UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

# EPIDEMIOLOGY OF DERMATITIS

## - A CHARACTERIZATION OF GENETIC PREDISPOSITION AND PERSONAL CONSEQUENCES

## PhD Thesis

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# Epidemiology of dermatitis – a characterization of genetic predisposition and personal consequences

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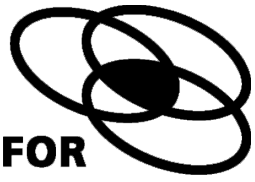

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**This PhD thesis is based on the following four manuscripts:**

The manuscripts will be referred to using their roman numerals throughout the thesis.

**I** Heede NG, Thyssen JP, Thuesen BH, Linneberg A, Johansen JD. *Anatomical patterns of dermatitis in adult filaggrin mutation carriers.* Journal of the American Academy of Dermatology (2015) 72: 440-8.

**II** Heede NG, Thyssen JP, Thuesen BH, Linneberg A, Johansen JD. *Predictive factors of self-reported hand eczema in adult Danes: a population-based cohort study with 5-year follow-up.* British Journal of Dermatology (2016) 175: 287–295.

**III** Heede NG, Thyssen JP, Thuesen BH, Linneberg A, Szecsi PB, Stender S, Johansen JD. *Health-related quality of life in adult dermatitis patients by filaggrin genotype.* Submitted for publication in contact dermatitis (2016).

**IV** Heede NG, Thyssen JP, Thuesen BH, Linneberg A, Szecsi PB, Stender S, Menné T, Johansen JD. *Hand eczema, atopic dermatitis, and filaggrin mutations in adult Danes: a registry-based study including risk of disability pension.* Submitted for publication in British Journal of Dermatology (2016).

# PREFACE

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With appreciation,

Nina Glasser Heede

Gentofte, 12 September 2016

## ABBREVIATIONS

The following are listed alphabetically; some abbreviations are used only in tables and figures:

| | |
|---|---|
| **AD** | Atopic dermatitis |
| **CI** | Confidence interval |
| **DLQI** | Dermatology life quality index |
| ***FLG*** | Filaggrin gene |
| **FLGmut** | Filaggrin mutation carrier |
| **FLGwt** | Filaggrin wild type |
| **HE** | Hand eczema |
| **HRQoL** | Health-related quality of life |
| **OR** | Odds ratio |

# SUMMARY

## Background and aims

Atopic dermatitis and hand eczema are widespread in the general population with an estimated one-year prevalence in adults of 2–15% and 10%, respectively. Loss-of function filaggrin gene (*FLG*) mutations are also common in Northern European populations and the prevalence in the Danish population is around 8%. Filaggrin deficiency has been shown to result in impaired skin barrier integrity and the *FLG* mutations are further identified as the strongest genetic factor for the development of atopic dermatitis. Additionally, *FLG* mutations have been found to be predictive factors of persistent hand eczema in individuals with atopic dermatitis; the interplay between hand eczema, *FLG* mutations and atopic dermatitis is, however, still to be elucidated.

The personal and societal consequences of dermatitis are substantial and include reduced quality of life, increased healthcare costs and, in the worst case, sick leave, job change, rehabilitation and/or disability pension. It is currently unknown whether *FLG* mutation carriers, who often experience severe and persistent disease, experience worse consequences than do individuals without *FLG* mutations.

The overall objective of the thesis was to investigate the epidemiology of dermatitis and look into the role of genetic pre-disposition, defined by *FLG* mutations, and personal consequences. In detail the aims were:

- To investigate the epidemiology of dermatitis in the general population including prevalence, anatomical localization and association with *FLG* mutations (Manuscript I).
- To investigate incidence and predictive factors of hand eczema in the general adult population (Manuscript II).
- To characterize the adult dermatitis patient with and without *FLG* mutations focusing on health-related quality of life (HRQoL), skin characteristics and comorbidity (Manuscript III).
- To investigate occupational consequences and previous work in risk occupations among the adult population with or without dermatitis and *FLG* mutations (Manuscript IV).

## Methods

This thesis builds on data from two populations: I) a population-based cohort study with a 5-year follow-up called “Health2006” and II) a cross-sectional study of adult dermatitis patients included with atopic dermatitis and/or hand eczema. Data from participants from both populations were transferred to Statistics Denmark and linked to central registries for information about socio-economy, occupation, and social benefits. In addition, all participants completed the same questionnaire about skin symptoms and

dermatitis. Hand eczema, for both populations, was self-reported whereas a history of atopic dermatitis was defined by the UK criteria in the general populations and was clinician diagnosed in the patients. All participants were genotyped for three of the most common Northern European loss-of-function *FLG* mutations, which together constitute 83% of the total risk alleles associated with atopic dermatitis: R501X, 2282del4, and R2447X. Manuscripts I and II are based on data from the Health2006 population and Manuscript III is based on data from the patient population. Manuscript IV is based on data from both populations and from Statistics Denmark.

## Results

The overall estimated lifetime prevalence of unspecified dermatitis in the general population was 37.8%. We also found that *FLG* mutations were associated with dermatitis on the hands and feet in individuals with atopic dermatitis (Manuscript I). In our analyses investigating predictive factors of hand eczema in adult Danes in the general population, we found that a history of atopic dermatitis predicts both incident and persistent hand eczema (odds ratio (OR) = 9.0; 95% confidence interval (CI) 5.6–14.4 and OR = 3.0; 95% CI 1.7–5.2, respectively). In contrast, *FLG* mutations predicted only persistent hand eczema in individuals with atopic dermatitis and were not associated with incident hand eczema in adults, suggesting that *FLG* mutations as a predictive factor for hand eczema decrease with time. Lastly, contact sensitization was also associated with persistent hand eczema (OR = 2.5; 95% CI 1.2–5.0), independently of a history of atopic dermatitis (Manuscript II). In relation to HRQoL, we found that patients with atopic dermatitis (± hand eczema) and *FLG* mutations reported reduced HRQoL when compared with patients with *FLG* wild type suggesting that this subgroup of patients might experience an additional challenge in their everyday life (Manuscript III). Lastly, we found that self-reported dermatitis, particularly in individuals with *FLG* mutations, was significantly associated with receiving disability pension in the general population. However, the primary diagnosis for awarding disability was unknown (Manuscript IV).

## Conclusions

Taken together, our results indicate that *FLG* mutation carriers with atopic dermatitis are a subgroup of individuals who stand out on several parameters. The parameters are biologically manifested by increased prevalence of foot dermatitis and increased persistence of hand eczema, psychologically manifested by reduced HRQoL, and socially manifested by the finding that self-reported dermatitis was associated with receiving disability pension, particularly in individuals with *FLG* mutations. These findings points towards *FLG* mutations predisposing to increased severity, highlighting the need for increased skin awareness in this subgroup.

## DANSK RESUMÈ (SUMMARY IN DANISH)

### Baggrund og formål

Atopisk eksem og håndeksem er hyppige hudsygdomme i den danske befolkning og har en estimeret 1-års prævalens blandt voksne på henholdsvis 2-15 % og 10 %. Mutationer i genet, der koder for hudproteinet filaggrin, er også hyppige. Omkring 8 % af den danske befolkning har mindst én mutation i filaggrin genet (*FLG*) hvilket betyder, at de personer har en delvis eller total filaggrinmangel. Filaggrinmangel medfører en nedsat funktion af hudbarrieren, som er den barriere, der beskytter huden imod påvirkninger fra omgivelserne. *FLG* mutationer har yderligere vist sig at være en stærk genetisk risikofaktor for udvikling af atopisk eksem. Derudover er *FLG* mutationer blevet identificeret som en risikofaktor for håndeksem, dog kun blandt personer, der har haft atopisk eksem. Samspillet mellem *FLG* mutationer, atopisk eksem og håndeksem er komplekst og er endnu ikke fuldt belyst.

Eksem har både store personlige og samfundsmæssige konsekvenser, da sygdommen er associeret med reduceret livskvalitet, øget forbrug af sundhedsydelser og i de værste tilfælde, sygefravær, jobskifte, revalidering og/eller førtidspension. Det er endnu uvist hvorvidt personer med *FLG* mutationer, der ofte oplever svær og vedvarende sygdom også oplever større konsekvenser sammenlignet med personer uden *FLG* mutationer.

Det overordnede formål med denne Ph.d.-afhandling var, at undersøge epidemiologien af eksem blandt voksne danskere, både blandt befolkningen og eksempatienter, og se på samspillet med genetisk disponering, defineret med *FLG* mutationer, og personlige konsekvenser. De enkelte formål var:

- At lave en epidemiologisk undersøgelse af eksem i den generelle befolkning inklusiv prævalens, anatomisk lokalisation og samspil med *FLG* mutationer (Manuskript I).
- At undersøge incidensen og prædiktive faktorer for håndeksem i den generelle voksne befolkning (Manuskript II).
- At karakterisere voksne eksempatienter, med eller uden *FLG* mutation, med fokus på sygdomsrelateret livskvalitet, hudkarakteristika og komorbiditet (Manuskript III).
- At undersøge arbejdsrelaterede konsekvenser og historie i risikoerhverv blandt voksne danskere, med og uden *FLG* mutationer (Manuskript IV).

### Metode

Denne afhandling er baseret på data fra to populationer: I) et populationsbaseret kohortestudie med en 5-års opfølgning kaldet ”Helbred2006” og II) en tværsnitsundersøgelse af voksne eksempatienter inkluderet med atopisk eksem og/eller håndeksem. Data fra begge populationer er derudover blevet overført til Danmarks Statistik, og linket til centrale registre for information omkring socioøkonomi, beskæftigelse og

sociale ydelser. Alle deltagere udfyldte ydermere et spørgeskema omkring hudsymptomer og eksem. Håndeksem for begge populationer var selvrapporteret, hvorimod diagnosen omkring atopisk eksem var defineret ved hjælp af UK kriterierne i befolkningen og var lægediagnosticeret blandt patienterne. Alle deltagerne blev derudover genotypet for tre af de mest almindelige *FLG* mutationer i Nordeuropa, der tilsammen repræsenterer 83 % af risikoallelerne for atopisk eksem; R501X, 2282del4 og R2447X. Manuskript I og II er baseret på data fra 'Helbred2006' populationen mens Manuskript III er baseret på data fra patientpopulationen. Manuscript IV er baseret på data fra begge populationer og fra Danmarks Statistik.

## Resultater

Den samlede livstidsprævalens for uspecificeret eksem i den danske befolkning var 37,8 %. Derudover fandt vi, at *FLG* mutationer disponerede særligt til eksem på hænder og fødder blandt personer med tidligere atopisk eksem (Manuskript I). I vores analyser omkring prædiktive faktorer for håndeksem fandt vi, at tidligere atopisk eksem prædikterer både incident og persisterende håndeksem hos voksne (henholdsvist, OR = 9.0; 95%CI 5.6–14.4 og OR = 3.0; 95%CI 1.7–5.2). Derimod prædikterede *FLG* mutationer kun persisterende håndeksem i personer med tidligere atopisk eksem, og var ikke associeret med incident håndeksem blandt voksne hvilket indikerer, at *FLG* mutationer udspiller deres rolle tidligt i livet. Ydermere fandt vi, at allergisk sensibilisering var associeret med persisterende håndeksem uafhængigt af tidligere atopisk eksem (OR=2.5; 95%CI 1.2–5.0) (Manuskript II). I forhold til sygdomsrelateret livskvalitet fandt vi, at patienter med atopisk eksem (± håndeksem) og *FLG* mutationer rapporterede reduceret livskvalitet sammenlignet med patienter uden *FLG* mutationer hvilket indikerer, at denne undergruppe af patienter særligt kan opleve udfordringer i deres dagligdag (Manuskript III). Derudover fandt vi, at selv-rapporteret eksem, særligt blandt personer med *FLG* mutationer, var associeret med at være førtidspensionist i den generelle befolkning. Den primære diagnose for at have fået tildelt førtidspension var dog ukendt i vores analyse (manuskript IV).

## Konklusion

Alt I alt indikerer vores resultater at personer med *FLG* mutationer og tidligere atopisk eksem er en særlig undergruppe som adskiller sig på flere parametre; biologisk i form af øget prævalens af eksem på hænder og fødder og psykisk i form af reduceret hudspecifik livskvalitet. Derudover fandt vi også en social parameter idet selvrapporteret eksem var særligt associeret med førtidspension blandt deltagere med *FLG* mutationer. Disse fund peger imod at *FLG* mutationer disponerer til øget sværhedsgrad af eksem og øger behovet for viden omkring hudsymptomer blandt disse personer.

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# 1. INTRODUCTION

Dermatitis is the largest disease group within dermatology and comprises several disease manifestations, the most prevalent ones being atopic dermatitis and contact dermatitis, which can be present at any localization, e.g. hand eczema, facial dermatitis or foot dermatitis. The focus on dermatitis has increased markedly through the last decades. This is reflected in the increasing number of scientific publications focusing on various aspects of the disease including the underlying biology, diagnosis, treatment, and personal consequences. The importance of skin barrier integrity and its role in dermatitis has been widely discussed, and the theories suggesting a genetic predisposition to dermatitis were revolutionized in 2006 with the identification of the *FLG* mutations.[1]

This thesis, entitled, "*Epidemiology of dermatitis – a characterization of genetic predisposition and personal consequences*" focuses on dermatitis in the general population and in patients and looks into the interplay between atopic dermatitis, hand eczema, and *FLG* mutations as well as personal consequences including HRQoL, socioeconomic measures and history in risk occupations. The background for the four manuscripts included in the thesis is introduced in the following sections.

## 1.1 Filaggrin and its role in the skin barrier

The skin is the body's first line of defence against invading pathogens and external stimuli from the environment. The epidermis is 0.05–1 mm thick and is the outer layer of the skin, which can be further divided into the basal layer, spinous layer, granular layer and stratum corneum.[2] In short, epidermal cells (95% keratinocytes) divide in the basal layer and move upwards while differentiating to ultimately end up in stratum corneum, the outermost layer of the epidermis, as corneocytes without nuclei and cytoplasmic organells.[2] The terminal differentiation is a continuous process and renewal of the epidermis takes approximately 1 month in humans.[3]

In 1977, the filaggrin protein was purified for the first time from the stratum corneum of rat epidermis and was named "stratum corneum basic protein".[4] Four years later, the protein was renamed as filaggrin as a consequence of its unique biological function of aggregating intermediate filaments (filament aggregating protein), particularly keratins.[5] Filaggrin derives from a large precursor protein called profilaggrin. Profilaggrin is a major component of the keratohyalin granules, which are organelles found in the granular layer of the epidermis.[6] During the late stages of terminal differentiation, where transition from the granular to the terminally differentiated cornified cells occurs, profilaggrin is dephosphorylated and proteolyzed into multiple filaggrin monomers in the epidermis.[7,8] The profilaggrin molecule is processed

into 10–12 filaggrin monomers.[9] The functional filaggrin monomers hence align keratin filaments to form the interfilamentous matrix in stratum corneum.[10] Subsequently, filaggrin degradation results in the formation of free amino acids and their derivatives, including, for example 2-pyrrolidone-5-carboxylic acid and trans-urocanic acid, which are part of the natural moisturizing factors ensuring epidermal hydration, photoprotection and maintenance of the acid mantle.[11]

Chromosome 1 is our largest chromosome and contains about 8% of the entire human genomic information. *FLG* is located within the epidermal differentiation complex on chromosome 1 region 1q21.[12] Mutations in the *FLG* are among the most prevalent single-gene mutations identified to date.[Reviewed in 13] Up to 10% of the population with Northern European origin are heterozygous carriers of a loss-of-function mutation within the *FLG*, resulting in a 50% reduction of expressed protein.[14,15] Figure 1 illustrates filaggrin, and profilaggrin, expression in the different layers of the epidermis for an individual with normal filaggrin expression (A) and a patient with total filaggrin deficiency (B).

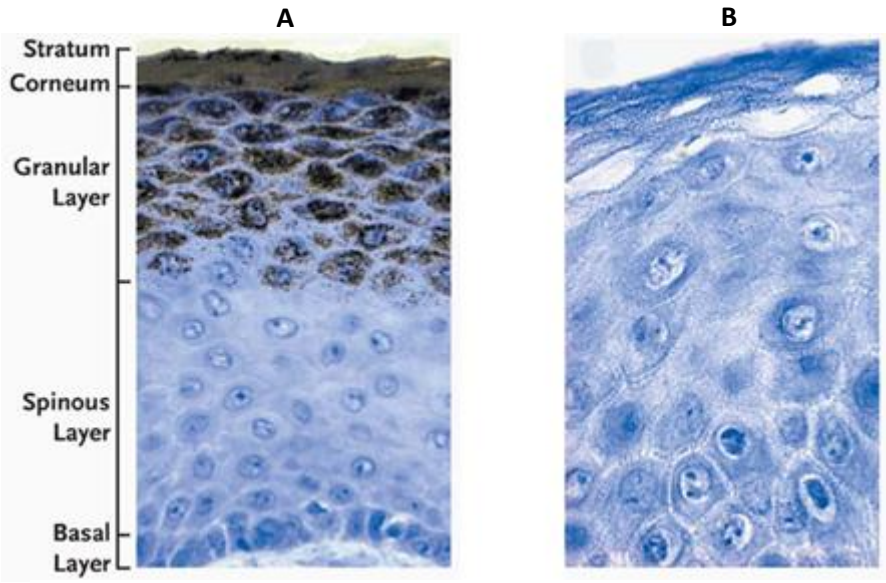


**Figure 1: Filaggrin stain of the epidermis**
Immunohistochemical staining for profilaggrin and/or filaggrin shows staining in the granular layer and stratum corneum (panel A) in contrast to the staining from a patient homozygous for loss-of-function mutations in the filaggrin gene (panel B). Reproduced with permission from Irvine *et al*., 2011, N Eng J Med,[13] Copyright Massachusetts Medical Society.

Including the two initially reported mutations (R501X and 2282del4), more than 50 additional mutations have been identified throughout the profilaggrin molecule, of which many European-specific and Asian-specific mutations exist.[13,16] The three mutations R501X (39%), 2282del4 (41%) and S2447X (3%) together constitute 83% of the total risk alleles associated with development of atopic dermatitis.[13]

The filaggrin protein has been shown to have numerous functions in epidermis, and mutations in the *FLG* have been shown to result in an impaired skin barrier associated with both skin and allergic diseases. This is presented in the following sections.

## 1.2 Atopic dermatitis

Atopic dermatitis is a highly pruritic inflammatory skin condition with chronic or recurrent episodes of dermatitis. The clinical manifestations of the condition are thought to vary with age, but all stages are characterized by pruritus and xerosis. In infancy, lesions usually emerge on the cheeks and scalp. Later in childhood, and through adolescence and adulthood, lesions are usually localized to flexures, the neck and the dorsal aspects of the limbs. [Reviewed in 17]

An increase in both prevalence and incidence of atopic dermatitis has been reported since World War II.[18,19] Today, the lifetime prevalence of atopic dermatitis is estimated to be around 20% in Denmark, in Western Europe and in the United States.[20-24] The proportion of adults experiencing symptoms of atopic dermatitis is uncertain but persistency of the disease into adulthood and late onset is common.[25-27] A meta-analysis from 2016 found that 80% of childhood atopic dermatitis did not persist beyond the age of eight years and that less than 5% of cases of childhood atopic dermatitis persisted 20 years after diagnosis.[28] In contrast, a prospective Danish cohort study with a 15-year follow-up reported persistence of atopic dermatitis in 50% of those diagnosed in school age.[27] Another Danish study found that the 1-year prevalence of atopic dermatitis was 14.3% in adults aged 30–89 years and that the prevalence decreased with increasing age.[29] In 1996, Herd and colleagues reported that adults over 16 years made up 38% of all patients with atopic dermatitis and proposed a future cohort effect as a consequence of the rising prevalence.[25] In line with this, a recent cross-sectional cohort study found that it was not until the age of 20 years that 50% of patients had at least one lifetime episode of a 6-month symptom- and treatment-free period.[26] Thus, atopic dermatitis is not only a childhood disease but can be considered as a life-long phenotype.

