were chosen as they encompassed a broad array of inflammatory markers thought to be relevant in inflammatory skin disease. Further, the panels had been previously employed in AD and psoriasis populations, which enabled a comparison of our results. Although the chosen panels, comprising of 266 proteins, did not cover the entire plasma proteome, the diversity and high number of markers enabled a more explorative approach. Several other factors are associated with systemic inflammation such as CVD and increased age, which were considered in our analyses. However, information on other relevant factors such as smoking status, and body mass index were not collected at inclusion, which is a limitation. For both *Manuscript II* and *III* it should be noted that these were cross-sectional examinations of circulating biomarkers that did not include profiling of corresponding skin proteins. Therefore, the source of the inflammatory plasma proteins cannot be determined. Incorporation of analyses on the corresponding proteins from skin were planned. However, profiling of one Olink panel from skin yielded poor results with the majority of markers being under the limit of detection. Therefore, no additional Olink panels were profiled from skin, and the results from the one profiled panel were deemed unsuitable for analyses.

In *Manuscript II*, patients were categorized into three groups: AD with active lesions, CHE with no history of AD, and CHE with previous AD. This categorization method considered both the AD status, and the extent of eczema (with active AD generally affecting a larger body surface area than isolated CHE). Patients with active AD were not further sub-stratified based on the co-occurrence of CHE. This was chosen as it would be difficult to attribute any systemic changes specifically to

CHE, given the presence of concurrent lesions elsewhere on the body. It can be discussed whether previous AD is relevant for current CHE. We chose to make this distinction, as AD history could indicate underlying genetic predispositions and a generally impaired skin barrier, as described in the background section of this thesis. In line with this, we also found a higher prevalence of FLG gene mutations in $CHE^{PREVIOUS\_AD}$ patients (36.4%) than in CHE^{NO_AD} patients (7.5%). Also, hypothetically, a history of AD could be associated with a different systemic inflammatory profile than seen in individuals without.

In *Manuscript III*, only patients with CHE without concomitant active AD were included. This was chosen to avoid potential confounding of AD lesions at other body sites. The patients were stratified both according to aetiology and unique clinical subtype. Opting to include only patients with unique clinical subtypes led to smaller yet more homogeneous subgroups. Larger, homogenous sub-groups would increase the validity of the results. In the DCDG classification of clinical subtypes, it is characterized that hyperkeratotic HE does not evolve into psoriasis.[20] The patients included with this subtype in *Manuscript III* had no history of psoriasis nor any other clinical signs of psoriasis at inclusion. Due to the cross-sectional nature of the study, it cannot be ruled out that their disease would evolve into psoriasis at some point.

6.4 Manuscript IV

In *Manuscript IV* we initially conducted broader investigations of 220 skin biopsies collected from palms, dorsa, and arms. We chose to exclude samples taken from other body sites from analyses (e.g., from the back, trunk, wrists, or legs) as few samples were collected from these sites.

We performed unsupervised analyses and found that anatomical sample site contributed to sample data variance with palm skin in particular being unique.

Thus, we decided to conduct site-matched comparisons, focusing on the 54 lesional and 16 healthy palm samples. The majority of samples from dorsum hand skin were collected from patients with AD, which impeded investigations of transcriptomic differences across subtypes, the main aim of *Manuscript IV*. Therefore, these samples were not further investigated in *Manuscript IV*.

As previously described, a limitation of our analysis is the absence of paired lesional versus non-lesional comparisons within palm skin. The non-lesional samples in our cohort were exclusively collected from arm skin. While we considered including paired lesional palm versus non-lesional

arm comparisons, the significant inherent differences between palm and arm skin, as observed in our preliminary analyses, would make it challenging to conclusively attribute any gene-expression variations to eczema. To avoid any such potential misinterpretations, we chose not to include such comparisons in *Manuscript IV*.

Over 60,000 genes were profiled from RNA-seq. As described, we included the 25,441 genes with entrez gene ID's, meaning that the analyses were limited to genes that were well-documented. This approach facilitated the interpretation of DEGs between contrasts and subsequent pathway analysis. However, potentially important genes that are yet to be annotated could be overlooked through this method.