A genetic component for the development of atopic dermatitis has been suspected since the 1980s.[19] In 2006, loss-of-function variants within the *FLG* were identified as the strongest risk factors for the development of atopic dermatitis.[1] Today, numerous studies have confirmed this association and meta-analyses have estimated the overall OR to range from 3.12 to 4.78.[30,31] Despite the presence of *FLG* mutations being the strongest known risk factor for atopic dermatitis, around half (46%) of the individuals heterozygous for *FLG* mutations will not develop signs of dermatitis, underlining that environmental factors are important.[32] A strong association between *FLG* mutations and both early-onset and persistent disease has however been shown.[33-35] In addition, the rate of children “growing out’’ of their atopic dermatitis has been reported to be much lower among individuals with *FLG* mutations.[32] Genome-wide association studies

later identified new risk loci for atopic dermatitis, among others, stressing the importance of epidermal barrier function and immune dysregulation in disease pathogenesis.[36,37]

### *1.2.1 Comorbidity associated with atopic dermatitis and filaggrin mutations*

Atopic dermatitis is further considered to be the first clinical manifestation of the atopic march, which describes the phenomenon in persons with early onset of atopic dermatitis of increased risk of developing allergic rhinitis and/or asthma, and possible food allergy.[38] Approximately 70% of patients with severe atopic dermatitis will develop allergic rhinitis or asthma later in life.[39] The relevance of using the term 'atopic march' has, however, been questioned recently as a consequence of different time patterns in developing atopic dermatitis, asthma and allergic sensitization.[40,41]

Interestingly, *FLG* mutations have been found to be risk factors for rhinitis,[31] asthma,[30,42] and food allergy[43], primarily in co-occurrence with atopic dermatitis. In support of this, it has been hypothesized that an impaired skin barrier can provide entry for environmental allergens and thereby function as a route of primary sensitization.[44-46] Notably, *FLG* mutations were recently found not to be associated with food and aeroallergen sensitization in adults without concomitant atopic dermatitis.[47] Moreover, results from population studies have shown that *FLG* mutation carriers are at increased risk of developing early-onset and persistent hand eczema, but only in co-occurrence with atopic dermatitis.[48]

In contrast to the well-known association between *FLG* mutations and ichthyosis vulgaris,[**1,49**] only few studies have investigated associations between *FLG* mutations and skin cancer. It is widely recognised that UV exposure is an indirect cause of skin cancer—non-melanoma skin cancer (squamous cell carcinoma and basal cell carcinoma); malignant melanoma; and pre-stages to non-melanoma skin cancer, actinic keratosis.[50] Experimental data have indicated that filaggrin deficiency alone can impair the epidermal barrier function, resulting in increased UV sensitivity in human skin models, most likely as a consequence of reduced levels of *trans*-urocanic acid.[51] However, previous studies investigating the association between filaggrin and skin cancer are ambiguous. A population-based cohort study found no association between *FLG* mutations and squamous cell carcinoma or malignant melanoma,[52] whereas another study suggested that complete filaggrin deficiency is associated with squamous cell carcinoma.[53] Further, no association between *FLG* mutations and basal cell carcinoma was found when comparing the proportion of *FLG* mutation carriers in patients with basal cell carcinoma with that of the general population.[54]

### *1.2.2 Acquired filaggrin deficiency*

When investigating the role of *FLG* mutations in dermatitis, acquired filaggrin deficiency must be highlighted as an important modulator. Elevated levels of inflammatory cytokines have been shown to affect filaggrin expression and can result in an acquired filaggrin deficiency.[55-61] As $T_H2$-associated cytokines

are one of the hallmarks of acute atopic dermatitis, the initial studies investigated the effect of IL-4 and IL-13 on filaggrin expression.[55] More recently, a whole panel of cytokines associated with atopic dermatitis, including IL-17A, IL-22, IL-25 (IL-17E), IL-31, and TNF-alpha, have been associated with acquired filaggrin deficiency in *in vitro* studies.[55-61]

Figure 2 illustrates the complex relationship between impaired barrier function, immunologic hyper-reactivity and acquired filaggrin deficiency.

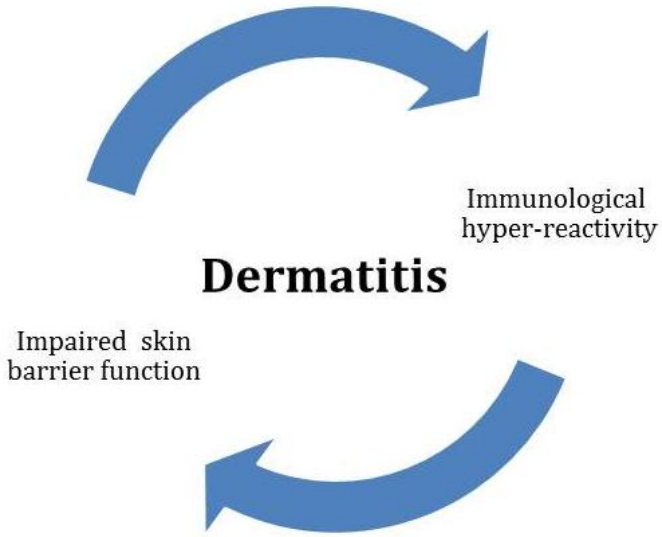


**Figure 2:** Illustration of the interplay between impaired skin barrier function and immunological hyper-reactivity seen in patients with atopic dermatitis and with hand eczema.

Thus research investigating immune dysregulation in patients with atopic dermatitis has shown that acquired filaggrin deficiency is common, which suggests that disease severity affects filaggrin expression, irrespective of *FLG* genotype. Filaggrin expression was also found to be down regulated in nonlesional skin of adult patients with atopic dermatitis.[56] Lastly, prolonged use of topical corticosteroids can reduce epidermal filaggrin levels.[62]

Research regarding the immunological profile of hand eczema is limited and studies focusing on acquired filaggrin deficiency in hand eczema are sparse. Nevertheless, it has been reported that skin barrier dysfunction also plays a key role in the pathogenesis of chronic hand eczema which, among others proteins, results on down-regulation of the filaggrin protein.[63]

## 1.3 Hand eczema – a frequent disease manifestation

Hand eczema is an inflammatory skin condition with various clinical manifestations including erythema, cell infiltration, hyperkeratosis, oedema and vesicles. Moreover, secondary signs exist, such as scaling, hyperkeratotic areas, skin fissures and bacterial infections primarily with *Staphylococcus aureus.*[64]
Often, morphology and symptoms vary over time. In general, hand eczema often starts as acute dermatitis characterized by erythema, oedema and vesicles, which can develop into chronic dermatitis characterized by hyperkeratosis, infiltrations and fissures.[64]

Hand eczema is a multifactorial condition that usually develops as a consequence of repeated or prolonged contact with irritant and/or allergic compounds (contact dermatitis). It particularly affects individuals with a history of atopic dermatitis and women.[64-66] Most cases of contact dermatitis manifest as hand eczema and factors frequently involved in the aetiology are wet work, detergents, sensitizing chemicals, regular use of occlusive gloves and exposure to food proteins.[64] The pathogenesis of hand eczema is complex, challenging the traditional clinical distinction between irritant, allergic and atopic phenotypes. Thus atopic dermatitis can also be manifested on the hands in adults. Frequent sub-diagnoses, or combinations of sub-diagnoses, have been proposed based on data from 319 patients from 10 European patch test clinics.[67] Here, irritant contact dermatitis (21.5%) was the most frequent subtype, followed by allergic contact dermatitis (15.2%), irritant contact dermatitis + allergic contact dermatitis (15.2%), vesicular hand eczema (9.3%), atopic hand eczema + irritant contact dermatitis (7.8%) and atopic hand eczema (5.8%).[67]

The life-time prevalence of hand eczema in the adult Scandinavian general population has been found to range between 15% and 21.8%,[68,69] and the 1-year prevalence is estimated to be nearly 10%, while the point prevalence was estimated to be 4%, in a review mainly based on European studies.[70] The incidence of hand eczema varies within age groups and the incidence rate of self-reported hand eczema has been reported to peak among young woman aged 20–29 years and subsequently decrease with age.[71] The fact that hand eczema has repeatedly been shown to be a female-dominated condition[21,69,72] has been explained by gender differences in domestic and occupational exposures to irritants and allergens, rather than by susceptibility differences between men and woman.[70,73] However, individual susceptibility to hand eczema has been found to be associated with atopic dermatitis. Rystedt and colleagues showed already in 1985 that patients with persistent and recurrent atopic dermatitis had an increased risk of developing hand eczema in adulthood.[74] Atopic dermatitis has since been confirmed as a risk factor for hand eczema, also in prospective cohorts of adolescence from the general population.[66,75] Other risk factors for hand eczema, apart from female sex and atopic dermatitis, include contact allergy, wet work, xerosis, and a high use of tobacco.[65,70,76-79] As mentioned, *FLG* mutation carriers with atopic dermatitis have been found to have an

increased risk of persistent hand eczema;[48] however, the interplay between hand eczema, *FLG* mutations and atopic dermatitis remains to be elucidated.

### *1.3.1 Occupational hand eczema*

Work-related skin diseases are the most prevalent condition reported to the Danish National Board of Industrial Injuries. In 2014, 2,889 cases of occupational skin diseases were reported, of which 1,616 were recognized (55.9%).[80] Because most occupational skin diseases manifest as occupational hand eczema, occupational hand eczema is the most frequently recognized industrial injury and represents a substantial expense to society.[81,82]

The epidemiology of occupational contact dermatitis has long been in focus. A review from 1999 found that the annual incidence rate of the disease was the highest among hairdressers (194 cases per 10,000 employees/year), followed by bakers (64 cases per 10,000 employees/year).[83] Moreover, the median induction period for occupational skin diseases in different professions showed that hairdressers, food industry workers, health service workers and metal workers belong to high risk professions.[83] Apart from hairdressing and health-care work, other female-dominated occupations involving extensive wet work, such as cleaning or catering, are characterized as occupations with a high risk of hand eczema.[Reviewed in 84] A recent population-based study investigating water exposure in high-risk occupations showed that more than 50% of individuals working in service occupations, including kitchen assistants, cleaners, restaurant workers and hairdressers, report water exposure for more than 2 hours a day.[85] Individuals working in occupations in the health-care sector and the construction sector had equally high proportions of water exposure.[85] As mentioned, atopic dermatitis is the most pronounced risk factor for hand eczema, and skin atopy has been estimated to double the risk of the disease in occupations where hand eczema is common.[86] In relation to skin barrier, research has shown that *FLG* mutation carriers with childhood hand eczema tend to choose occupations with a low risk of exposure to irritants.[87] This finding suggests that individuals with *FLG* mutations forfeit occupations with a high risk of dermatitis, such as wet work. Moreover, *FLG* mutations have been found to be associated with a high prevalence of contact dermatitis in construction workers.[88] However, no studies have investigated the prevalence of *FLG* mutation carriers working in a risk occupation, either among patients or in the general population.

## 1.4 Consequences of dermatitis

The personal consequences of having dermatitis, both atopic dermatitis and hand eczema, are far-reaching and include financial consequences, work-related consequences, increased use of healthcare services and decreased quality of life. Thus the overall “cost” of having dermatitis can be considerable.

### *1.4.1 Societal healthcare costs of dermatitis*

The economic burden of atopic dermatitis in the United States is estimated to range from $364 million to $ 3.8 billion (€324 million to €3.4 billion)[I].[89,90] In 1999, a German study also investigated the cost of having atopic dermatitis and found that the annual cost per patient was €2462[II] and that the estimated annual cost to society was €3.57 billion[II].[91] In addition, a recent systemic review investigating cost-of-illness studies in hand eczema found that the mean annual total cost per patient ranged from €1712 to €9792 (direct cost per patient: €521 to €3829 and indirect cost per patient: €100 to €6846) whereof occupational hand eczema patients had indirect costs up to 70% of the total costs, mainly because of sick days.[92] Thus, dermatitis represents substantial expense to both the individual and society.

An association between the severity of hand eczema and medical consultations has previously been shown.[93] Here, 63.4% of individuals with hand eczema reported medical consultations because of the disease and 15.6% reported more than five consultations.[93] Further, atopic dermatitis has been associated with increased healthcare costs,[94] and the risk of reporting more than one medical consultation due to hand eczema was increased in individuals with a history of atopic dermatitis (OR = 3.0; 95% CI 1.4–6.4).[93] To date, no studies have investigated whether individuals with a genetically impaired skin barrier have an increased use of healthcare services. Information about the use of health-care services among *FLG* mutation carriers is unknown but might be valuable since they have an increased risk of developing both atopic disorders and hand eczema and represent a considerable proportion of the population.

### *1.4.2 Socioeconomic consequences of dermatitis including work-related consequences*

Socioeconomic status can be estimated by several variables including income, family status, occupational status and degree of education. Research has shown that both increased educational level and income are associated with a better health status.[95] For patients with hand eczema, associations between middle-income households and increased prevalence of hand eczema have been suggested.[96] Moreover, reports have shown that men who live alone tend to report hand eczema more often than those who live with someone.[96] Hand eczema is further known to have widespread work-related consequences including productivity loss, sick leave, job change, rehabilitation benefits and disability pensions.[97-100] In 2005, Meding and colleagues reported that the proportion of patients with hand eczema, diagnosed in a population-based study, who experienced far-reaching consequences including extended sick leave, disability pension and changes of occupation, was about 5%.[100] It is currently unknown whether persons with *FLG* mutation have different job-related consequences compared with non-mutation carriers.

[I] The rate 1 USD = 0.89 Euro has been used to calculate the amount (11 September 2016).
[II] The original study reports an amount in German mark (DM). The rate: 1 DM = 0.51 Euro has been used.

In individuals with atopic dermatitis, associations between high parental education level and increased risk of atopic diseases in the offspring have been suggested.[101,102] Atopic dermatitis has also been associated with increased work loss when compared with controls.[103] However, epidemiological evidence targeting the relationship between socioeconomic status and dermatitis is limited[101] and the potential socioeconomic consequences of having a genetically impaired skin barrier are unknown. Figure 3 summaries the widespread consequences of having dermatitis.


**Figure 3:** Illustration of the widespread consequences associated with dermatitis (eczema). **Blue arrows** represent known consequences of dermatitis while **red dotted arrows** represent the unknown consequences of having a genetically disrupted skin barrier.

### *1.4.3 Dermatitis and health-related quality of life*

HRQoL can be estimated using the Dermatology Life Quality Index (DLQI) introduced in 1994 by Finlay and colleagues as the first skin-specific instrument to assess HRQoL.[104] The DLQI has been used to measure HRQoL in patients with both atopic dermatitis and hand eczema where increased severity has been found to be associated with greater impairment in DLQI score.[105-107] While several studies have found hand eczema to be associated with reduced HRQoL,[107] the association between hand eczema and depression and anxiety is ambiguous. A Danish study from 2006 including 758 patients with occupational hand eczema found no association between occupational hand eczema and depression,[108] whereas a German study from 2012 found a high prevalence of both anxiety and depression in the study population of patients with occupational hand eczema.[109] Higher anxiety level and increased DLQI scores were associated with female sex.[109] A multicentre study from 2015 also found that depression and anxiety were associated with hand eczema and atopic dermatitis, suggesting that there is an additional burden of having skin diseases.[110] In line with this, it has repeatedly been shown that individuals with atopic dermatitis report an increased prevalence of both depression and anxiety.[111,112] It is currently unknown whether the psychological impact of dermatitis differs between *FLG* mutations carriers and non-mutation carriers.

In light of the extensive literature investigating the biological consequences of having *FLG* mutations, it is warranted to investigate the epidemiology of dermatitis with focus on genetic predisposition and personal consequences.

# 2. METHODS: STUDY POPULATIONS AND REGISTERS

This PhD thesis builds on data from two populations: I) a population-based cohort study with a 5-year follow-up called "Health2006" and II) a cross-sectional study of adult dermatitis patients included with atopic dermatitis and/or hand eczema. Data from participants from both populations (the Health2006 cohort and patients) were transferred to Statistics Denmark and linked to central registries for information about socio-economy, occupation, and social benefits. In addition, all participants completed questionnaires about skin symptoms and dermatitis and were genotyped for three of the most common Northern European loss-of-function mutations in the profilaggrin gene which together constitute 83% of the total risk alleles associated with atopic dermatitis; R501X, 2282del4, and R2447X.[1,13,113]

## 2.1 The Health2006 cohort

The Health2006 population was established between 2006 and 2008 and has previously been described in detail.[114] Participants in the Health2006 study were adults aged 18 to 69 years, with Danish citizenship who were born in Denmark. Participants were recruited as a random sample of the population. All participants lived in one of the 11 municipalities[III] in the south-western part of the Copenhagen County.[114] The study comprised 3471 individuals (participation rate: 44.7%) and was designed to investigate research questions dealing with lifestyle-related chronic diseases in Denmark, including skin health. All participants completed detailed questionnaires, including questions on skin health, dermatitis, and co-morbidities, and underwent a general health examination at the Research Centre for Prevention and Health. In addition, blood samples were collected for *FLG* genotyping, and type IV allergy was estimated by patch testing for contact allergens incorporated in panel 1 and 2 from the standardised ready-to-apply Thin-layer Rapid Use Epicutaneous (TRUE)-test (Mekos Laboratories, Hillerød, Denmark).[114] Finally, type I allergy was estimated by measuring allergen-specific IgE in serum. All samples were analysed for specific IgE to birch, grass, cat and house dust mite (ALK-Abello A/S, Hørsholm, Denmark). The 5-year follow-up was completed between 2011 and 2013 and included questions similar to those at baseline. In total, 2308 individuals were included in the follow-up study (participation rate at follow-up 66.5%).

## 2.2 The dermatitis patient population

The dermatitis population was established in 2014 as part of this thesis. In total, 1119 adult dermatitis patients (> 18 years) were invited to participate in the study. Of these individuals, 520 with atopic dermatitis and/or hand eczema were included (participation rate 46.5%); however, one did not return the questionnaire. Clinical diagnoses of atopic dermatitis and/or hand eczema were scored by the attending dermatologist and were registered in the patient's file along with the internationally recognized MOAHLFA-

[III] Albertslund, Ballerup, Brøndby, Glostrup, Herlev, Høje Taastrup, Hvidovre, Ishøj, Ledøje-Smørum, Rødovre and Vallensbæk

index (Male, Occupational dermatitis, history of Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, and Age above 40 years)[115] which is registered routinely for all patients who are patch tested. The patch test database at Gentofte Hospital contains clinical data on all patients examined during 1985–2016. All patients were seen in the clinic between 2006 and 2012. In the clinic, the diagnosis of atopic dermatitis represents a lifetime prevalence and is based on family history, flexural dermatitis and typically childhood onset. Hand eczema is a clinical diagnosis made according to the guideline of the Danish Dermatological Society.[116] The diagnoses was made by the attending doctor and was recorded in the database together with results from patch testing (European baseline series, and relevant additional test series according to exposure analysis, in accordance with the European patch test guidelines),[117] and skin prick tests testing type I allergies. 97.3% of the population were Danish citizens. Patients collected genomic DNA by a buccal swap (Isohelix, Harrietsham, United Kingdom) and completed a questionnaire about skin health and dermatitis. Epidata was used as the data entry module for questionnaire data.[118] A random check was performed on 10% of the questionnaires entered in Epidata and showed an error rate of 0.28%.The project was reported to, and approved by, the Danish Data Protection Agency.

## 2.3 The Danish registries

To address the personal consequences of having dermatitis, data from participants from both populations were transferred to Statistics Denmark and linked to central registries for information about socio-economy, occupation, and social benefits. Statistics Denmark administers an extensive database of register data that enables various research possibilities. Pseudo anonymised micro data, at individual level, are available in different registers, which are updated yearly. Participants were linked to the Danish National Registers using their unique personal identification numbers (CPR numbers). Data in this dissertation originate from two separate data extractions including 4584 individuals: 3471 individuals from the Health2006 cohort and 1119 individuals from the patient population, of whom 520 were included in the study (participation rate 46.5%). The total number of participants only adds up to 4584 because 6 of the participants in the Health2006 population were also invited to the patient study; 5 chose to participate. These 6 individuals were deleted as participants in the patient population in Manuscript IV and appear only once in the analyses.

In the first data extraction, the general population was linked to the Danish registers in 2006 and the patients were linked in 2013. The following variables were retrieved: family status, occupational status, highest completed education, and income. In a second data extraction, made by Statistics Denmark, a retrospective linkage was performed investigating whether participants had a 'history in a risk-occupation' during 1994–2013.

**Yearly variables:** Family status, occupational status, education, and income were used to evaluate socioeconomic position. Exact information regarding all variables was retrieved, for both populations, by linkage to the National Danish Registers during the inclusion year: 2006 for the Health2006 population and 2013 for the patient population, respectively. The Danish National Registry of Population Statistics includes information regarding family type and country of origin. The National Danish Registry of Personal Income contains information regarding gross income and information regarding whether a person is employed, unemployed or outside the labour force. The Danish Education Register contains information about the highest completed education (this variable is updated each 1 October) and information about ongoing education.

**Complete tailored data extraction from Statistics Denmark during 1994–2013:** Previous work in a risk occupation was investigated for all individuals in the work active period (16–64 years) using the Danish Registers on Labour Market Affiliation, which contain information on economic and employment conditions.[119] The registry is an annual labour market statistic based on the individual's connection to the labour market estimated on the last working day in November. Statistics Denmark looked into data files, tracking occupations back for as long as possible, from 1994 to 2013. The occupational classification was based on DISCO-88[120] (1994–2009) and DISCO-08[121] (from 2010 and onwards) codes. The changes in DISCO codes are due to a change in the international standard classification of occupation system.

## 2.4 Ethical statement

All participants in both populations signed a written informed consent form before inclusion. The ethics committee of the Capital Region of Denmark approved the Health2006, baseline (KA-20060011) and follow-up (H-3-2011-081), studies and the patient population (H-1-2013-127). Both studies were further approved by the Danish Data Protection Agency.

## 2.5 Statistical analyses

All statistical analyses were performed using SAS software (SAS, Version 9.3 for Windows, SAS Institute Inc., Cary, NC, USA). Statistical analyses for dichotomous variables were made using the Chi Square Test, Fishers Exact Test, or Cochran-Armitage (Exact) Trend Test. Continuous variables were presented as median scores with 25$^{th}$ and 75$^{th}$ percentiles. Non-parametric variables were tested for group difference using Kruskal-Wallis Test. Level of significance was set at $P < 0.05$. Multivariate logistic regression models were used to adjust for confounders (for example sex, age group and atopic dermatitis) when investigating associations between a dependent variable and selected explanatory variables. Associations were expressed as OR with 95% CI. Figures were made with SAS, GraphPad Prism version 6.07 for Windows (GraphPad software, La Jolla, CA, USA) or with NodeXL for Excel.[122]

# 3. OBJECTIVES OF THE PHD THESIS

This thesis is based on epidemiological data from the adult general population and adult dermatitis patients.

The overall objective of the thesis was to estimate the prevalence of dermatitis in the general population, and look into the role of genetic pre-disposition, defined by *FLG* mutations, and personal consequences of the conditions.

In more detail, the aims were:

- To investigate the epidemiology of dermatitis in the general population including prevalence, anatomical localization and association with *FLG* mutations (Manuscript I).

- To investigate incidence and predictive factors of hand eczema in the general adult population (Manuscript II).

- To characterize the adult dermatitis patient with and without *FLG* mutations focusing on HRQoL, skin characteristics and comorbidity (Manuscript III).

- To investigate occupational consequences and previous work in risk occupations among the adult population with and without dermatitis and *FLG* mutations (Manuscript IV).

# 4. RESULTS AND MANUSCRIPTS

This section summarises key findings related to the stated aims. The original manuscripts are included after each aim. Manuscripts I and II are based on data from the Health2006 cohort, and Manuscript III is based on data from the patient population. Manuscript IV is based on data from both populations as well as from Statistics Denmark.

## 4.1 Epidemiology of dermatitis in the general population

The overall estimated lifetime prevalence of dermatitis in the Health2006 cohort was 37.8% (not presented in Manuscript I). The fact that more than one third of the general population report a lifetime prevalence of dermatitis confirms dermatitis as being a prevalent disease affecting individuals from both sexes and all age groups.

In Manuscript I we evaluated prevalence, anatomical localization and association with *FLG* mutations. Table 1 is a simplified summary of Tables I and II in Manuscript I and illustrates that hand eczema is the most frequent localization of dermatitis among participant who both participated in the baseline and follow-up studies, followed by dermatitis at the combined localization abdomen, chest or back, and third, facial dermatitis. The prevalence of atopic dermatitis was found to be 9.4% when using of the UK criteria.

**Table 1:** Lifetime prevalence of dermatitis at different localizations at baseline.

| Lifetime prevalence | Percent (%) |
|---|---|
| **Hand eczema (HE)** | |
| *Lifetime prevalence of HE reported at baseline* | 20.9 |
| *Lifetime prevalence of HE both at baseline and follow-up (Table II)* | 14.2 |
| **Abdomen, chest or back** | 11.8 |
| **Facial dermatitis** | 10.6 |
| **Atopic dermatitis** | 9.4 |
| **Axillae** | 8.0 |
| **Foot dermatitis** | 6.7 |

In total, 8.0% were carriers of at least one mutation in the *FLG* and 70% of the homozygous *FLG* mutation carriers had a history of atopic dermatitis. The frequency of foot dermatitis and persistent hand eczema in the general population was associated with *FLG* genotype ($P = 0.014$ and $P < 0.001$, respectively). However, when stratifying for *FLG* genotype and a history of atopic dermatitis, we found that *FLG* mutations affected only the lifetime prevalence of foot dermatitis and persistent hand eczema in participants with a history of atopic dermatitis. Thus the atopic phenotype showed to be a more important factor than *FLG* genotype in relation to dermatitis at different localizations.

# Anatomical patterns of dermatitis in adult filaggrin mutation carriers

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***Background:*** Common filaggrin (*FLG*) null mutations are associated with severe and early onset of atopic dermatitis (AD). To date, few studies have investigated anatomical patterns of dermatitis and none has been conducted in the general population.

***Objective:*** We evaluated patterns of dermatitis in an adult general population stratified by *FLG* genotype.

***Methods:*** Data from a population-based cohort study with a 5-year follow-up were used. This study included 2143 participants aged 18 to 72 years. Information about dermatitis on the hands; feet; face; axillae; and abdomen, chest, or back was obtained by use of questionnaires. Participants were genotyped for common *FLG* mutations. A history of AD was defined by the United Kingdom Working Party's diagnostic criteria.

***Results:*** The frequency of foot dermatitis in the general population was associated with *FLG* genotype ($P$ = .014). However, when stratification of *FLG* genotype and AD was performed, we found that *FLG* mutations increased the prevalence (odds ratios) of foot dermatitis (odds ratio 10.41; 95% confidence interval 5.27-20.60) and persistent hand dermatitis (odds ratio 17.57; 95% confidence interval 8.60-35.89) only in participants with AD.

***Limitations:*** Potential misclassification and recall bias are study limitations.

***Conclusion:*** *FLG* mutations affected the lifetime prevalence of hand and foot dermatitis in participants with a history of AD. (J Am Acad Dermatol 2015;72:440-8.)

***Key words:*** atopic dermatitis; epidemiology; filaggrin; foot dermatitis; genotype; hand dermatitis; population study.

Loss-of-function mutations within the filaggrin (*FLG*) gene are associated with a dysfunctional skin barrier and are considered the strongest genetic risk factors for the development of atopic dermatitis (AD).[1-4] The *FLG* gene is located within the

*Abbreviations used:*

AD: atopic dermatitis
FLG: filaggrin
OR: odds ratio

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epidermal differentiation complex on chromosome 1q21.[5] To date, 49 truncating mutations in the pro-FLG molecule have been reported and variation among European-specific and Asian-specific mutations exists.[6] Upon normal gene expression, the pro-FLG molecule is dephosphorylated and proteolyzed into FLG monomers, which help to align keratin filaments in the stratum corneum.[7,8] FLG degradation products are part of the natural moisturizing factors, which provide epidermal hydration, photoprotection, and maintenance of the acid mantle.[9] Hence, *FLG* mutation carriers show significantly reduced levels of natural moisturizing factors and higher transepidermal water loss when compared with controls.[10]

About 10% of the population with Northern European origin is a heterozygous carrier of an *FLG* mutation.[11,12] *FLG* mutations convey major susceptibility to severe and early-onset AD that persists into adulthood.[13] Results from cross-sectional population studies have further demonstrated that *FLG* mutations are associated with fissured skin on the hands and that the combination of AD and *FLG* mutation is associated with early onset and persistent hand dermatitis.[14,15] Apart from the distinct phenotype of hand dermatitis,[16] a strong positive association between dry skin and *FLG* mutations has been reported in adults from the general population[17] and in adult patients with dermatitis.[18] Anatomical localizations of dermatitis stratified by *FLG* mutation status were investigated in a prospective birth cohort of Danish children during their first 7 years of life.[19] *FLG* mutations were associated with a specific endotype of AD primarily characterized by predilection to exposed skin areas of the body, in particular the hands and cheeks.[19] However, associations between *FLG* mutations and dermatitis on other body parts in the general adult population have been only sparsely investigated. In this study, we characterized patterns of self-reported dermatitis on the hands; feet; face; axillae; or abdomen, chest, or back in the general population stratified by *FLG* genotype and AD.

**CAPSULE SUMMARY**

- Filaggrin mutations are the strongest known genetic determinants of atopic dermatitis.
- In this general population of Danish adults, filaggrin mutations affected the lifetime prevalence of persistent hand dermatitis and foot dermatitis in persons with atopic dermatitis.
- This knowledge might help dermatologists to identify patients with filaggrin mutations.

## METHODS

### Study population

During June 2006 through June 2008, a cross-sectional population study including 3471 persons was conducted in the southwestern part of Copenhagen. The Health2006 cohort was established to investigate the epidemiology of chronic diseases in adult Danes and has been described in more detail elsewhere.[20] The sampling area has been used for decades and has previously been found to be representative of the total Danish population in regard to age, sex, and marital status.[21] Participants were aged 18 to 72 years and were all Danish citizens born in Denmark. The cohort was drawn as a random sample of the population obtained through the Danish Central Personal Register, Ministry of Internal Affairs. Participants attended a general health examination and completed questionnaires. Five-year follow-up examinations were conducted between 2011 and 2013. The follow-up examinations included 2308 participants (participation rate 66.5%). The study was approved by the ethics committee of Copenhagen County (KA-20060011). Written informed consent was obtained from all participants.

### *FLG* genotyping

Genotyping for the mutations R501X, 2282del4, and R2447X was performed as previously described.[22] Successful genotyping was obtained for 96% of the samples. FLG mutation status was noted as wild type, heterozygous, or homozygous/compound heterozygous.

### Questionnaire

All participants completed questionnaires on health, lifestyle, and socioeconomic factors. The questions about dermatitis were introduced by the following description of dermatitis: "Dermatitis is an itchy skin disorder showing redness, dryness, and possibly bladders and exudation. Dermatitis remains on the same area of the body for some time." The following question about hand dermatitis was asked at baseline and follow-up: "Have you ever had hand dermatitis?" Participants who gave an affirmative answer were further asked "Have you had hand dermatitis within the past 12 months?" The baseline questionnaire further asked the multiple choice question: "Have you ever had dermatitis on other locations" (feet; face; axillae; abdomen, chest or back; or other locations)?

A history of AD was defined by the United Kingdom Working Party's diagnostic criteria as a history of an itchy skin condition plus a minimum of 2 of 4 minor criteria.[23] The major criterion was an itchy skin condition and the minor criteria were: (1) a history of involvement of the skin creases, (2) a history of asthma or hay fever, (3) a history of general dry skin, and (4) onset before the age of 2 years.[23]

### Definitions of exposure and outcome variables

Participants were grouped into a skin barrier variable according to their history of AD and *FLG* genotype: ($FLG_{wt}$/-AD) participants with no *FLG* mutations (wild type) and no AD, ($FLG_{mut}$/-AD) participants with *FLG* mutations (heterozygous or homozygous/compound heterozygous) and no AD, ($FLG_{wt}$/+AD) participants with no *FLG* mutations but with AD, and ($FLG_{mut}$/+AD) participants with both *FLG* mutations and AD. Dermatitis on the following localizations was used as outcome variables: hands; feet; face; axillae; or abdomen, chest, or back. The follow-up design in this study enabled differentiation of persistent and occasional hand dermatitis. Participants who gave affirmative answers to the questions about lifetime prevalence and 1-year prevalence of hand dermatitis, both at baseline and follow-up, were grouped as persistent cases. Participants who reported hand dermatitis, both at baseline and follow-up, but did not fulfill the criteria for being grouped as persistent cases were grouped as participants with occasional hand dermatitis.

### Statistical analyses

We included 2143 participants (92.9%) in this analyses; 165 participants were omitted because of missing answers in the dermatitis questions, an unsuccessful *FLG* test, or an undeterminable history of AD. We included only participants who had completed the follow-up questionnaire. Descriptive statistics were performed to summarize and compare self-reported dermatitis among the 4 different skin barrier groups. The $\chi^2$ test or Fisher exact test was used for dichotomous variables. A Cochran-Armitage trend test was used to evaluate differences across the 3 different genotypes. Logistic regression models adjusted for sex and age group (baseline age: 18-35, 36-55, and 56-72 years) were used to calculate odds ratios (OR) and 95% confidence intervals. All statistical analyses were performed using software (SAS, Version 9.3 for Windows, SAS Institute Inc, Cary, NC). An interaction between *FLG* mutations and AD was found for the outcome variable hand dermatitis and foot dermatitis. Networks diagrams were constructed with NodeXL for Excel.[24]

## RESULTS

We included 2143 participants from the general population to evaluate patterns of dermatitis in adult Danes. Table I shows the differences among participants entering the follow-up survey and those who were lost to follow-up. The mean age of the participants was significantly higher among participants in the follow-up survey ($P < .001$, $t$ test). Moreover, nonparticipants had a higher prevalence of *FLG* mutations and AD and they reported significantly more dry skin and hand and foot dermatitis.

Characteristics of the study group and frequencies of dermatitis stratified by *FLG* genotype are shown in Table II. The prevalence of AD in wild type, heterozygous, and homozygous or compound heterozygous carriers was 8.2%, 18.8%, and 70.0%, respectively. Moreover, an *FLG* genotype—dependent association with foot and hand dermatitis was observed ($P < .01$, trend test).

In this follow-up study, the lifetime prevalence of hand dermatitis in wild type, heterozygous, and homozygous or compound heterozygous carriers was 13.4%, 20.6%, and 70.0%, respectively ($P = .001$, trend test). When subdividing participants with hand dermatitis (n = 305) into occasional (n = 207) or persistent (n = 98) hand dermatitis, only persistent hand dermatitis showed a significant trend value with *FLG* genotype ($P < .001$, trend test). Notably, significant differences between heterozygous and homozygous or compound heterozygous *FLG* mutation carriers were found only for occasional hand dermatitis and foot dermatitis ($P = .003$, Fisher exact test).

Table III shows the frequencies and ORs of dermatitis localizations stratified by AD and *FLG* genotype. *FLG* mutations had no effect on dermatitis on any of the localizations in participants with no history of AD. In contrast, the ORs of all the included localizations were increased more than 3-fold when comparing participants with a history of AD but no *FLG* mutations ($FLG_{wt}$/+AD) with the reference group without AD and no *FLG* mutations ($FLG_{wt}$/-AD). A difference in OR = 3.03 (95% confidence interval 1.89-4.90) was found when comparing foot dermatitis as reported by participants with AD but no *FLG* mutations ($FLG_{wt}$/+AD) with those without AD and no *FLG* mutations ($FLG_{wt}$/-AD). Furthermore, the OR increased to 10.41 (95% confidence interval 5.27-20.60) when comparing self-reported foot dermatitis in participants with both AD and *FLG* mutations ($FLG_{mut}$/+AD) with participants without AD and no *FLG* mutations ($FLG_{wt}$/-AD). Notably, a significant interaction between *FLG*

**Table I.** Characteristics of participants and nonparticipants in the 5-year follow-up study (N = 3471)

| | Participants, N = 2308 | Nonparticipants, N = 1163 | $\chi^2$ Test for significance |
|---|---|---|---|
| Characteristics of the study group | | | |
| Mean age, y (SD)* | 50.0 (12.5) | 48.0 (14.0) | $P < .001$§ |
| Male sex, % (n/N) | 45.8 (1058/2308) | 42.6 (495/1163) | $P = .067$ |
| *FLG* mutation carriers,† % (n/N) | 8.0 (178/2226) | 10.8 (121/1120) | $P = .007$ |
| R501X (heterozygous), n | 57 | 50 | |
| 2282Del4 (heterozygous), n | 94 | 58 | |
| R2447x (heterozygous), n | 16 | 12 | |
| R501X (homozygous), n | 3 | - | |
| R2447X (homozygous), n | - | 1 | |
| 2282Del4 (homozygous), n | 4 | - | |
| Compound (heterozygous), n | 4 | - | |
| Dry skin lifetime prevalence, % (n/N) | 13.8 (312/2266) | 17.6 (199/1130) | $P = .003$ |
| Lifetime prevalence of dermatitis | | | |
| Atopic dermatitis,‡ % (n/N) | 9.4 (212/2253) | 12.0 (135/1127) | $P = .020$ |
| Hands (total), % (n/N) | 20.9 (478/2287) | 23.6 (270/1142) | $P = .067$ |
| Feet, % (n/N) | 6.7 (161/2308) | 9.0 (105/1163) | $P = .032$ |
| Face, % (n/N) | 10.6 (245/2308) | 9.2 (107/1163) | $P = .192$ |
| Axillae, % (n/N) | 8.0 (185/2308) | 8.3 (96/1163) | $P = .808$ |
| Abdomen, chest or back, % (n/N) | 11.8 (273/2308) | 11.4 (132/1163) | $P = .679$ |

At follow-up, 94.5% of the baseline population was invited; 65 participants were either dead or had immigrated.
*FLG*, Filaggrin.
*Age at baseline.
†Genotyping for FLG mutations (R501X, 2282del4, and R2447X).
‡Defined by 1 major criterion and 2 of 4 minor criteria.[23]
§*t* Test is used to evaluate differences in mean age.

**Table II.** Characteristics of the study group stratified by filaggrin genotype (N = 2143)

| | Wild type, n = 1968 | HET, n = 165 | HOM, n = 10 | Cochran-Armitage trend test | Fisher exact test (HET vs HOM) |
|---|---|---|---|---|---|
| Characteristics of the study group | | | | | |
| Genotype frequencies, % (n/N)* | 91.8 (1968/2143) | 7.7 (165/2143) | 0.5 (10/2143) | | |
| Mean age, y (SD)† | 50.0 (12.5) | 49.6 (12.0) | 51.1 (16.0) | | |
| Male sex, % (n) | 46.1 (908) | 44.9 (74) | 60 (6) | $P = .892$ | $P = .516$ |
| **Frequency of dermatitis** | **n = 1968** | **n = 165** | **n = 10** | | |
| Atopic dermatitis % (n)‡ | 8.2 (161) | 18.8 (31) | 70.0 (7) | $P < .001$ | $P = .001$ |
| Hands (total) % (n) | 13.4 (264) | 20.6 (34) | 70.0 (7) | $P = .001$ | $P = .002$ |
| Occasional hand dermatitis % (n) | 9.5 (186) | 9.7 (16) | 50.0 (5) | $P = .334$ | $P = .003$ |
| Persistent hand dermatitis % (n) | 4.0 (78) | 10.9 (18) | 20.0 (2) | $P < .001$ | $P = .320$ |
| Feet % (n) | 6.7 (132) | 9.7 (16) | 50.0 (5) | $P = .014$ | $P = .003$ |
| Face % (n) | 10.6 (208) | 9.7 (16) | 30.0 (3) | $P = .955$ | $P = .080$ |
| Axillae % (n) | 7.6 (149) | 12.1 (20) | 10.0 (1) | $P = .037$ | $P = 1.000$ |
| Abdomen, chest, or back % (n) | 11.7 (231) | 15.2 (25) | 20.0 (2) | $P = .155$ | $P = .654$ |

The differences in self-reported dermatitis are evaluated across the 3 genotype groups using Cochran-Armitage trend test. Fisher exact test is used to evaluate differences between HET and HOM.
*HET*, Heterozygous carriers; *HOM*, homozygous or compound heterozygous carriers.
*Genotyping for filaggrin mutations (R501X, 2282del4, and R2447X).
†Age at baseline.
‡Defined by 1 major criterion and 2 of 4 minor criteria.[23]

mutations and AD was found for the extremities, hand and foot dermatitis, but not for facial dermatitis.