A major strength of *Manuscript IV* is the inclusion of skin palm biopsies from 54 patients. This large sample size enabled meaningful subgroup analyses on CHE subtypes, something that has not previously been investigated from skin biopsies. In addition, the only previous study to have profiled the transcriptome of (vesicular) HE from biopsies[12], focused only on the epidermis, making *Manuscript IV* the first to investigate the transcriptome of both the epidermis and dermis. Importantly, the dermis contains a diverse composition of immune cells of importance to the immunological skin barrier.[85] Further, the employment of IPA enabled an in-depth investigation and characterization of the dysregulated immune response in CHE. Validation of markers at protein level as well as investigations of the spatial distribution of these markers in skin would have provided more comprehensive insights into the pathophysiology of CHE.

7. General Discussion

Prevalence, severity, and chronicity of hand eczema in the Danish general population

The prevalence of HE has been extensively studied in the past. We found a total adult 1-year prevalence of 13.3%, aligning with previous Danish studies[30,33,61,65], but surpassing the pooled estimate found for adults in a meta-analysis (9.7%,)[3], and the estimates found in a recent Dutch study (7.6%) performed in 2020.[35] Our study and the Dutch study, similar in participant demographics, methodology, and study period, revealed a notable discrepancy in HE 1-year prevalence (13.3% vs. 7.6%). This difference could suggest a higher prevalence of HE in Denmark or an increased awareness of HE among the Danish population. Different circumstances during the COVID-19 pandemic could also have a say. We found that 82.6% of those with HE reported chronic disease, and that this was mainly caused by people reporting multiple HE eruptions and not a duration over three months. This estimate was higher than what had been reported in two other studies.[30,35] However, between study comparisons are complicated by the lack of standardized questions to assess CHE. The majority reported mild disease and one third moderate-severe disease, which is in accordance with findings from other populations.[3]

These results highlight that HE poses a significant disease burden in Denmark. Particularly, high risk individuals with chronic and moderate-severe disease encompass a large proportion of affected individuals. This questions whether adequate preventive and management measures are in place for individuals with HE in Denmark.

Overall health, sick leave, and quality of life

We found that individuals with HE reported poorer overall health than those without, which is in line with previous findings.[34,51] Further, they reported more sick leave (for any reason) than those without HE, which has not been previously examined. These findings could indicate an increased disease associated socio-economic burden. However, there is a complex interplay between health-status, socio-economic factors, and the risk for- or impact of HE.[51,122,123] Some of these factors, for example educational attainment, were not taken into consideration in analyses, whereas others, such as income, were. One possibility is that HE directly contributes to the deterioration of health perception and sick leave. Alternatively, or more likely in addition, individuals with poorer health or those in lower socio-economic classes might be at increased risk of HE development, e.g., by

engaging in manual labour. The poorer health status and higher sick leave rates could be a result of their overall life circumstances rather than HE alone. No larger differences in number of general practitioner consultations (for any reason) were seen between individuals with or without HE. This raises the question of whether individuals with HE are less likely to seek medical attention for their condition, a topic that warrants further investigation.

We employed the QOLHEQ in a general population setting for the first time and found that one third with current HE experienced moderate to very strong HRQoL impairment because of HE and that the factors most strongly associated with decreased HRQoL were moderate-severe HE, chronic HE, and occupational HE, and female sex. The results cannot be compared to other population-based studies, but chronic and severe HE have previously been associated stronger HRQoL impairment in selected populations.[45,48] These results highlight the need for targeted interventions aimed at individuals suffering from chronic, moderate-severe, and occupational HE in the Danish general population.