Many of the participants in the Health2006 cohort reported dermatitis on several localizations. The descriptive network diagrams in Fig 1 demonstrate the connection between different anatomical localizations of dermatitis in participants with AD. The percentages in brackets next to the localization refer

**Table III.** Frequencies of self-reported dermatitis on the following anatomical localizations: hands; feet; face; abdomen, chest, or back; or axillae OR of self-reported dermatitis adjusted for sex and age group (N = 2143)

| | Participants without AD | | Participants with AD | | Statistics |
|---|---|---|---|---|---|
| Dermatitis localization | $FLG_{wt}$/−AD, n = 1807 | $FLG_{mut}$/−AD, n = 137 | $FLG_{wt}$/+AD, n = 161 | $FLG_{mut}$/+AD, n = 38 | $\chi^2$ Test for group difference |
| **Hands; feet; face; axillae; or abdomen, chest or back** | | | | | |
| Hands (total) % (n) | 11.5 (207) | 11.7 (16) | 35.4 (57) | 65.8 (25) | $P < .001$ |
| Occasional hand dermatitis % (n) | 8.1 (147) | 7.3 (10) | 24.2 (39) | 29.0 (11) | $P < .001$ |
| Persistent hand dermatitis % (n) | 3.3 (60) | 4.4 (6) | 11.2 (18) | 36.8 (14) | $P < .001$ |
| Feet % (n) | 5.9 (107) | 4.4 (6) | 15.5 (25) | 39.5 (15) | $P < .001$ |
| Face % (n) | 8.1 (146) | 5.8 (8) | 38.5 (62) | 29.0 (11) | $P < .001$ |
| Axillae % (n) | 6.3 (114) | 7.3 (10) | 21.7 (35) | 29.0 (11) | $P < .001$ |
| Abdomen, chest, or back % (n) | 10.1 (182) | 9.5 (13) | 30.4 (49) | 36.8 (14) | $P < .001$ |
| **Self-reported dermatitis adjusted for sex and age group** | $FLG_{wt}$/-AD OR (95% CI) | $FLG_{mut}$/-AD OR (95% CI) | $FLG_{wt}$/+AD OR (95% CI) | $FLG_{mut}$/+AD OR (95% CI) | ***P* value for interaction between *FLG**AD** |
| Hands (total) | 1 | 1.00 (0.58-1.72) | 4.01 (2.80-5.76) | 15.23 (7.62-30.42) | $P = .004$ |
| Occasional hand dermatitis | 1 | 0.87 (0.44-1.69) | 3.24 (2.16-4.87) | 4.60 (2.22-9.53) | $P = .354$ |
| Persistent hand dermatitis | 1 | 1.32 (0.56-3.11) | 3.93 (2.23-6.92) | 17.57 (8.60-35.89) | $P = .038$ |
| Feet | 1 | 0.72 (0.31-1.67) | 3.03 (1.89-4.90) | 10.41 (5.27-20.60) | $P = .005$ |
| Face | 1 | 0.69 (0.33-1.44) | 6.39 (4.42-9.23) | 4.52 (2.18-9.36) | $P = .960$ |
| Axillae | 1 | 1.14 (0.58-2.24) | 3.69 (2.41-5.67) | 5.99 (2.88-12.47) | $P = .511$ |
| Abdomen, chest, or back | 1 | 0.93 (0.51-1.68) | 3.44 (2.36-5.00) | 5.04 (2.54-9.97) | $P = .345$ |

Each of the localizations was analyzed separately.
Participants with no *FLG* mutations and no history of AD are references ($FLG_{wt}$/-AD).
*AD*, Atopic dermatitis; *CI*, confidence interval; $FLG_{mut}$, filaggrin mutation; $FLG_{wt}$, filaggrin wild type; *OR*, odds ratio.

to the prevalence of dermatitis among the participants in each group. The lines between different localizations refer to the frequency of concomitant dermatitis. Thicker lines indicate higher frequency whereas the circles show the overall prevalence of dermatitis on the given location. Separate patterns of self-reported dermatitis were detected when stratifying by *FLG* genotype. For example, only 5.0% of the participants without *FLG* mutations but with AD reported a history of persistent hand and foot dermatitis in contrast to 18.4% of the participants with both *FLG* mutations and AD.

## DISCUSSION

In this population-based cohort study, with a 5-year follow-up, investigating anatomical localizations of dermatitis in the adult Danish population, we found that distinct patterns of dermatitis could be identified when stratifying by *FLG* mutations. We found that dermatitis on the hands, on the feet, and in the axillae was significantly associated with *FLG* genotype ($P < .040$, trend test), with homozygous or compound heterozygous carriers reporting the highest frequencies. Overall, the hands were the most common site of dermatitis in this population. The lifetime prevalence of hand dermatitis in the Health2006 cohort has previously been published.[14] Our results regarding *FLG* mutations and persistent hand dermatitis are in line with the previous literature[25,26] but are based on answers from the 5-year follow-up survey, enabling stratification on the persistence of hand dermatitis.

The associations between *FLG* mutations and dermatitis on other body parts in the general adult population have only been sparsely investigated. To our knowledge, this study is the first to report an increased lifetime prevalence of foot dermatitis in individuals with *FLG* mutations. Moreover, 18.4% of the participants with *FLG* mutations and with AD reported a lifetime prevalence of both foot and persistent hand dermatitis. A previous study, performed on children of mothers with asthma, showed an association between *FLG* mutations and dermatitis on exposed areas of the body such as cheeks and hands.[19]

Notably, *FLG* mutations did not increase the frequency of dermatitis on any of the localizations

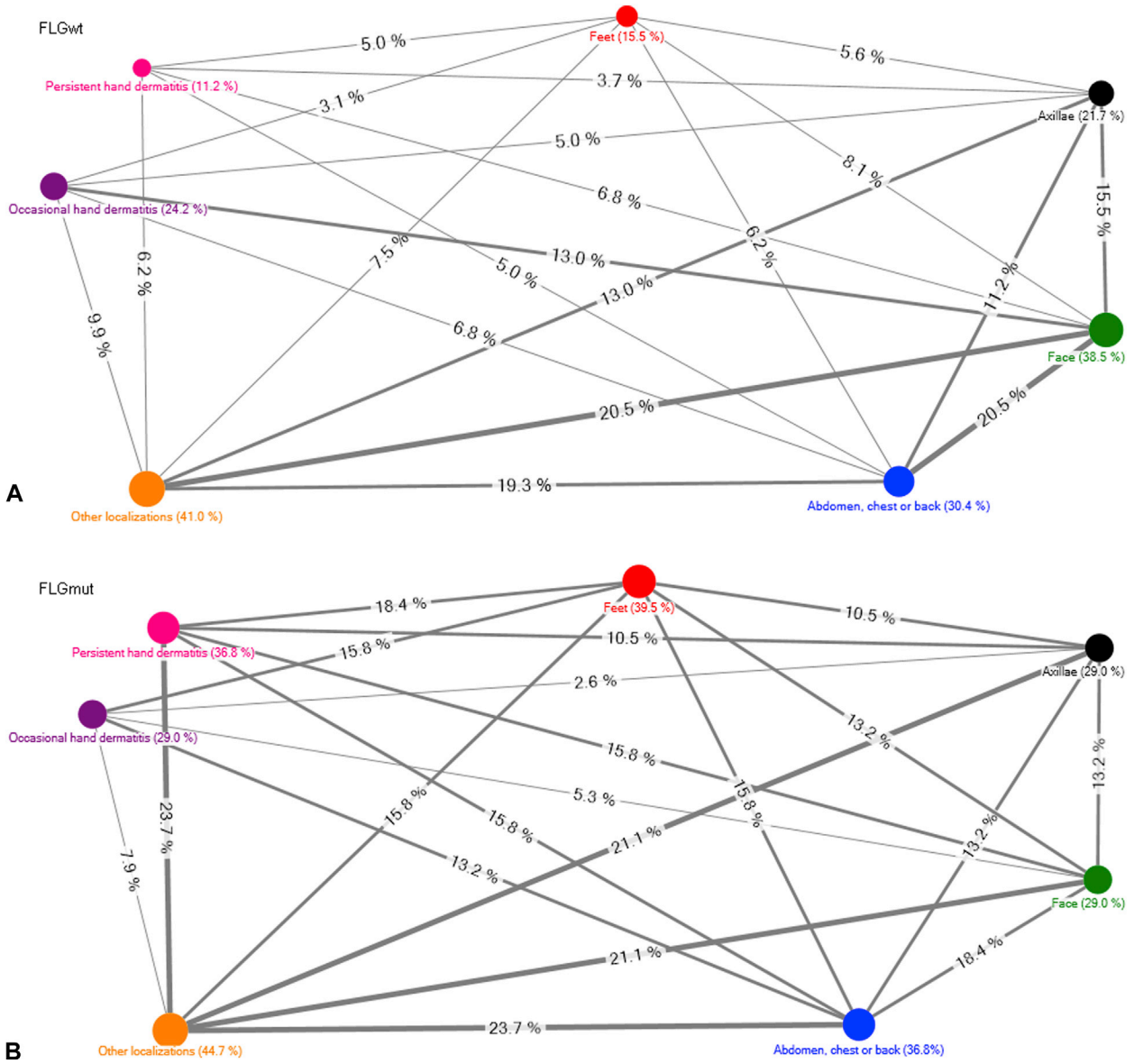


**Fig 1.** Network diagrams showing dermatitis localizations in participants with a history of atopic dermatitis (N = 199). **A**, filaggrin (*FLG*) wild type carriers (n = 161). **B**, *FLG* mutation carriers (n = 38). Circles show the prevalence of dermatitis at the given location. For example, 15.5% of the participants without *FLG* mutations reported foot dermatitis (**A**) in contrast to 39.5% of the participants with *FLG* mutations (**B**). Lines show frequencies of concomitant dermatitis. Thicker lines indicate higher frequency and the percentages are reported in clams. In all, 5.0% of the participants without *FLG* mutations reported persistent hand and foot dermatitis (**A**) in contrast to 18.4% of the participants with *FLG* mutations (**B**). Likewise, 6.2% of the participants without *FLG* mutations reported both a history foot dermatitis and dermatitis on abdomen, chest, or back (**A**) in contrast to 15.8% of the participants with *FLG* mutations (**B**).

in individuals without previous AD. These results are similar to those of Visser et al,[27] who suggested that the influence of *FLG* mutations on hand dermatitis in Dutch apprentice nurses differed between individuals with and without AD. Visser et al[27] showed that the highest risk of hand dermatitis during traineeships was found among participants with concomitant *FLG* mutations and AD (OR 3.6).

The strength of this study is the use of an unselected population-based cohort with individual *FLG* genotype. We analyzed 3 of the most common *FLG* mutations in Europe, which, taken together,

constitute 83% of the total risk alleles associated with development of AD: R501X (39%), 2282del4 (41%), and R2447X (3%).[6]

As nonparticipants in the follow-up had more AD and reported significantly more hand and foot dermatitis at baseline than the included participants, we do not expect that our results are overestimated; however, they may be underestimated.

One of the limitations of this study is missing variables that were addressed by only analyzing complete cases (92.9% of the available participants were included). Another limitation is the use of self-reported data and the risk of recall bias. We estimated the lifetime prevalence of AD according to the United Kingdom Working Party's criteria, which have a sensitivity of 69% to 85% and a specificity of 96%, depending on validation setup.[23] The validation has, however, mainly been performed in children, which could potentially be a source of misclassification.[28] Moreover, Moberg et al[29] have shown that several factors such as disease activity in adult life, disease severity, and who noticed the disease in childhood may influence how well adults remember childhood AD.

The question about hand dermatitis within the past 12 months has been validated among car mechanics, dentists, and office workers.[30] Meding and Barregård[30] reported a sensitivity of 53% to 59% and a specificity of 96% to 99%. Validation of self-reported hand dermatitis has further been demonstrated in Danish hairdressing apprentices with an overall sensitivity of 70.3% and specificity of 99.8%.[31] To increase the sensitivity, we only included participants who confirmed their lifetime prevalence of hand dermatitis in the follow-up questionnaire (70.5% of the participants).

The question regarding dermatitis on other localizations has not been validated, which could lead to potential misclassification. Facial dermatitis could be confused with other disorders such as seborrheic dermatitis, psoriasis, or acne, whereas foot dermatitis could be confused with tinea pedis. Moreover, self-reported dermatitis could be a clinical manifestation of contact dermatitis with varying genetic susceptibility according to the etiology. An association between *FLG* mutations and contact sensitization to nickel has been suggested.[11,17] However, other studies have found that irritant contact dermatitis and contact allergy are associated with *FLG* mutations but only in the presence of atopy.[32,33] Hence, we do not suspect that misclassification would be differentially related to *FLG* genotype. Finally, the participation rate was higher among the older age groups[34] but because age differences were not dependent on genotype, we do not consider this to affect the outcome of the study**.** The prevalence of *FLG* mutations in general populations have been shown to be latitude dependent[35] and the types of mutations have been shown to be region specific.[36] Participants in the Health2006 cohort were all Danish citizens born in Denmark, which could reduce the generalizability to persons born outside Denmark. In addition, the questions about dermatitis were part of a larger questionnaire, which is why prior power calculations for the dermatitis questions were not performed, and the results should therefore be regarded as explorative. Confirmation of the presented results in a cohort of well-characterized dermatologic patients will be needed to confirm the clinical relevance of the association between *FLG* mutations and foot dermatitis. Validation in a cohort of well-characterized patients would also enable investigation of both atypical distributions and potential differences between the extrinsic and intrinsic type of AD.

*FLG* genotyping is currently gaining more attention as a diagnostic tool to understand the etiology of disease when treating patients in dermatologic clinics. Palmar hyperlinearity[37] and dry skin[38] are currently used as indications of FLG haploinsufficiency. In our study the OR of foot dermatitis, among participants with AD, was increased more than 3-fold when participants also carried *FLG* mutations.

We suggest that knowledge about previous foot dermatitis in individuals with AD could aid dermatologists in identifying potential *FLG* mutation carriers. Knowledge about genetic predisposition to dermatitis could also help dermatologists to increase patients' disease understanding. In addition, early identification of atopic individuals with concomitant *FLG* mutations is likely to help optimize patient care and preventive measures because these individuals have an increased risk of developing occupational irritant contact dermatitis,[32] asthma,[2,3,39] and food allergies.[40]

In conclusion, an association between *FLG* mutations and foot dermatitis was found in participants with a history of AD and was the strongest in homozygous or compound heterozygous carriers. *FLG* mutations did not affect the lifetime prevalence of dermatitis in participants with no history of AD suggesting that the atopic phenotype is the most important factor.

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## 4.2 Predictive factors of hand eczema

We investigated predictive factors of hand eczema in adult Danes from the general population (Manuscript II). We used data from 2270 individuals who had answered questions about hand eczema at both baseline and follow-up (98.4% of the follow-up population).

Table 2 is a simplified summary of Table 3 in Manuscript II. Here, we confirm that a history of atopic dermatitis is the strongest predictive factor of hand eczema. Even though the CIs overlap in the three groups, the strongest association was observed with persistent hand eczema showing an OR = 8.95; 95% CI 5.55–14.43. Moreover, we found that a history of atopic dermatitis also predicted incident hand eczema in adults (OR = 2.97; 95% CI 1.71–5.16).

**Table 2:** Logistic regression models for the association between hand eczema and baseline variables. Each variable was analysed separately and adjusted for age group and sex**.**

| Lifetime prevalence | Hand eczema group | Odds ratio (95% confidence interval) |
|---|---|---|
| **Atopic dermatitis** | Never | 1 |
| | Incident | 2.97 (1.71 – 5.16) |
| | Non-persistent | 4.76 (3.38 – 6.70) |
| | Persistent | 8.95 (5.55 – 14.43) |
| **Filaggrin mutation carriers** | Never | 1 |
| | Incident | 0.52 (0.21 – 1.29) |
| | Non-persistent | 1.22 (0.80 – 1.85) |
| | Persistent | 3.07 (1.82 – 5.19) |
| **Contact sensitization** *(23 allergens without nickel)* | Never | 1 |
| | Incident | 1.58 (0.74 – 3.37) |
| | Non-persistent | 1.77 (1.10 – 2.85) |
| | Persistent | 2.49 (1.24 – 5.02) |

In contrast, *FLG* mutations were found to be associated with persistent hand eczema only, suggesting that the effect of *FLG* mutations as a predictive factor for hand eczema decreases with time. Other predictive factors, including contact allergy, were investigated.

We confirmed previous studies showing associations between contact sensitization (23 allergens without nickel) and persistent hand eczema, but in this cohort the association was also found to be significant in participants without a history of atopic dermatitis (OR = 2.84; 95% CI 1.24–6.50). Manuscript II thus adds new knowledge to the interplay between atopic dermatitis, *FLG* mutations and hand eczema in the general population.

EPIDEMIOLOGY

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# Predictive factors of self-reported hand eczema in adult Danes: a population-based cohort study with 5-year follow-up*

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## Summary

*Background* Information about predictive factors of hand eczema is crucial for primary prevention.
*Objectives* To investigate predictive factors of hand eczema in adult Danes from the general population.
*Methods* Participants from a cross-sectional 5-year follow-up study in the general population, aged 18–72 years (n = 2270), completed questionnaires about skin health and were grouped into four hand eczema groups: 'never', 'incident', 'nonpersistent' and 'persistent'. Multiple logistic regression models adjusted for age group and sex were used to evaluate associations with baseline variables. The participation rate for the follow-up study was 66·5% (29·7% of the participants originally invited to the baseline study).
*Results* A history of atopic dermatitis (AD) was associated with both persistent and incident hand eczema [odds ratio (OR) 9·0, 95% confidence interval (95% CI) 5·6–14·4 and OR 3·0, 95% CI 1·7–5·2, respectively]. Thus, even in adulthood, a history of AD should be considered as a predictor of incident hand eczema. While filaggrin gene (*FLG*) null mutations were not associated with incident hand eczema, a statistically significant association was observed with persistent hand eczema (OR 3·1, 95% CI 1·8–5·2). Finally, contact sensitization (23 allergens without nickel) was also associated with persistent hand eczema (OR 2·5, 95% CI 1·2–5·0), independently of a history of AD.
*Conclusions* This study confirms a history of AD as the strongest predictor of persistent hand eczema. We additionally found that a history of AD was associated with incident hand eczema in adults, in contrast to *FLG* mutations, which were associated only with persistent hand eczema in individuals with a history of AD, and not with incident hand eczema. Our study adds new knowledge to the interplay between AD, *FLG* mutations and hand eczema in the adult general population.

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### Conflicts of interest
None declared.

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### What's already known about this topic?

- Atopic dermatitis (AD), female sex, contact sensitization and wet work are known risk factors for hand eczema.
- Knowledge about predictive factors of hand eczema in the adult general population is limited.

### What does this study add?

- A history of AD predicts both incident and persistent hand eczema in adults.
- Filaggrin gene (*FLG*) null mutations strongly predict persistent hand eczema, but the association with incident hand eczema in adults is nonsignificant.

- Contact sensitization is significantly associated with persistent hand eczema, also in participants without AD.

In Denmark, hand eczema is the most commonly recognized occupational skin disease, with widespread societal costs.[1,2] The lifetime prevalence of hand eczema in the adult general Scandinavian population ranges between 15·0% and 21·8%,[3,4] whereas the worldwide 1-year prevalence, based mainly on European studies, has been estimated at nearly 10%.[5]

Hand eczema is a multifactorial condition that typically develops following repeated or prolonged contact with allergic and/or irritant compounds, and it particularly affects individuals with a history of atopic dermatitis (AD).[6–8] However, the pathogenesis of hand eczema is complex, and despite the traditional clinical distinction between the irritant, allergic and atopic phenotypes, mixed forms are common.

In 1985, Rystedt showed that patients with persistent or recurrent AD had a significantly higher risk of having hand eczema in adulthood.[9] This association has later been confirmed in various studies, most recently in prospective cohorts of adolescence from the general population.[10,11] Other risk factors for hand eczema include female sex, contact allergy, wet work, xerosis and tobacco smoking.[5,7,8,12–14] Pertinently, loss-of-function mutations within the filaggrin gene (*FLG*) have been identified as genetic factors that increase the risk and persistence of hand eczema, in subjects with AD.[15,16] However, no studies have investigated whether *FLG* mutations are predictors of incident hand eczema in adults.

Recent studies have suggested that cumulative exposure to irritants impairs skin barrier integrity,[17] and that an inherited skin barrier impairment due to *FLG* mutations appears to increase allergen penetration.[18] In addition, skin barrier dysfunction has recently been shown to play an essential role in the pathogenesis of chronic hand eczema, for example by downregulation of barrier genes including *FLG*, *FLG2* and hornerin.[19] However, the interplay between hand eczema and endogenous factors such as AD and *FLG* mutations, as well as exogenous factors including exposure to contact allergens and irritants, is not completely understood.

Most recent studies investigating predictors of hand eczema in the general population have focused on adolescents.[10,11,20,21] Thus, research focusing on predictors of hand eczema in the adult general population is warranted. In this 5-year follow-up study, we aimed to characterize the association between baseline variables and self-reported hand eczema in the adult Danish general population, stratified by persistence of hand eczema.

## Materials and methods

### The Health2006 cohort

A cross-sectional population-based cohort study including 3471 participants aged 18–72 years was performed in the south-western part of Copenhagen, between 2006 and 2008 (participation rate 44·7%). The cohort was drawn as a random sample of the general population using the Danish Central Personal Register. All participants were Danish citizens born in Denmark.[22] Five-year follow-up examinations including 2308 participants (follow-up participation rate 66·5%) were performed between 2011 and 2012.[23] At both time points, participants attended general health examinations and completed questionnaires regarding chronic diseases, including skin health.

### Ethics

All participants signed a written informed consent form prior to inclusion. The ethics committee of the Capital Region of Denmark approved the baseline (KA-20060011) and follow-up (H-3-2011-081) studies.

### Defining the hand eczema outcome variable using baseline and follow-up data

The questions about hand eczema were introduced by the following description of eczema: 'Eczema is an itchy skin disorder showing redness, dryness, and possibly blisters and exudation. Eczema remains on the same area of the body for some time'. At baseline and follow-up, participants were asked, 'Have you ever had hand eczema?' Participants who gave an affirmative answer were further asked, 'Have you had hand eczema within the past 12 months?' Based on their answers, participants were grouped into a 'hand eczema variable' consisting of four groups: (i) participants never reporting hand eczema ('no hand eczema'); (ii) participants reporting hand eczema (lifetime and/or 12-month prevalence) only at follow-up but not at baseline ('incident hand eczema'); (iii) participants reporting hand eczema within the past 12 months at both baseline and follow-up ('persistent hand eczema'); and (iv) participants reporting hand eczema at baseline but who did not qualify to be grouped as persistent or incident cases ('nonpersistent hand eczema').

### Definition of the questionnaire variables at baseline

Lifetime prevalence of self-reported food allergy was investigated using the question, 'Has a doctor ever told you that you have/have had food allergy? (Similarly for self-reported hay fever and asthma.) Self-reported generalized xerosis was determined as follows: 'Have you ever had dry skin all over your body?' A question regarding skin symptoms due to use of cosmetic products was as follows: 'Have you ever experienced skin redness, rash and itch caused by exposure to cosmetics, moisturizers, lotions or such related products?' A history of

AD was defined by the U.K. Working Party's diagnostic criteria with a major criterion of a history of an itchy skin condition plus a minimum of two of four minor criteria.[24] The minor criteria were: (i) a history of involvement of the skin creases; (ii) a history of asthma or hay fever; (iii) a history of general dry skin; and (iv) onset before the age of 2 years.[24] At follow-up the participants were also asked about itchy skin within the last 12 months: 'Have you within the past 12 months experienced itchy skin where you have rubbed and scratched a lot?'

### Biological measurements at baseline

#### Filaggrin genotyping

Genotyping for the three most common filaggrin mutations (R501X, 2282del4 and R2447X) was performed as previously described.[25] A positive filaggrin mutation status was defined as heterozygote or homozygote/compound heterozygote.

#### Patch test

Participants were patch tested at baseline with panels 1 and 2 of the ready-to-use TRUE Test® (Mekos Laboratories, Hillerød, Denmark) as previously described.[26] Participants applied the patch tests to the upper back 2 days prior to the health examination. All reactions were read by healthcare professionals on day 2. Readings were in accordance with the guideline from the International Contact Dermatitis Research Group.[27] A positive patch test was defined as at least one positive reaction (+, ++ or +++).

#### Measurements of allergen-specific IgE

Blood samples were analysed for serum-specific IgE against four of the most common inhalation allergens in Denmark: birch, grass, cat and house-dust mite (*Dermatophagoides pteronyssinus*) (ADVIA Centaur® Specific IgE assay; Siemens, Deerfield, IL, U.S.A.).[28] A positive test was defined as a positive reaction (> 0·35 kU $L^{-1}$) to at least one of the four allergens tested.

### Statistics

We included 2270 participants (98·4%) in the present analyses; 38 participants had been omitted due to missing data on the hand eczema question. We analysed only complete cases who participated in the follow-up examination, thereby enabling stratification on persistence of hand dermatitis. The incidence rate was calculated as the number of incident cases (n = 133) divided by the population at risk during the 5 year follow-up period (participants reporting no hand eczema or incident hand eczema, n = 1791), thus the incidence rate = 133/(1791 × 5 years).

Descriptive statistics were used to present and compare various baseline determinants among the four different hand eczema groups. The $\chi^2$-test or Fisher's exact test was used for dichotomous variables. Multiple logistic regression models adjusted for sex and age group (baseline age 18–35, 36–55 and 56–72 years) were used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs). Multiple logistic regression models with stratification for participants without AD were made with 2019 participants; 208 participants with AD were omitted together with 43 having an indeterminable history of AD.

All statistical analyses were performed using SAS version 9.3 for Windows (SAS Institute Inc., Cary, NC, U.S.A.). Statistical significance was defined as $P < 0·05$.

## Results

In total, 2270 of the 2308 follow-up participants were included in this analysis (98·4%). Figure 1 illustrates a flow diagram of the participants in the baseline and follow-up studies.

The 1-year prevalence of hand eczema at follow-up was 9·5%. The cumulative incidence rate of hand eczema during the 5-year period was 14·8 per 1000 years, 95% CI 12·6–17 (female: 16·1 per 1000 years and male: 13·5 per 1000 years).

The characteristics of the study population are outlined in Table 1. The participants were divided into four hand eczema groups. During the 5-year follow-up period 133 participants reported incident hand eczema and 108 participants reported persistent hand eczema. Moreover, 371 participants were classified as having nonpersistent hand eczema and 1658 participants reported no hand eczema. All three groups with hand eczema reported significantly more itchy skin within the last 12 months than participants never reporting hand eczema ($P < 0·001$, $\chi^2$-test). The highest prevalence of itchy skin was found among participants with persistent hand eczema (75·7%) and the lowest prevalence among participants who never reported hand eczema (27·9%). Moreover, only 9·0% of the participants in the group reporting nonpersistent hand eczema reported hand eczema within the past 12 months at follow-up, in contrast to 57·3% of the participants reporting incident hand eczema.

Associations between baseline variables and the persistence of hand eczema are illustrated in Table 2. The life-

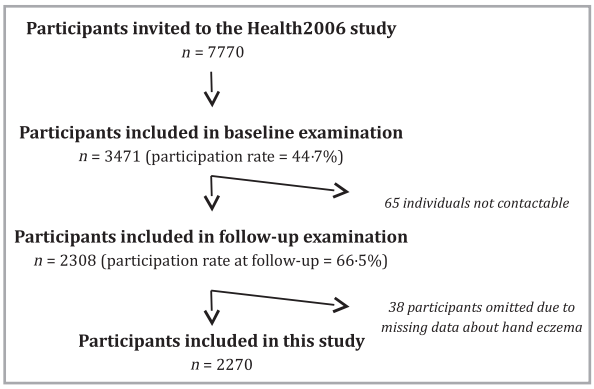


**Fig 1.** Participant flow diagram of the Health2006 cohort.

**Table 1** Characteristics of the population at follow-up (2270 participants). Participants are divided into no, incident, nonpersistent or persistent hand eczema (HE)

| | No HE (n = 1658) | Incident HE (n = 133) | Nonpersistent HE (n = 371) | Persistent HE (n = 108) | P-value ($\chi^2$-test) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | No vs. incident | No vs. nonpersistent | No vs. persistent | Incident vs. persistent |
| Sex (female), n (%) | 854 (51·5) | 75 (56·4) | 240 (64·7) | 59 (54·6) | 0·28 | < 0·001 | 0·53 | 0·78 |
| Age (years), median (range) | 56·4 (24·2–76·5) | 52·0 (25·7–74·0) | 54·9 (24·7–76·3) | 55·3 (25·0–76·0) | [c] | [c] | [c] | [c] |
| Hand eczema (within the past 12 months at follow-up), n/N (%)[a] | – | 75/131 (57·3) | 33/365 (9·0) | 108/108 (100) | – | – | – | < 0·001 |
| Itchy skin (past 12 months), n/N (%)[b] | 450/1615 (27·9) | 80/130 (61·5) | 152/363 (41·9) | 81/107 (75·7) | < 0·001 | < 0·001 | < 0·001 | 0·02 |

[a]n = 2262. [b]n = 2215. [c]All-group comparison of median age by Kruskal–Wallis test: P = 0·01.

time prevalence estimates of AD, self-reported generalized xerosis, self-reported asthma, self-reported hay fever, self-reported food allergy and 'redness, rash or itch related to use of cosmetic products' were all significantly higher in participants with either incident or persistent hand eczema, when compared with participants without hand eczema ($P \leq 0{\cdot}027$, Fisher's exact test). Contact sensitization was investigated using the TRUE Test containing 24 allergens. In total, 215 participants were sensitized to at least one allergen. Nickel sulfate was the most frequent allergen, with 123 participants having a positive patch test. The results for contact sensitization are shown as total frequencies of all the 24 allergens, for 23 allergens (without nickel sulfate) and for nickel sulfate separately. The prevalence rates of contact sensitization (excluding nickel sulfate) and *FLG* mutations were significantly higher among participants with persistent hand eczema than in participants without hand eczema ($P = 0{\cdot}021$ and $P < 0{\cdot}001$, respectively, Fisher's exact test).

Notably, significant differences between the incident and persistent hand eczema groups were found for *FLG* mutations, AD and 'redness, rash or itch related to use of cosmetic products' ($P \leq 0{\cdot}021$, Fisher's exact test).

Table 3 shows multiple logistic regression models for nine of the significant baseline variables identified in Table 2. Each variable was analysed separately and the analyses were adjusted for age group and sex. A subanalysis was made for participants who did not report a history of AD (n = 2019). Variables incorporated into the U.K. criteria (self-reported generalized xerosis, hay fever and asthma) were not included in the stratified analyses.

A strong association between a history of AD and persistent hand eczema was found, making AD the most prominent predictive factor of persistent hand eczema (OR 9·0, 95% CI 5·6–14·4). However, a history of AD was also associated with nonpersistent hand eczema (OR 4·8, 95% CI 3·4–6·7) and incident hand eczema (OR 3·0, 95% CI 1·7–5·2) when compared with participants who never reported hand eczema. In addition, the nonoverlapping CIs indicate a significant difference between the associations with AD and incident and persistent hand eczema, respectively. In contrast, the CIs between the remaining variables overlap, which excludes exact separation between the groups with incident and persistent hand eczema.

Overall, contact sensitization was found to be a predictive factor for hand eczema. Of note, contact sensitization was also found to be associated with persistent hand eczema in participants without AD (OR 2·8, 95% CI 1·2–6·5). In more detail, 14 participants with persistent hand eczema were sensitized to at least one allergen. The percentage of participants with persistent hand eczema, who had no AD and had at least one positive patch test, was 14% (10 of 69), in contrast to 12% (four of 33) of the participants with persistent hand eczema but with AD.

In total, 173 participants had *FLG* mutations, of whom 39 (22·67%) had a history of AD according to the U.K. criteria

**Table 2** Frequencies reporting the association between hand eczema (HE) and baseline variables. The three different P-values represent Fisher's exact test performed on 'never vs. incident', 'never vs. persistent' and 'incident vs. persistent' HE. Total participants = 2270

| | Never HE (n = 1658) | Incident HE (n = 133) | Nonpersistent HE (n = 371) | Persistent HE (n = 108) | P-value (Fisher's exact test) Never vs. incident | Never vs. persistent | Incident vs. persistent |
|---|---|---|---|---|---|---|---|
| Allergen specific IgE (n = 2257)[a] | 283 (23·2) | 38 (29·0) | 98 (26·5) | 24 (22·9) | 0·14 | 1·00 | 0·30 |
| Contact sensitization (all 24 allergens) (n = 2162)[b] | 141 (8·9) | 14 (11·0) | 46 (13·0) | 14 (13·6) | 0·42 | 0·12 | 0·69 |
| (i) Contact sensitization (23 allergens) | 64 (4·1) | 8 (6·3) | 26 (7·3) | 10 (9·7) | 0·25 | 0·02 | 0·46 |
| (ii) Nickel sulfate (1 allergen) | 85 (5·4) | 7 (5·5) | 26 (7·3) | 5 (4·9) | 0·84 | 1·00 | 1·00 |
| Filaggrin mutation carriers (n = 2189)[c] | 117/1606 (7·3) | 5/127 (3·9) | 31/354 (8·8) | 20/102 (19·6) | 0·21 | < 0·001 | < 0·001 |
| Atopic dermatitis (n = 2227)[d] | 81/1632 (5·0) | 18/130 (13·9) | 76/359 (21·1) | 33/106 (31·1) | < 0·001 | < 0·001 | 0·002 |
| Self-reported generalized xerosis (n = 2240) | 249/1636 (15·2) | 32/132 (24·2) | 114/366 (31·2) | 35/106 (33·0) | 0·009 | < 0·001 | 0·15 |
| Self-reported food allergy (n = 2231)[e] | 58/1630 (3·6) | 15/131 (11·5) | 31/364 (8·5) | 16/106 (15·1) | < 0·001 | < 0·001 | 0·44 |
| Self-reported hay fever (n = 2246)[e] | 259/1644 (15·8) | 31/132 (23·5) | 83/364 (22·8) | 35/106 (33·0) | 0·03 | < 0·001 | 0·11 |
| Self-reported asthma (n = 2248)[e] | 132/1642 (8·0) | 21/132 (15·9) | 49/367 (13·4) | 24/107 (22·4) | 0·005 | < 0·001 | 0·24 |
| Redness, rash or itch related to use of cosmetic products (n = 2258) | 695/1649 (42·2) | 77/132 (58·3) | 236/369 (64·0) | 79/108 (73·2) | < 0·001 | < 0·001 | 0·02 |

Values are n (%) or n/N (%). [a]Allergen specific IgE against birch, grass, cat and house-dust mite. [b]All 24 allergens in the TRUE Test (215 participants sensitized to at least one allergen). (i) TRUE Test with at least one positive reaction, without nickel sulfate; (ii) TRUE Test positive for only nickel sulfate; 16 participants are in both groups, which is why the total number of positive tests is 231. [c]Genotyping for filaggrin mutations (R501X, 2282del4 and R2447X). [d]Atopic dermatitis was defined by the U.K. Working Party's criteria, with one major criterion and two of four minor criteria. [e]'Has a doctor ever told you that you have/have had. . .?'

**Table 3** Logistic regression models for the association between hand eczema (HE) and baseline variables. Each variable was analysed separately and adjusted for age group and sex

| Variable | HE group | All participants (n = 2270) OR adjusted for age group and sex (95% CI) | Stratification: participants without AD (n = 2019) OR adjusted for age group and sex (95% CI) |
|---|---|---|---|
| AD (n = 2227)[a] | Never | 1 | – |
| | Incident | 2·97 (1·71–5·16) | – |
| | Nonpersistent | 4·76 (3·38–6·70) | – |
| | Persistent | 8·95 (5·55–14·43) | – |
| Self-reported food allergy (n = 2231; non-AD, n = 1994)[b] | Never | 1 | 1 |
| | Incident | 3·42 (1·87–6·26) | 4·05 (2·11–7·77) |
| | Nonpersistent | 2·39 (1·51–3·77) | 1·76 (0·98–3·17) |
| | Persistent | 5·03 (2·76–9·16) | 2·91 (1·20–7·10) |
| Redness, rash or itch related to use of cosmetic products (n = 2258; non-AD, n = 2011) | Never | 1 | 1 |
| | Incident | 1·85 (1·27–2·68) | 1·72 (1·15–2·56) |
| | Nonpersistent | 2·22 (1·75–2·83) | 2·04 (1·56–2·67) |
| | Persistent | 3·91 (2·49–6·13) | 3·45 (2·05–5·82) |
| Self-reported asthma (n = 2248; non-AD, n = 2007)[b] | Never | 1 | – |
| | Incident | 2·07 (1·25–3·42) | – |
| | Nonpersistent | 1·67 (1·17–2·38) | – |
| | Persistent | 3·34 (2·04–5·47) | – |
| Filaggrin mutation carrier (n = 2189; non-AD, n = 1946)[c] | Never | 1 | 1 |
| | Incident | 0·52 (0·21–1·29) | 0·51 (0·19–1·42) |
| | Nonpersistent | 1·22 (0·80–1·85) | 0·88 (0·52–1·49) |
| | Persistent | 3·07 (1·82–5·19) | 1·29 (0·55–3·06) |
| Self-reported generalized xerosis (n = 2240; non-AD, n = 2005) | Never | 1 | – |
| | Incident | 1·77 (1·16–2·71) | – |
| | Nonpersistent | 2·38 (1·83–3·09) | – |
| | Persistent | 2·75 (1·79–4·24) | – |
| Self-reported hay fever (n = 2246; non-AD, n = 2006)[b] | Never | 1 | – |
| | Incident | 1·57 (1·03–2·40) | – |
| | Nonpersistent | 1·55 (1·17–2·06) | – |
| | Persistent | 2·59 (1·69–3·98) | – |
| Contact sensitization (all 24 allergens) (n = 2162; non-AD, n = 1925)[d] | Never | 1 | 1 |
| | Incident | 1·18 (0·65–2·12) | 1·00 (0·51–1·99) |
| | Nonpersistent | 1·30 (0·91–1·87) | 1·20 (0·79–1·82) |
| | Persistent | 1·49 (0·82–2·73) | 1·76 (0·86–3·58) |
| Contact sensitization (23 allergens) (n = 2162; non-AD, n = 1925)[e] | Never | 1 | 1 |
| | Incident | 1·58 (0·74–3·37) | 1·21 (0·47–3·09) |
| | Nonpersistent | 1·77 (1·10–2·85) | 1·70 (0·98–2·95) |
| | Persistent | 2·49 (1·24–5·02) | 2·84 (1·24–6·50) |

OR, odds ratio; CI, confidence interval; hand eczema (HE). [a]AD was defined by the U.K. Working Party's criteria with one major criterion and two of four minor criteria. [b]'Has a doctor ever told you that you have/have had . . .?'. [c]Genotyping for filaggrin null mutations (R501X, 2282del4 and R2447X). [d]Contact sensitization based on all 24 allergens in the TRUE Test. [e]Contact sensitization based on 23 allergens in the TRUE Test (without nickel sulfate).

and 133 (77·32%) did not (one participant had an undeterminable AD history). A significant association between *FLG* mutations and persistent hand eczema was seen (OR 3·1, 95% CI 1·8–5·2). However, the association was nonsignificant in participants without AD (OR 1·3, 95% CI 0·6–3·1).