Systemic traces of hand eczema

We found that severe to very severe CHE with no history of AD was associated with systemic type1/type2 immune activation and that circulating biomarkers correlated with clinical CHE disease severity (*Manuscript II*). The detection of numerous inflammatory markers in the systemic circulation of patients with severe CHE, despite its confinement to a small body site, suggests a systemic impact of severe CHE and supports the consideration of systemic therapies for these patients, as noted by Roessner and Wittman in a commentary on *Manuscript II*.[124] Chronic inflammatory skin diseases, such as AD, have been associated with various comorbidities, as a result of chronic inflammation.[125] Our findings further give rise to the question of whether patients with severe CHE without AD are also at increased risk of such comorbidities. Further, these findings also underscore the effectiveness of the analysis techniques employed, for which several markers can be profiled from very small amounts of plasma. Our findings on systemic inflammation in moderate-severe, but not mild AD are in line with previous reports.[104–107] The findings from the AD patients in *Manuscript II* not only corroborate previously identified biomarkers in other populations within a Northern European context but also validate the results observed in CHE patients without AD. This is of importance as systemic biomarkers in CHE patients without concomitant AD had not been previously investigated. Our results further show the association of age with expression of several inflammatory markers in both patients with AD and non-AD CHE.

An association that has previously been reported in AD populations.[104] The identification of shared systemic type 2 inflammation in non-AD CHE and AD suggests that interventions targeting type 2 inflammation in non-AD CHE could be effective. This notion is supported by several case reports and a recent phase II dupilumab trial.[57,126–129] Additionally, we observed increased expression several other markers in non-AD CHE including Th1 associated CXCR3 ligands CXCL9-11, which suggested a systemic Th1 skewing in non-AD CHE.

In *Manuscript III* we examined the inflammatory plasma signature according to aetiological and clinical CHE subtypes. In line with findings from non-AD CHE in *Manuscript II,* we found that very severe ACD CHE was associated with mixed type 1/ type 2 inflammatory blood signature. These findings align with previous findings from ACD skin noting a prominent Th1 activation, although cutaneous Th2 activation has been reported to be dependent on the contact allergen.[86] The nature of the systemic immune activation observed ACD patients in our cohort, whether specific to contact allergens or a general response to various contact allergies, is uncertain, as our study included patients with a range of different contact allergies. Further, although not examined in HE populations, ACD has previously been associated with systemic immune activation in other clinical populations.[95,96] The correlations between circulating inflammatory biomarkers and HECSI score were stronger in ACD CHE (*Manuscript III*) than seen for the non-CHE group in *Manuscript II*. And notably, levels of several CXCR3 ligands showed positive and strong correlations with HECSI score in ACD patients. These findings are consistent with those from ACD skin which demonstrate increased gene expression of CXCR3 ligands.[93] This supports the significant involvement of CXCR3 signalling and type 1 inflammation in ACD.

The patient pool with a unique ICD diagnosis was too small to stratify by clinical disease severity, and future studies are needed for the identification of any potential systemic biomarkers associated with this aetiology. We did not find any distinguishing circulating markers between ICD and ACD. However, this comparison was complicated by the fact that a limited number of patients had a unique ICD diagnosis.

The main finding from the analyses on the clinical subtypes in *Manuscript III* was that hyperkeratotic CHE was associated with increased expression of numerous inflammatory and CVD risk proteins, with highest significance found for Th1 and TNF associated markers. Further, this subtype could be discriminated from both chronic fissured and vesicular CHE by showing increased expression of CCL19 and CXCL9-10. These findings of a rather psoriasiform systemic footprint

align with studies from skin, showing a larger resemblance of hyperkeratotic CHE to psoriasis than to other CHE subtypes.[115–117]

Based on the results from *Manuscripts II and III* we cannot determine the source of the inflammatory plasma proteins. This uncertainty stems from the cross-sectional nature of the studies and the fact that these markers were not also analysed in skin samples. Additionally, there may be other contributing factors, not accounted for in our analyses, that could have influenced the systemic inflammation observed in the described groups, as mentioned in the methodological consideration section of this thesis.