'Skin rash related to use of cosmetic products' and self-reported food allergy were associated with hand eczema, also in participants with no history of AD. As xerosis, hay fever and asthma are criteria incorporated into the definition of AD, the associations with hand eczema were determined only when analysing all participants. Figure 2 summarizes the predictive factors found to influence persistent hand eczema.

## Discussion

In this population-based cohort study with 5-year follow-up, we aimed to evaluate the complex relationship between hand eczema in adults and predictive factors such as AD, FLG mutations, asthma, hay fever, food allergy, xerosis, specific inhalation allergens and contact sensitization. Of these predictive factors, we found that a history of AD was the strongest predictive factor for adult hand eczema.

Our data confirmed previous literature showing that a history of AD strongly predicted persistent hand eczema (OR 9·0, 95% CI 5·6–14·4).[9,10,20] Moreover, a history of AD was

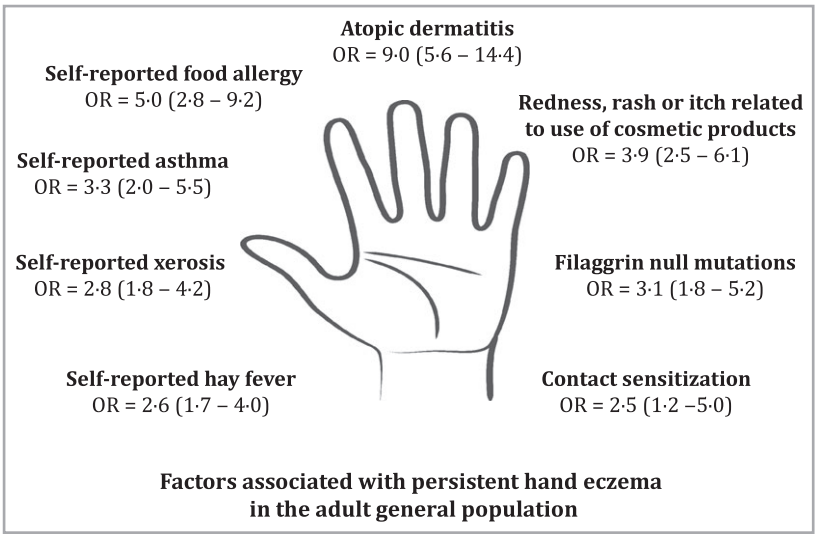


**Fig 2.** Illustration of factors associated with persistent hand eczema in adult Danes from the general population. The odds ratios (ORs) and 95% confidence intervals are adjusted for age group and sex, and were retrieved by comparing participants reporting persistent hand eczema with participants who never reported hand eczema. All variables refer to a self-reported lifetime prevalence, except for filaggrin null mutations and contact sensitization (23 allergens, without nickel sulfate).

associated with incident hand eczema in adults from the general population (OR 3·0, 95% CI 1·7–5·2), which, to our knowledge, has not been shown previously. The hypothesis that a smaller proportion of cases of AD would be detected among patients with incident hand eczema when compared with persistent cases has been suggested previously,[20] but Johannisson *et al.* found no significant difference in the proportion of childhood eczema among their group with incident hand eczema in a longitudinal cohort study of young adults. However, they did find a higher proportion of individuals reporting childhood eczema among participants with hand eczema at both baseline and follow-up (similar to that in our persistent group).[20]

In addition to a history of AD, several other factors were identified as predictors of incident hand eczema in adults. The ORs for incident hand eczema were increased in relation to self-reported generalized xerosis, self-reported asthma, self-reported hay fever, self-reported food allergy and 'redness, rash or itch related to use of cosmetic products', when compared with participants without hand eczema. However, because of overlapping CIs, no clear distinction can be made between the three hand eczema groups. A previous study performed in a general population concluded that female sex, a history of AD, and asthma/hay fever were associated with incident hand eczema only in participants aged < 30 years.[29] Our regression analyses were adjusted for age group and sex, but because our data build on measures of lifetime prevalence we were not able to investigate whether the association was significant only in participants aged < 30 years. The association between asthma and hay fever as a predictor of hand eczema should therefore be interpreted with caution.

Of note, 'redness, rash or itch related to use of cosmetic products' was associated with all groups of hand eczema. In addition, the association between incident hand eczema and a lifetime prevalence of 'redness, rash or itch related to use of cosmetic products' was still significant when analysing only participants without a history of AD, when compared with participants who never reported hand eczema (OR 1·7, 95% CI 1·2–2·6). Information about the aetiology of the symptoms is missing in this set-up, and it is possible that some participants report symptoms of allergic contact dermatitis and others of irritant contact dermatitis. However, no statistical difference was found when comparing the frequency of contact sensitization in the incident group with that in the group reporting no hand eczema.

In contrast, a significantly higher prevalence of contact sensitization (to 23 allergens without nickel sulfate) was found among participants with persistent hand eczema when compared with participants without hand eczema ($P = 0·021$, Fisher's exact test). The association between persistent hand eczema and contact sensitization is known,[30] but in this cohort the association was also found to be significant in participants without a history of AD (OR 2·8, 95% CI 1·2–6·5). The association between *FLG* mutations and persistent hand eczema in individuals with a history of AD has previously been published using data from the Health2006 cohort.[15, 31] However, the current article includes data from a 5-year follow-up examination, which enables investigation of the association between *FLG* mutations and incident hand eczema.

In this study we report that *FLG* mutations are not associated with incident hand eczema in adults. The fact that *FLG* mutations were associated only with persistent hand eczema could suggest that the effect of the mutations, as a risk factor for hand eczema, decreases with time. Despite conflicting experimental results in the origin of the downregulation of FLG, two experimental studies have suggested that *FLG* muta-

tions exert an effect early in life, but that the effect might be neutralized with increasing age.[32,33]

Advantages of this study include the follow-up design and the use of an unselected cohort of adult individuals. When using questionnaire-derived data the most important limitations are the risk of recall bias and misclassification. In addition, the 2308 participants enrolled in the follow-up examination constitute only 29·7% of the originally invited subjects, which could introduce a selection bias. However, the follow-up participation rate was 66·5% of the participants included in the baseline study. The differences between participants and nonparticipants in the 5-year follow-up study have previously been published, showing that participants in the follow-up study reported significantly less AD ($P = 0{\cdot}020$, $\chi^2$-test) and slightly less hand eczema (20·9%) than the nonparticipants (23·6%) ($P = 0{\cdot}067$, $\chi^2$-test).[33] Thus, our results might be an underestimation.

Moreover, there was no clinical examination by a dermatologist to confirm the hand eczema diagnoses or to evaluate potential subtypes of hand eczema. Nevertheless, the question about hand eczema within the past 12 months has been validated among car mechanics, dentists and office workers, resulting in a sensitivity of 53–59% and a specificity of 96–99%.[34] Validation has further been conducted in Danish hairdressing apprentices, with an overall sensitivity of 70·3% and specificity of 99·8%.[35] However, no validation has been conducted in a follow-up design, and our definition of incident hand eczema builds upon two lifetime prevalence measures, which might increase the risk of recall bias, and gives no information about when the symptoms began during the 5 year follow-up period. Overall, the total 1-year prevalence of hand eczema was 9·5% at follow-up, which is in agreement with the average 1-year prevalence of 10% reviewed by Thyssen *et al.*[5]

The U.K. criteria have previously been validated.[24,36] However, a recent study concluded that recall bias for childhood AD is a significant problem in retrospective questionnaire studies evaluating a history of AD.[37] A risk of recall bias affecting the association between AD and hand eczema should therefore be highlighted. Moreover, Mortz *et al.* also reports that recall bias regarding asthma and allergic rhinitis was less than for AD, possibly as a result of the later debut of these diseases.[37] It is however not possible to make a direct comparison between the study by Mortz *et al.*[37] and our study because of the differences in age and study design.

Lastly, our study set-up enabled patch test readings for contact sensitization only on day 2. The European patch test guideline recommends an occlusion time of 2 days,[38] but the risk of false-negative results is increased when reading only on day 2.[39] The prevalence of contact sensitization to nickel was shown separately in Table 2 to increase the transparency of our data, because nickel was the most frequent allergen. In addition, the risk of allergic contact sensitization to nickel has been suggested to be increased in *FLG* mutation carriers,[40] possibly due to the nickel-binding properties of the filaggrin protein.[41] However, the lowest frequency of contact sensitization to nickel was found in the group with persistent hand eczema, which had the highest prevalence of *FLG* mutation carriers (19·6%).

Our data show that contact sensitization (23 allergens without nickel) is associated with persistent hand eczema, also for participants without a history of AD. This result, together with the high prevalence of 'redness, rash or itch related to use of cosmetic products', stresses the need for primary prevention by reducing exposure to common allergens in cosmetic products, such as fragrances and isothiazolinones. In addition, we suggest that education about skin health and skin protection in the general adult population is needed, irrespective of age, and especially in individuals with AD, who have an increased risk of hand eczema throughout life. Despite our data suggesting that the effect of *FLG* mutations, as a genetic marker for hand eczema, is associated with persistent hand eczema only, a further understanding of skin barrier function is still vital. This is supported by the fact that the hands of individuals with *FLG* mutations have a distinct phenotype.[42] Frequent use of emollients has been suggested to improve the skin barrier[43] and to be useful in the therapy of hand eczema.[44,45] In line with this, our data identified the highest prevalence of generalized xerosis among the participants reporting persistent hand eczema. Notably, individual skin counselling has already proven effective against hand eczema in healthcare workers.[46]

In conclusion, we found that a history of AD predicted incident hand eczema, but that *FLG* mutations had no effect on incident hand eczema in the general adult population. Finally, contact sensitization was associated with persistent disease in participants with and without a history of AD, which altogether provides new knowledge to the research field investigating the interplay between hand eczema, AD, contact sensitization and *FLG* mutations.

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## 4.3 Health-related quality of life among adult filaggrin mutation carriers

We established a population of adult patients to estimate the proportion of *FLG* mutation carriers in a mixed population of patients diagnosed with atopic dermatitis and/or hand eczema. We found, that out of the 520 patients included in this population, 17.0% were *FLG* mutation carriers. Specifically, 18.8% of patients included with atopic dermatitis only were *FLG* mutations carriers, 8.9% of the patients included with hand eczema only had *FLG* mutations, and 33.1% of the patients included with both atopic dermatitis and hand eczema were carriers of at least one mutation in the *FLG*.

HRQoL was measured using the DLQI questionnaire. While *FLG* mutations were significantly associated with reduced HRQoL, no association with self-reported anxiety or depression was identified. Notably, the highest median DLQI score, reflecting greater impairment, was reported by patients having both *FLG* mutations and atopic dermatitis.

Figure 4 illustrates that 19.7% of patients with both atopic dermatitis and *FLG* mutations reported a 'large or extremely large', impact on patient's life which was twice the prevalence found in patients with atopic dermatitis but *FLG* wild type (9.6%). Thus patients with both atopic dermatitis and *FLG* mutations stand out on several parameters including reduced HRQoL, which highlights the need for increased focus on this subset of patients.

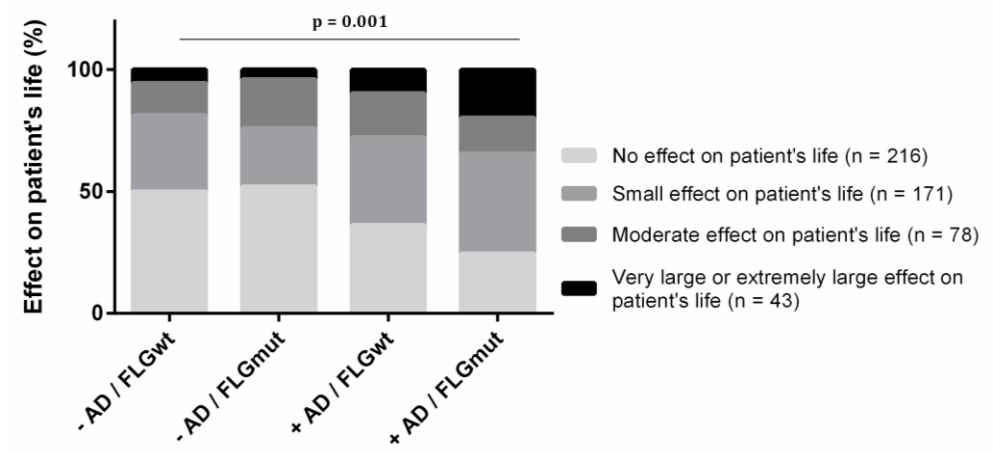


**Figure 4:** Subdivision of the health-related quality of life effect on patient's life, measured by DLQI, stratified by combined filaggrin genotype and history of atopic dermatitis. The two categories "very large effect on patient's life" and "extremely large effect on patient's life" representing DLQI >10 are pooled.

Lastly, we identified an association between *FLG* genotype and self-reported actinic keratosis with the highest prevalence reported by homozygous individuals (wildtype 2.2%, heterozygote carriers 9.0%, and homozygote/compound heterozygote 16.7% (Cochran-Armitage Exact Trend Test *P* = 0.003)).

# Health-related quality of life in adult dermatitis patients by filaggrin genotype

**Running head:** Quality of life by filaggrin genotype

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## ABSTRACT

**Background:** Information concerning health-related quality of life (HRQoL) and comorbidities of adult dermatitis patients stratified by loss-of-function mutations in the filaggrin gene (*FLG*) is limited.

**Method:** This cross-sectional study included patients diagnosed with atopic dermatitis and/or hand eczema (N=520). Patients completed questionnaires about dermatitis, skin symptoms, HRQoL, and comorbidities including actinic keratosis, as well as atopic and mental disorders.

**Results:** *FLG* mutations (R501X, 2282del4, and R2447X) were identified in 16.9% and were significantly associated not only with atopic dermatitis, but also independently with skin fissures on the fingers and heels, as well as self-reported actinic keratosis. While *FLG* mutations were significantly associated with reduced HRQoL, measured by the Dermatological Life Quality Index (DLQI), no association with self-reported anxiety or depression was identified. Notably, the highest median DLQI score, reflecting greater impairment, was reported by patients having both *FLG* mutation and atopic dermatitis. Overall, 19.7% of patients with both atopic dermatitis and *FLG* mutations reported a 'large or extremely large', impact on patient's life; twice the prevalence in patients with atopic dermatitis and *FLG* wild type (9.6%).

**Conclusion:** The prominent proportion of atopic dermatitis patients with common *FLG* mutations stands out on reduced HRQoL, stressing the need for increased focus on this subset of patients.


**Keywords:** Filaggrin mutations, atopic dermatitis, hand eczema, health-related quality of life, actinic keratosis.

## INTRODUCTION

Research addressing the etiological role of skin barrier impairment in dermatological disease has intensified over the last decade following the discovery of loss-of-function mutations in the filaggrin gene (*FLG*) and its influence on atopic dermatitis (1). Atopic dermatitis is a chronic pruritic inflammatory skin condition that is estimated to affect 10-20% of school children in Western Europe and in the United States (2). While many children outgrow their atopic dermatitis, persistence into adulthood is surprisingly common (3,4). Independent of age, patients with atopic dermatitis may experience repercussions including increased need of healthcare (5), anxiety (6), depression (7), and decreased health-related quality of life (HRQoL). HRQoL has frequently been measured by the Dermatological Life Quality Index (DLQI) (8), where increasing disease severity is associated with greater impairment in HRQoL (9,10). Moreover, associations between *FLG* mutations and atopic disorders such as asthma (11,12), allergic rhinitis (13), and food allergy (14) respectively, primarily in co-occurrence with atopic dermatitis, have been shown.

Hand eczema is an equivalent common skin condition with a lifetime prevalence ranging between 15% and 21.8% in the adult general population (15,16). Hand eczema severity correlates with a reduced HRQoL, measured by DLQI (17), and increased frequency of sick-leave and job change are known consequences (18). Hand eczema can be a clinical manifestation of atopic dermatitis, but is in many cases an independent disease provoked by environmental exposure to allergenic and/or irritant compounds. While a recent European multicenter study found no increased risk of contact allergy in individuals with atopic dermatitis from the general population (19), patients with atopic dermatitis have repeatedly been shown to have a compromised skin barrier function resulting in an increased reactivity to skin irritants (20-22). The biological interplay between contact dermatitis, atopic dermatitis and *FLG* mutations remain to be fully elucidated, but it has been shown that *FLG* mutations increase the risk of developing hand eczema (irritant contact dermatitis (23) and combined irritant and allergic contact dermatitis (24)), often with a chronic course, and in particular in individuals with a history of atopic dermatitis (25). A direct association, independent of atopic dermatitis, between *FLG* mutations and hand eczema has only been identified in one study (26). Last, *FLG* mutations have recently been found to be associated with contact dermatitis in construction workers, most strongly in individuals with an atopic predisposition (27).

Despite the dramatic increase in our understanding of the pathogenic role of *FLG* mutations in dermatitis patients, only few studies have made thorough clinical characterizations of adult dermatitis patients investigating both *FLG* mutation status, skin symptoms, and dermatological and non-dermatological comorbidities. The objective of this study was to analyse dermatitis, skin symptoms, HRQoL, and comorbidities in adult *FLG* mutation carriers, to evaluate the incentive for extra care among this group of patients.

## MATERIALS AND METHODS

### Study population

The study included patients who had been examined at our tertiary Dermatology Department at Gentofte Hospital between 2006 and 2012. Patients were diagnosed with atopic dermatitis and/or hand dermatitis and were all ≥ 18 years. Patients received a written invitation and contacted the Department if they were interested to get more information about the study. Interested patients were verbally informed about the study and completed the questionnaire and collected genomic DNA by a buccal swap (Isohelix, Harrietsham, United Kingdom). The participation rate was 46.5 % (520/1119). Of the 520 included patients; one patient did not return the questionnaire. Written informed consent was obtained for all participants. The study was approved by the Ethics committee of Copenhagen County (H-1-2013-127).

### Clinical diagnoses and patch test

Clinical diagnoses of atopic dermatitis and/or hand eczema were scored by the attending dermatologist and were registered in the patient' file along with the internationally recognized MOAHLFA-index (Male, Occupational dermatitis, history of Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, and Age above 40 years) (28) which is registered routinely for all patients who are patch tested. Patch tests (European baseline series and relevant additional test series according to exposure analysis) were applied on the upper back and occluded for two days in accordance with The European patch test guidelines (29). Readings were carried out recommending readings at day 2, day 3 or day 4 and day 7. Results were registered in The National Database of Contact Allergy from where the dataset was extracted.

### Genotyping

*FLG* genotyping was performed at the Department of Clinical Biochemistry at Gentofte Hospital (30). A positive test was defined for heterozygote, and homozygote or compound heterozygote mutation carriers. Genotyping was conducted for three of the most common Northern European loss-of-function mutations in the profilaggrin gene which together constitute 83% of the total risk alleles associated with atopic dermatitis; R501X, 2282del4, and R2447X (1,31,32).

#### *Extraction of genomic DNA from buccal swaps*

Buccal swaps were stored at -80$^{o}$C until DNA extraction was performed with JetQuick kit (Genomed, Löhne, Germany) according to manufactures' instructions. Purified genomic DNA was stored at -20$^{o}$C before allele-specific PCR was performed.

***Allele-specific PCR, microbead-specific hybridization and detection***

We used a slightly modified version of the method which has previously been described in detail (30). Briefly, regions covering the *FLG* mutations were amplified by multiplexed, allele-specific asymmetric PCR using tagged primers. The single stranded PCR products hence underwent tag-specific hybridization to microbeads which were analyzed on Bio-Plex 200 (Bio-Rad) flow cytometer using Bio-Plex Manager software, version 5.0 (30).

**Questionnaire**

The following definition of dermatitis was listed prior to the questions about dermatitis: ''*dermatitis is an itchy skin disorder showing redness, dryness, and possibly bladders and exudation. Dermatitis remains on the same area of the body for some time*.'' Questions used in the questionnaire are listed in Table S1. The Dermatological Life Quality Index by Finley et al., 1994 was used to address skin specific quality of life (8).