Molecular profile of hand eczema skin

The findings of palm specific transcriptomic signatures both for healthy and eczema affected skin are supported by the findings Wiedemann et al. (healthy palm skin)[130], and Hu et al. (lesional AD palm skin compared to other body sites).[131] This highlight the importance of considering anatomical sample site when performing molecular comparisons in CHE studies. It is well known that palmoplantar skin is structurally different than non-palmoplantar skin, e.g., it has a thicker epidermis, lack of pigmentation and hair, and a unique expression of K9.[130,132] Wiedemann et al. further showed that healthy palmoplantar skin has an altered, dampened immune environment as compared to non-palmoplantar skin.[130] We also found low expression of immune signalling markers in healthy palm skin. One other study has investigated the transcriptome of CHE (vesicular HE) from biopsies, and they also described very low expression levels of immune signalling markers in CHE.[12] However, a direct comparison between our results and those of Voorberg et al. is complicated by the fact that Voorberg et al. exclusively profiled the biopsied epidermis, whereas we performed bulk RNA seq on full-thickness biopsies which also include the dermis.

We characterized a heterogeneous dysregulated immune system in lesional CHE compared to healthy palm skin. Dominating activated immune pathways included Th1 and Th2 and top upstream regulators were molecules associated with these pathways as well as with IL-1 signalling. In our analyses on subtypes, we found that the lesional molecular signature was primarily shared across subtypes categorized both by AD status, and by unique aetiology. The main clinical implications of these findings are, that treatments targeting these characterized shared activated pathways and upstream regulators might be effective options for many CHE patients, regardless of AD status and aetiology.

As summarized in the background section of this thesis, other studies have also pointed to a shared immune profile between non-AD CHE and AD-CHE as well as between vesicular HE and AD at other body sites. The successful cross-over application of dupilumab, originally developed for AD, in treating non-AD CHE[57] further highlights an important role of IL-4 signalling in the pathophysiology of non-AD CHE. Importantly, in *Manuscript IV* we show that IL-4 is indeed among the top significant upstream regulators across subtypes.

We did not find any distinguishing skin markers between ACD and ICD. However, we did note some differences in pathway and upstream regulator activity between the subtypes. Several patch test studies have investigated potential biomarkers to distinguish allergic from irritant reactions and proposed various discriminating biomarkers, as described in the background section of this thesis. However, no biomarkers have been consistently replicated across studies. Our inability to replicate these previous suggested biomarkers may stem from differences in sampling methods and molecular profilings and, importantly, between experimentally induced acute eczema at a previously unexposed and different body site (the back) and the more complex, chronic eczema seen on the hands in the clinic. Importantly, and as previously described, it is difficult to completely rule out an irritant aggravating component in both ACD and AD CHE, and misclassification to some extent cannot be ruled out.

Investigating the distinctions between acute and chronic forms of contact dermatitis (ACD and ICD) in both clinical and experimental settings could deepen our insight into the immune processes underlying these conditions. It could be hypothesized that significant variances between ACD and ICD primarily manifest during the early, acute stages of these diseases. However, as these conditions progress to their chronic stages, the differences between ACD and ICD may become less pronounced, suggesting a convergence in the immunological response. Another important factor, which was not considered in *Manuscript IV*, is the potential effect of microbiome alterations on the molecular profile of CHE. Severe HE in particular has been associated with increased *S. aureus* colonization[112], and most patients included in *Manuscript IV* had severe to very severe CHE.

Common findings from skin and blood

Although we did not leverage omics from different tissues in the manuscripts included in this thesis, some common findings were noted between investigations from skin (*Manuscript IV*) and blood (*Manuscripts II-III*). We found evidence of both local and systemic activations of Th1 and Th2 type immunity in CHE. Further, we observed an increased expression of CXCR3 ligands (CXCL9-11) in

both skin (all subtypes) and blood (specifically in very severe non-AD CHE, very severe ACD CHE, and hyperkeratotic CHE). CXCR3 ligands are traditionally considered linked to Th1-driven inflammation. However, IL-4 has been shown to enhance keratinocyte expression of CXCR3 ligands in contact dermatitis.[133] Thus, the role of CXCR3 signaling in CHE needs further exploration. In particular, it remains to be investigated whether the effect of IL-4 inhibition on hyperkeratotic CHE,[129] and other non-AD CHE subtypes[57], can be partially linked to the modulation of CXCR3 signaling pathways.