**Statistics**

Statistical analyses for dichotomous variables were made by use of the Chi Square Test, Fishers Exact Test, or Cochran-Armitage Exact Trend Test. DLQI median scores and $25^{th}$ and $75^{th}$ percentiles were presented based on responses from 515 participants. Non-parametric Kruskal-Wallis Test for group difference was performed. Level of significance was set at $P < 0.05$. We made sub-analyses of the DLQI scores using relative percentages obtained by adding up the total scores in each of the six questions and divide the result by the number of patients with the likely maximum score of 6 (3 in the work/school and treatment category) $((n_{very\_much} \times 3 + n_{a\_lot} \times 2 + n_{a\_little} \times 1) / (n_{total} \times 6)) * 100\%$. Epidata was used as data entry module (33) and all statistical analyses were performed using SAS software (SAS, Version 9.3 for Windows, SAS Institute Inc., Cary, NC, USA). Figures were made in SAS or GraphPad Prism version 6.07 for Windows (GraphPad software, La Jolla, CA, USA).

**RESULTS**

**Patient characteristics**

Characteristics of the patients' dermatitis were registered by use of the MOAHLFA-index (28). The MOAHLFA-index and patch test results for the 1,119 invited patients are shown in Table 1. Table 1a shows, that included participants (n=520) were significantly older, and had a higher prevalence of atopic dermatitis, than non-participants (n=599) ($P < 0.001$, Chi square Test). In contrast, participants had a significant lower point prevalence of hand eczema (80.4%) and fewer cases had occupational relevance of dermatitis (43.9%) when compared with non-participants (86.7% and 55.5%, ($P \leq 0.005$, Chi square Test), respectively). Stratification for the MOAHLFA-index, among the 520 included patients, with the three inclusion diagnoses (atopic dermatitis, hand eczema, or atopic dermatitis and

hand eczema) is presented in Table 1b and shows that there was no sex difference between the groups, but that participants in the group with 'hand eczema only' were significantly older ($P < 0.001$, Kruskal-Wallis Test).

**Filaggrin gene mutation carriers reported an increased prevalence of skin symptoms, including skin fissures in acral areas**

To examine skin symptoms of *FLG* mutation carriers, all participants were genotyped for three common *FLG* mutations. A successful genotype was determined for 98.6% of the included patients. The prevalence of *FLG* mutations carriers was significantly higher in the group of patients diagnosed with both atopic dermatitis and hand eczema (33.1%), followed by the group with atopic dermatitis only (18.8%) and finally the hand eczema group (8.9%) ($P< 0.001$, Chi Square Test, Table 2a).

Because the MOAHLFA-index only specifies hand eczema at the time of patch testing, a questionnaire regarding skin symptoms and hand eczema was distributed. The question regarding self-reported lifetime prevalence of hand eczema demonstrated an overall sensitivity of 96.7% (three patients had missing values and 11 patients included with hand eczema reported "no hand eczema"). Table 2b shows stratification by combined genotype and history of atopic dermatitis. Patients with both *FLG* mutations and atopic dermatitis reported an increased point prevalence of hand eczema (71.0%) compared with atopic dermatitis patients who were *FLG* wild type carriers (53.6%)($P = 0.018$, Chi square Test). Moreover, skin fissures on the hands and heels were significantly more prevalent among *FLG* mutation carriers, independently of atopic dermatitis status ($P \leq 0.031$, Chi square Test). The highest prevalence was found among patients with hand eczema and *FLG* mutations whereof 76.0% reported fissures on the hands and 68.0% reported fissures on the heels. Last, atopic dermatitis patients with *FLG* mutations reported significantly more xerosis than atopic dermatitis patients with wild type *FLG* status, 80.7% and 59.6% ($P = 0.003$, Chi square Test), respectively.

Self-reported estimates of severity and current disease showed no significant difference among the four combinations of *FLG* genotype and atopic dermatitis phenotype in Table 2b. Among patients with both *FLG* mutation and atopic dermatitis, 5.0% reported that they no longer suffered from dermatitis, 63.3% reported an improvement, and 31.7% reported no effect or a worsening since they last visited our dermatological clinic.

**Patients with both filaggrin gene mutations and atopic dermatitis report decreased HRQoL**

To investigate whether *FLG* mutation carriers differed from non-mutation carriers in relation to HRQoL, we asked patients to complete the DLQI questionnaire, as well as, answering questions regarding lifetime prevalence of anxiety or depression. In total, 6.6% of the patients reported a lifetime prevalence of anxiety and 17.1% reported a lifetime prevalence of depression. Notably, no associations between *FLG* mutations and, respectively, anxiety or depression were found (Table 3).

DLQI scores stratified by inclusion diagnoses, age group, sex, *FLG* genotype, and combined genotype and atopic dermatitis phenotype are presented in Figure 1. Overall, the total DLQI score ranges between 0 and 30 where increased DLQI scores reflect reduced HRQoL. The following references were used for total DLQI sum: 0-1 'no effect on patient's life', ≥2 'small impact on patient's life', 5-10 'moderate effect on patients life', and > 10 'large or extreme effect on patient's life' (8). In total, 515 patients (99.2%) completed the DLQI questionnaire correctly. Patients with atopic dermatitis only (median (M) score ($25^{th}$ and $75^{th}$ percentiles)) (M 3 (1 - 7)), or combined atopic dermatitis and hand eczema (M 3 (1 - 6)) reported higher DLQI score than patients with hand eczema only (M 1 (0 - 4)(Figure 1a)). No significant sex difference was detected (Figure 1b), but the younger age group reported a lower DLQI score (Figure 1c) possibly influenced by the fact that patients with atopic dermatitis were, on average 10 years, younger than the patients with hand eczema (Table 2b).

Moreover, *FLG* mutation carriers reported significantly higher DLQI scores than non-mutation carriers ($P$ = 0.033, Kruskal-Wallis Test, Figure 1d). The highest median score was found for 61 patients with both *FLG* mutations and atopic dermatitis (M 5 (2-7)) (Figure 1e). In total, 59 patients no longer suffered from dermatitis (Table 2a). The association between *FLG* mutations and decreased HRQoL remained highly significant if the 59 participants who did not report dermatitis any longer were excluded.

Subdivision of the DLQI is shown in Figure 2. Most participants reported that "symptoms and feelings" affected their HRQoL. Participants with both *FLG* mutation and atopic dermatitis reported the highest scores in all categories, especially "symptoms and feelings" (35.8%), "daily activities" (18.8%), and "treatment" (18.6%) (Figure 2a). The overall association between DLQI and HRQoL, in this study population, shows that more participants with atopic dermatitis reported a 'very large' or 'extremely large' effect on patient's life (DLQI score >10) (Figure 2b). In total, 19.7% of the participants with both *FLG* mutation and atopic dermatitis reported an extremely large effect on patient's life.

**The highest prevalence of atopic comorbidities in adults is reported by patients with both atopic dermatitis and filaggrin gene mutations**

To fully characterize our study population, we investigated the prevalence of atopic diseases which, in addition, have both been associated with *FLG* mutations and reduced HRQoL. The overall prevalence of atopic dermatitis in this population consisting of patients with atopic dermatitis and/or hand eczema was 44.8%. Twenty-eight percent reported a lifetime prevalence of asthma, 42.7% reported rhinitis and 30.5% reported food allergy. The highest prevalence of all of the self-reported atopic diseases was reported by patients with both atopic dermatitis and *FLG* mutations (asthma 45.2%, rhinitis 62.9% and food allergy 41.0%). No *FLG* genotype dependent differences were however found for asthma, rhinitis or food allergy, irrespective of atopic dermatitis (Table 3). If we, instead of descriptive statistics, use a

logistic regression model we do, however, see a significant association between *FLG* mutations and both asthma (odds ratio (OR) = 1.81; 95% confidence interval (CI): 1.10 – 2.96) and rhinitis (OR = 1.70; 95%CI: 1.06 – 2.72). The significance is however lost for both associations when adjusting for age group, sex and atopic dermatitis.

**Association between filaggrin gene mutations and actinic keratosis**

We asked participants whether a doctor had ever told them that they have, or have had, actinic keratosis, non-melanoma skin cancer (squamous cell cancer or basal cell cancer), or malignant melanoma. The percentages of participants reporting a lifetime prevalence of non-melanoma skin cancer or melanoma skin cancer were, 1.8% and 0.8%, respectively. In total, 20 (4.0%) patients reported a lifetime prevalence of actinic keratosis. Patients reporting actinic keratosis were significantly older (mean age 60.4 (standard deviation (SD) 13.0)) than patient who did not report actinic keratosis (mean age 46.5 (SD 14.7)) ($P < 0.001$, Kruskal-Wallis Test). Of the 20 participants reporting actinic keratosis; three had a missing *FLG* genotype. However, a significant association between *FLG* mutations and actinic keratosis was found in patients with atopic dermatitis ($P = 0.022$) (Table 3). In total, 13 of the 20 patients (65.0%) reporting a history of actinic keratosis had a clinical atopic dermatitis diagnoses. When stratifying for heterozygote and homozygote/compound heterozygote carriers, the prevalence of actinic keratosis was: wild type 2.2 % (n = 9), heterozygote 9.0% (n = 7) and homozygote/compound heterozygote 16.7% (n =1) (Cochran-Armitage Exact Trend Test $P = 0.003$). If we assume that the three patients with missing *FLG* mutations status were all wild type carriers the association remained significant (data not shown).

## DISCUSSION

In this cross-sectional study, we showed that the prevalence of *FLG* mutations was 8.9% among adult patients with hand eczema but without atopic dermatitis; an estimate that is equivalent to estimates from the general Danish population (34). However, the prevalence of *FLG* mutations among patients with atopic dermatitis only was 18.8%, which is similar to the prevalence of 17.5% originally reported by Palmar and colleagues (1). Moreover, 33.1% of adult patients with both atopic dermatitis and hand eczema had at least one *FLG* mutation. Collectively, the results support the notion that *FLG* mutations primarily constitute a risk factor for hand eczema in individuals with atopic dermatitis (25,35)

Adult dermatitis patients with both *FLG* mutations and atopic dermatitis reported decreased HRQoL and differed in terms of daily impairment, when compared with atopic dermatitis patients that were *FLG* wild type. Hence, nearly 20% of patients with concomitant *FLG* mutation and atopic dermatitis reported that their disease affected their lives in a ‘large’ or ‘extremely large’ way and, furthermore, that the category with ‘symptoms and feelings’ had the greatest negative impact. It is well-known that

multiple skin disorders have detrimental effects upon HRQoL (8,36). Both asthma and rhinitis reduce HRQoL (37,38) which could potentially influence HRQoL, since both disorders were prevalent among patients with both atopic dermatitis and *FLG* mutations. The DLQI questionnaire is, however, skin specific and the strong association between reduced HRQoL and *FLG* mutations is strengthen by the higher DLQI score among homozygous and compound heterozygous individuals in the population (Figure 1d). Our result indicates that patient-centered management is important in atopic dermatitis patients with concomitant *FLG* mutations. Notably, neither depression nor anxiety was associated with *FLG* genotype or atopic dermatitis (Table 3), indicating that the difference is skin specific. In line with this, we observed no association between *FLG* genotype and generic HRQoL measured by the EQ-5D (39) (data not shown).

Patients in this study population were not included based on disease severity. In fact, 11.7% of the population reported that they no longer suffered from dermatitis, which we believe makes the results more comparable to patients with all severities of dermatitis. The decreased disease severity may also be reflected in the average DLQI scores which were lower than results from other studies investigating HRQoL in patients with atopic dermatitis (8,9) or hand eczema (17). We did however not exclude the 108 patients reporting a total DLQI score of zero in the analyses which lowers the average DLQI score.

We examined the putative association between selected dermatological manifestations and *FLG* mutations. We found significant associations between *FLG* mutations and a life time prevalence of skin fissures on the hand and heels (Table 2b). While fissures in the heels have never been linked to *FLG* genotype, fissured skin on the hands has previously been reported as a clinical marker used to identify *FLG* mutation carriers (26,40), together with palmar hyperlinearity (41) and xerosis (42). In line, a significantly higher prevalence of xerosis was reported by patients with both *FLG* mutations and atopic dermatitis (71.0%) when compared with atopic dermatitis patients with *FLG* wild type (53.6%). No association between xerosis and *FLG* mutations among patients without atopic dermatitis was found, a finding that is in line with a cohort study of children from the general population (43).

We found no association between *FLG* mutations and non-melanoma skin cancer or malignant melanoma. We did however show that self-reported actinic keratosis was more prevalent among *FLG* mutation carriers when compared with non-carriers (Table 3). Degradation of the filaggrin protein in the stratum corneum results in *trans*-urocanic acid (44); an absorber of UV radiation in the stratum corneum (45,46). Thus, the association between self-reported actinic keratosis and *FLG* genotype could possibly be a result of the reduced photoprotective capacity of filaggrin deficient skin, but likely also explained by repeated UV therapy to treat patients' dermatitis. The primary risk factor for actinic keratosis is UV radiation (linked to mutations in the p53 gene) (47), increasing age, male sex, and fair

skin (48). We found that the prevalence increased with age but found no sex difference. Because of the small number of cases, the association between *FLG* mutations and actinic keratosis should be regarded as explorative and needs to be confirmed. In addition, we wish to highlight that we did not make prior power calculations for each of the included comorbidities. However, if confirmed, it might be valuable to know patients' *FLG* genotype prior to light treatment of atopic dermatitis, and to increase awareness against extensive sun exposure, especially for the homozygous / compound heterozygous *FLG* mutation carriers. Thus, patients with atopic dermatitis and *FLG* mutations had significantly more dermatological manifestations when compared with patients with atopic dermatitis and *FLG* wild type.

The strengths of this study are the combination of clinical data, self-reported data, and *FLG* genotype. We did however only genotype the study population for three of the most common mutations, covering 83% of the identified loss-of-function mutations (32). When using self-reported data, recall bias, misclassification, and selection bias are potential limitations. The DLQI score however reflects how skin symptoms affected patients' life during the past week, thereby minimizing recall bias. In addition, we had no clinical severity score for atopic dermatitis or hand eczema, and no data on treatment in relation to systemic medicine and potential UV treatment. Last, we had 25 patients in the group with no atopic dermatitis and *FLG* wild type (patients with hand eczema) which only represents 4.8% of the study population.

In conclusion, *FLG* mutation carriers reported decreased HRQoL. Patients with both *FLG* mutation and a history of atopic dermatitis were mostly affected and nearly 20% of this subset of patients reported that their skin disease influenced their lives in a 'large' or 'extremely large' way, confirming that these patients need extra care on several parameters.

## CONFLICT OF INTEREST

None declared

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**Table 1**: Clinical characteristics by the MOAHLFA index[1] stratified by participants and non-participants (Table 1a) and stratified by inclusion diagnoses for the included patients (Table 1b).

| | a<br>All invited patients (n = 1119) | | | B<br>Included patients (n = 520) | | | |
|---|---|---|---|---|---|---|---|
| | **Participants**<br>(n = 520) | **Non-participants**<br>(n = 599) | ***P* value**<br>(Chi square) | **AD**<br>(n = 102) | **HE**<br>( n = 287) | **AD/HE**<br>(n = 131) | ***P* value**<br>(Chi square) |
| Male, % (n) | 26.2 (136) | 31.2 (187) | = 0.062 | 21.6 (22) | 30.0(86) | 21.4 (28) | = 0.090 |
| Occupational relevance of dermatitis, % (n) | 43.9 (228) | 55.9 (335) | **< 0.001** | 2.0 (2) | 61.0 (175) | 38.9 (51) | **< 0.001** |
| A history of atopic dermatitis, % (n) | 44.8 (233) | 35.6 (213) | **= 0.002** | 100 (102) | - | 100 (131) | . |
| Hand eczema, % (n) | 80.4 (418) | 86.6 (519) | **= 0.005** | - | 100 (287) | 100 (131) | . |
| Leg dermatitis, % (n) | 0.8 (4) | 0.3 (2) | = 0.425[2] | - | 1.4 (4) | - | . |
| Facial dermatitis, % (n) | 28.1 (146) | 18.2 (109) | **< 0.001** | 64.7 (66) | 12.2 (35) | 34.4 (45) | **< 0.001** |
| Age above 40 years, % (n) | 62.5 (325) | 42.4 (254) | **< 0.001** | 50.0 (51) | 73.5 (211) | 48.1 (63) | **< 0.001** |
| Age (median in years, min – max) | 49 (18-83) | 40 (18-86) | **< 0.001**[3] | 42 (18 - 83) | 52 (20 - 82) | 42 (19 -81) | **< 0.001**[3] |
| ≥ 1 positive patch test,[4] % (n) | 44.8 (233) | 35.6 (213) | **= 0.002** | 34.3 (35) | 50.5 (145) | 40.5 (53) | **= 0.009** |

AD; atopic dermatitis, HE; hand eczema;

[1]MOAHLFA-index: Male, Occupational relevance, Atopic dermatitis (lifetime prevalence), Hand eczema (point prevalence), Leg dermatitis (point prevalence), Facial dermatitis (point prevalence) and Age above 40 years. [2]Fisher Exact Test. [3]Kruskal-Wallis Test. [4] Patients were tested with the European baseline series and relevant supplements.

**Table 2:** Overview of filaggrin genotype, self-reported dermatitis, disease severity, and current state of dermatitis stratified by inclusion diagnoses (Table 2a), and combined filaggrin genotype and history of atopic dermatitis (Table 2b).

| | **a (n = 520)** | | | **b (n = 513)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **AD[1] (n = 102)** | **HE[2] (n = 287)** | **AD/HE (n = 131)** | **- AD / FLGwt n = 257 (0)** | **- AD / FLGmut n = 25 (1)** | **+ AD / FLGwt n = 169 (2)** | **+ AD / FLGmut n = 62 (3)** | ***P* value (Group 0 vs 1) (Chi square)** | ***P* value ( Group 2 vs 3) (Chi square)** |
| Filaggrin mutation carriers[4] (n= 513) \| (n = 513) (% (n)) | 18.8 (19) | 8.9 (25) | 33.1 (43) | 0 | 100 (25) | 0 | 100 (62) | - | - |
| Life time prevalence of hand eczema (n= 517) \| (n = 510) (% (n)) | 68.3 (69)[2] | 96.8 (276)[3] | 97.7 (128)[3] | 96.5 (246) | 100 (25) | 83.3 (140) | 90.3 (56) | = 1.000[10] | = 0.185 |
| Point prevalence of hand eczema (n = 512) \| (n = 505) (% (n)) | 41.0 (41) | 55.3 (156) | 71.5 (93) | 55.6 (140) | 56.00 (14) | 53.6 (89) | 71.0 (44) | = 0.966 | **= 0.018** |
| Life time prevalence of fissures on the fingers[5] (n = 493) \| (n = 487) (% (n)) | 35.8 (34) | 54.0 (147) | 53.2 (67) | 51.44 (125) | 76.0 (19) | 41.1 (65) | 57.4 (35) | **= 0.019** | **= 0.031** |
| Life time prevalence of skin fissures on the heels[6] (n = 491) \| (n = 485) (% (n)) | 33.0 (31) | 38.0 (103) | 40.5 (51) | 35.5 (86) | 68.0 (17) | 29.9 (47) | 55.7 (34) | **= 0.002** | **< 0.001** |
| Life time prevalence of xerosis (n = 512) \| (n = 505) (% (n)) | 69.0 (69) | 28.7 (81) | 63.1 (82) | 27.8 (70) | 36.0 (9) | 59.6 (99) | 80.7 (50) | = 0.385 | **= 0.003** |
| **Severity of dermatitis[7]** (n =512) \| (n =505) | | | | | | | | | |
| Severity of your dermatitis today (Score 1-10) (n = 505) (median ($25^{th}$ – $75^{th}$ p[8])) | 2.5 (1 – 5) | 2.0 (1 – 4) | 3.0 (2 – 5) | 2.0 (1 – 4) | 1.5 (0 – 4) | 3.0 (1 – 5) | 3.0 (2 – 5) | = 0.619[11] | = 0.536[11] |
| Severity of your dermatitis when its worst (Score 1-10) (n = 505) (median ($25^{th}$ – $75^{th}$ p[8])) | 9.0 (7 – 10) | 8.0 (6 – 10) | 9.0 (8 – 10) | 8.0 (6 – 10) | 8.0 (5.5– 9) | 9.0 (7– 10) | 9.0 (8– 10) | = 0.291[11] | = 0.193[11] |
| **Current overall state of dermatitis[9]** (n = 503) \| (n = 496) (% (n)) | | | | | | | | | |
| No longer dermatitis | 14.6 (14) | 14.7 (41) | 3,1 (4) | 13.6 (34) | 25.0 (6) | 8.6 (14) | 5.0 (3) | | |
| A lot or a little better | 55.2 (53) | 60.2 (168) | 60.2 (77) | 60.4 (151) | 54.2 (13) | 56.8 (92) | 63.3 (38) | = 0.313 | = 0.552 |
| The same, worse or a lot worse | 30.2 (29) | 25.1 (70) | 36.7 (47) | 26.0 (65) | 20.8 (5) | 34.6 (56) | 31.7 (19) | | |

AD; atopic dermatitis, HE; hand eczema, FLGwt; Filaggrin wild type, FLGmut; Filaggrin null mutation carrier

[1] Clinically diagnosed lifetime prevalence of atopic dermatitis. [2] The clinical hand eczema diagnosis was made at the time of patch-testing and represents a point prevalence. We therefore asked about lifetime prevalence of hand eczema in the questionnaire. [3] The specificity of self-reported hand eczema in this population was 96.7%. [4]Patients were genotyped for the three *FLG* mutations: R501X; 2282del4; and R2447X; 7 participants had a missing *FLG* genotype, [5] "Do you sometimes experience cracks on your fingertips?", [6]"Do you sometimes experience deep cracks in your heels or the tip of your toes"?, [7] "How will you rate your dermatitis on a scale from 0 - 10? (today and when it is worst)", [8]Median and $25^{th}$ – $75^{th}$ percentiles, [9]"How is your dermatitis today, compared to when you were in contact with Gentofte Hospital?", [10] Fisher's Exact Test, [11]Kruskal-Wallis Test.