8. Conclusions and Future Perspectives

Collectively, the results from *Manuscript I* show that HE is associated with a considerable burden, both for society and for the individuals afflicted by the disease. These findings indicate unmet needs for individuals with HE, in particular for high-risk groups including those with occupational, chronic, and severe disease. A recent Danish randomised trial found that early and specialized treatment of occupational contact dermatitis, inspired by a German-intervention programme, decreased eczema severity and increased the likelihood of having consulted a dermatologist at three months follow-up.[134] Further follow-up from this or other similar Danish studies is needed to show the long-term effects of these intervention measures, with the ultimate goal being to implement such measures in the Danish society and improve the prognosis and quality of life of those affected by occupational contact dermatitis. With regards to the many affected by chronic and moderate-to-severe HE, future studies are needed to shed light on the medical attention seeking behaviour among these individuals, as done by Hald et al. in 2006.[33] In 2006, a large proportion of those with moderate to severe HE were not seen by a dermatologist, which might negatively affect the prognosis of the disease.[33] Identifying and addressing personal and healthcare barriers to early and specialized treatment of HE in Denmark could potentially lead to a lightened personal and societal disease associated burden.

The molecular investigations of HE included in this thesis (*Manuscripts II-IV*) provide novel insights into both systemic and local molecular fingerprints of the disease. Collectively, a Th1 and Th2 skewed immune profile was characterized, both in skin (*Manuscript IV*) and in the systemic circulation of CHE patients with very severe disease (*Manuscripts II-III*). A distinct plasma-inflammatory signature of hyperkeratotic CHE was characterized (*Manuscript III*). However, no biomarkers were found to distinguish ACD from ICD neither in skin (*Manuscript IV*) nor in blood (*Manuscript III*). In accordance, the skin transcriptomic investigations of *Manuscript IV* revealed a broadly shared molecular endotype across CHE subtypes categorized both by AD status and by unique aetiology. Furthermore, key molecular disease drivers were found to be shared across these subtypes, underscoring potential targets for universal treatment strategies.

Future research is essential to deepen our understanding of the pathogenesis and molecular patterns of HE. While molecular studies on ACD, ICD, and AD provide valuable insights, the unique structural and immunological characteristics of palm skin and the constant exposure of hands to physical and environmental factors make a direct translation of these findings to a clinical CHE setting challenging.

Studies including larger homogenous groups of both clinical and aetiological HE subtypes which employ state of the art profiling methods such as proteomics or single cell RNA-seq might further advance our knowledge of the disease. A uniform characterization of HE characteristics across studies and subsequently sharing of data would increase comparability between studies and enable future meta-analysis derived molecular profiles of HE. Furthermore, a multi-omics approach including profiling from both skin and blood and the use of data-driven, unsupervised analyses might further enhance our knowledge on any disease associated endotypes. It is possible that future stratifications of HE patients, as proposed for patients with AD[108], will rely more on the key molecular disease drivers among each individual with HE. This could enable a more endotype specific therapeutic management (personalized medicine).

Below are some additional points that warrant further investigation:

- **Do circulating biomarkers correlate with HE severity over time?**
  Based on the findings from *Manuscript II and III* it would be interesting to investigate, whether circulating inflammatory biomarkers (e.g., CCL17, CCL13, MMP12, and CXCL9-11) correlate with clinical HE severity over time in a prospective study, for example in a clinical trial investigating response to a specific treatment.
- **CXCR3 signalling in HE**
  Increased expression of CXCR3 ligands CXCL9-11 was found both in skin and blood among several CHE subtypes (*Manuscripts II-IV*). Future studies are needed to elucidate the role of CXCR3 signalling in HE.
- **Acute versus chronic HE**
  Investigations of molecular differences between acute and chronic forms of HE would be valuable, particularly if investigated prospectively.
- **Genetic risk factors for HE**
  Genome wide association studies (GWAS) with large sample sizes could identify hitherto unknown genes associated with HE development, independent of AD history.

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