**Table 3:** Prevalence of dermatological and non-dermatological comorbidities: atopic dermatitis, asthma, rhinitis, food allergy, actinic keratosis, non-melanoma skin cancer, malignant melanoma, anxiety, and depression stratified by filaggrin genotype and history of atopic dermatitis.

| | **Total prevalence**[1] | **- AD / FLGwt n = 257 (0)** | **- AD / FLGmut n = 25 (1)** | **+ AD / FLGwt n = 169 (2)** | **+ AD / FLGmut n = 62 (3)** | ***P* value (Group 0 vs 1) (Fisher's Exact Test)** | ***P* value ( Group 2 vs 3) (Chi Square Test)** |
|---|---|---|---|---|---|---|---|
| Atopic dermatitis[2] (% ($n/n_{total}$)) | 44.8 (233/520) | N/A | N/A | 100 (169) | 100 (62) | **N/A** | **N/A** |
| Self-reported asthma (% ($n/n_{total}$)) | 28.0 (139/497) | 18.2 (44) | 19.1 (4) | 37.0 (61) | 45.2 (28) | = 1.000 | = 0.260 |
| Self-reported rhinitis (% ($n/n_{total}$)) | 42.7 (214/501) | 29.8 (73) | 27.3 (6) | 56.4 (93) | 62.9 (39) | = 0.804[4] | = 0.374 |
| Self-reported food allergy (% ($n/n_{total}$)) | 30.5 (151/495) | 22.0 (53) | 22.7 (5) | 40.9 (67) | 41.0 (25) | = 1.000 | = 0.986 |
| Self-reported actinic keratosis[3] (% ($n/n_{total}$)) | 4.03 (20/496) | 1.7 (4) | 4.6 (1) | 3.1 (5) | 11.3 (7) | = 0.354 | **= 0.022**[5] |
| Self-reported non-melanoma skin cancer (SCC/BCC) (% ($n/n_{total}$)) | 1.8 (9/498) | 1.6 (4) | 4.6 (1) | 0.6 (1) | 3.2 (2) | = 0.353 | = 0.185[5] |
| Self-reported melanoma skin cancer (% ($n/n_{total}$)) | 0.8 (4/495) | 0.8 (2) | N/A | 0.6 (1) | 1.6 (1) | = 1.000 | = 0.478[5] |
| Self-reported anxiety (% ($n/n_{total}$)) | 6.6 (33/498) | 6.9 (17) | 4.8 (1) | 6.2 (10) | 6.5 (4) | = 1.000 | = 1.000[5] |
| Self-reported depression (% ($n/n_{total}$)) | 17.0 (85/501) | 17.1 (42) | 9.1 (2) | 17.7 (29) | 19.4 (12) | = 0.547 | = 0.771 |

AD; atopic dermatitis, FLGwt; Filaggrin wild type, FLGmut; Filaggrin null mutation carrier, SCC; squamous cell cancer, BCC; Basal cell cancer

[1] Patients were genotyped for the three *FLG* mutations: R501X; 2282del4; and R2447X. 7 patients had a missing FLG genotype which can cause difference compared with the total prevalence.

[2] Clinically diagnosed lifetime prevalence of atopic dermatitis. [3] "Have a doctor ever told you that you have/have had actinic keratosis (precursor to skin cancer/ solar damages)?"

[4] Chi Square Test. [5] Fisher's Exact Test

# FIGURES

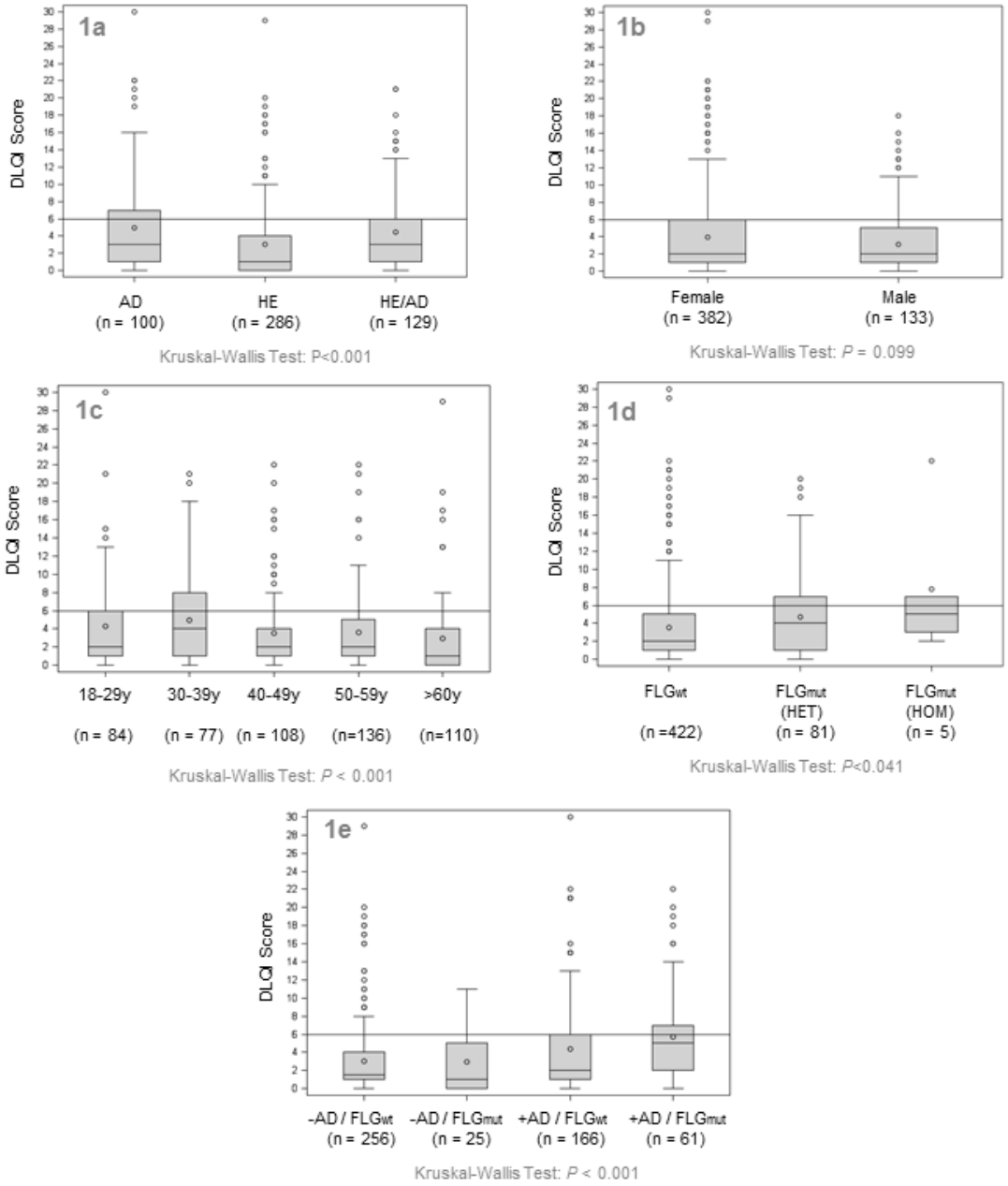


**Figure 1: DLQI scores stratified by inclusion diagnoses, sex, age group, *FLG* genotype, and combined *FLG* genotype and atopic dermatitis phenotype.** Total DLQI scores stratified by inclusion diagnoses (a), sex (b), age group (c), *FLG* genotype (d), and combined *FLG* genotype and atopic dermatitis phenotype (e). The DLQI values refer to HRQoL within the past week and ranges from 0 - 30. Higher DLQI values indicate lower HRQoL. Mean (diamond) and median (line) DLQI scores are shown within the box. The box represents the 25$^{th}$ and 75$^{th}$ percentile and the whiskers show the minimum observation and the maximum observation below the upper fence (1.5 interquartile range). Maximum observations are illustrated as circles. The horisontal line, DLQI = 6, represents a moderate effect on patient's life. A non-parametric Kruskal-Wallis Test shows overall statistical significance between groups in each figure. FLGwt; Filaggrin wild type, FLGmut; Filaggrin null mutation carrier; HET = heterozygous *FLG* mutation carriers; HOM = homozygous or compound heterozygous *FLG* mutations carriers; AD = atopic dermatitis; HE = hand eczema.

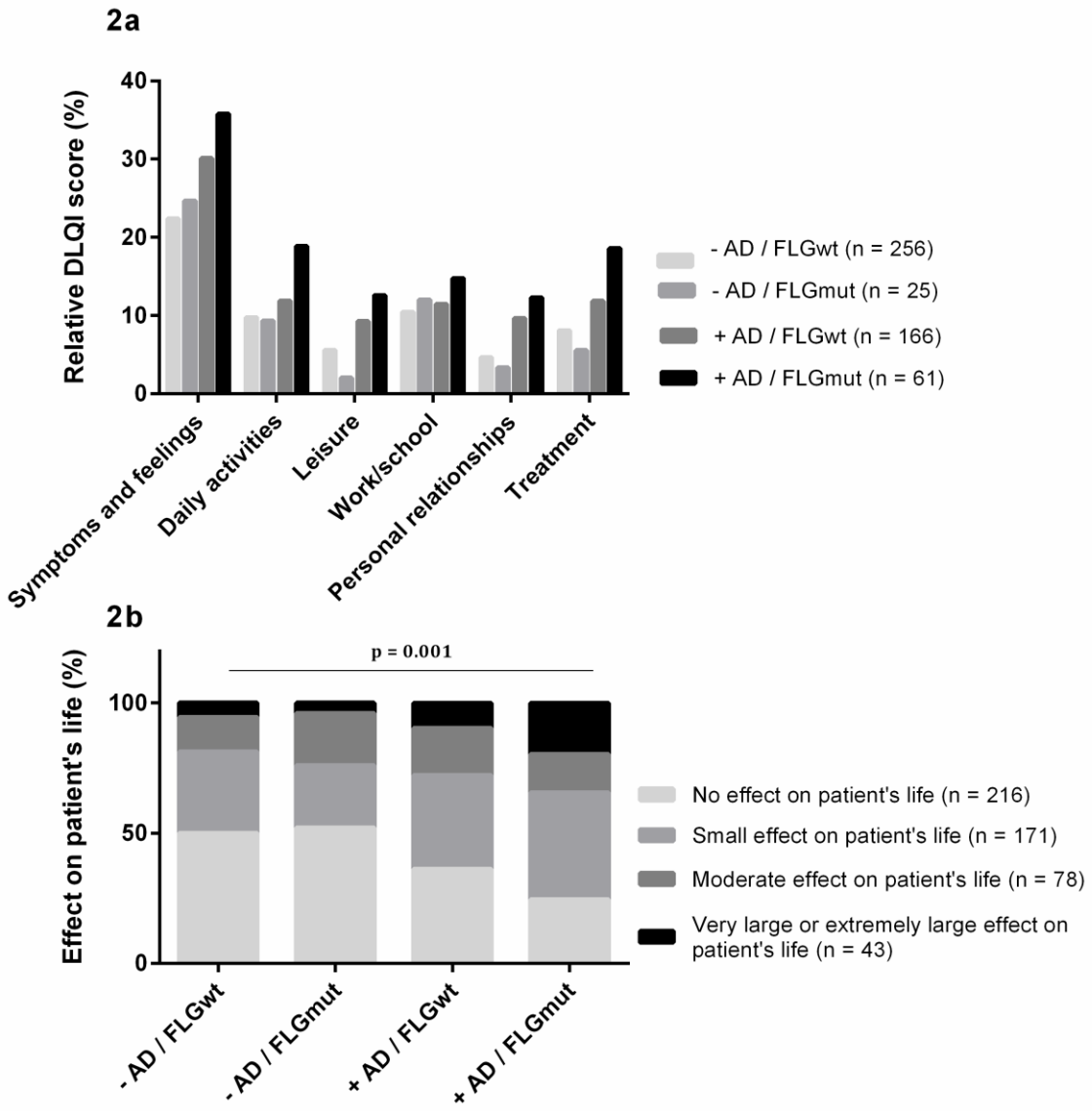


**Figure 2: Subdivision of the DLQI questionnaire**

The DLQI questionnaire can be subdivided into six categories defined by Finley and Kahn(8) (Figure 2a). The scores are relative percentages and can be compared with one another. Figure 2b show subdivision of the health-related effect on patient's life, stratified by combined filaggrin genotype and history of atopic dermatitis. The two categories "very large effect on patient's life" and "extremely large effect on patient's life" representing DLQI >10 is pooled in figure 2b.

# SUPPLEMENTARY MATERIAL

## Table S1: Questionnaire specification

<table>
<tr><th>Variable:</th><th>Question:</th></tr>
<tr><td>Lifetime prevalence of hand eczema</td><td>”Have you ever had hand eczema?” (yes /no)</td></tr>
<tr><td>Point prevalence of hand eczema*<br><br>The point prevalence was calculated based on the pooled answers in the first column: ”I have it now”.</td><td>On which location of your hands have you last had eczema? (Set one mark, in the table below, in the rows that are relevant to your eczema):
<table>
<tr><th></th><th>I have it now</th><th>3-12 months ago</th><th>More than 12 month ago</th></tr>
<tr><td>Fingers</td><td>☐</td><td>☐</td><td>☐</td></tr>
<tr><td>The finger tips</td><td>☐</td><td>☐</td><td>☐</td></tr>
<tr><td>Space between fingers</td><td>☐</td><td>☐</td><td>☐</td></tr>
<tr><td>Back of the hand</td><td>☐</td><td>☐</td><td>☐</td></tr>
<tr><td>Palm</td><td>☐</td><td>☐</td><td>☐</td></tr>
<tr><td>Wrist</td><td>☐</td><td>☐</td><td>☐</td></tr>
<tr><td>Forearm</td><td>☐</td><td>☐</td><td>☐</td></tr>
</table></td></tr>
<tr><td>Fissures on the fingers</td><td>“Do you sometimes experience cracks on your fingertips”?</td></tr>
<tr><td>Fissures on the heels</td><td>“Do you sometimes experience deep cracks in your heels or the tip of your toes”?</td></tr>
<tr><td>Xerosis</td><td>”Have you ever had dry skin all over your body?”</td></tr>
<tr><td>Self-reported asthma</td><td>”Have a doctor ever told you that you have/have had asthma?”</td></tr>
<tr><td>Self-reported rhinitis</td><td>”Have a doctor ever told you that you have/have had hay fever?”</td></tr>
<tr><td>Self-reported food allergy</td><td>”Have a doctor ever told you that you have/have had food allergy?”</td></tr>
<tr><td>Self-reported actinic keratosis</td><td>”Have a doctor ever told you that you have/have had actinic keratosis (precursor to skin cancer/ solar damages)?”</td></tr>
<tr><td>Self-reported non-melanoma skin cancer (BSS/SCC)</td><td>”Have a doctor ever told you that you have/have had skin cancer (basal cell cancer/ or spinocellulare cancer)?”</td></tr>
<tr><td>Self-reported melanoma skin cancer</td><td>”Have a doctor ever told you that you have/have had birthmark cancer (malignant melanoma)?”</td></tr>
<tr><td>Self-reported anxiety</td><td>”Have a doctor ever told you that you have/have had anxiety?”</td></tr>
<tr><td>Self-reported depression</td><td>”Have a doctor ever told you that you have/have had depression?”</td></tr>
<tr><td>Current overall state of dermatitis</td><td>”How will you score your dermatitis today compared to when you were in contact with the Dermatological Department at Gentofte Hospital?”<br>(No longer dermatitis, a lot better, a little better, the same, a little worse, a lot worse, don’t-know)</td></tr>
<tr><td>Self-rated severity of dermatitis (VAS scale)*</td><td>“How will you rate your dermatitis on a scale from 0 - 10?”<br>(set one mark in each of the figures)
<table>
<tr><th>Today</th><th>When it is worst</th></tr>
<tr><td>☐ 10 (Severe dermatitis)</td><td>☐ 10 (Severe dermatitis)</td></tr>
<tr><td>☐ 9</td><td>☐ 9</td></tr>
<tr><td>☐ 8</td><td>☐ 8</td></tr>
<tr><td>☐ 7</td><td>☐ 7</td></tr>
<tr><td>☐ 6</td><td>☐ 6</td></tr>
<tr><td>☐ 5</td><td>☐ 5</td></tr>
<tr><td>☐ 4</td><td>☐ 4</td></tr>
<tr><td>☐ 3</td><td>☐ 3</td></tr>
<tr><td>☐ 2</td><td>☐ 2</td></tr>
<tr><td>☐ 1</td><td>☐ 1</td></tr>
<tr><td>☐ 0 No dermatitis</td><td>☐ 0 No dermatitis</td></tr>
</table></td></tr>
</table>

*Question adapted from the Nordic Occupational Skin Questionnaire (NOSQ-2002)

## 4.4 Occupational consequences of having dermatitis and filaggrin mutations

When investigating occupational status in the general population, stratified by dermatitis, we found that the proportion of disability pension was increased among participants with both hand eczema and atopic dermatitis. Table 3 shows the association between disability pension and different explanatory variables and is a simplified illustration of Table 2 in Manuscript IV.

**Table 3:** Logistic regression analyses with 'disability pension' as dependent variable and *FLG* genotype or combined dermatitis and *FLG* genotype as explanatory variables are shown. OR = Odds ratio; 95 CI = 95% confidence interval; FLGwt = filaggrin wild type; FLGmut = filaggrin mutation carrier; HE = hand eczema; AD = atopic dermatitis. Age group (18–40, 41–55, and > 56 years).
N = 2772

| **Explanatory variables** | **Percent on disability pension (n=103)** *% (n)* | **Crude** *OR (95% CI)* | **Adjusted** *OR (95% CI)* |
|---|---|---|---|
| Filaggrin genotype | | | |
| *Wild type* | 3.45 (87) | 1 (reference) | 1 (reference) |
| *Mutation carrier* | 6.40 (16) | 1.91 (1.10 – 3.32) | 1.79 ( 1.02 – 3.15)* |
| Dermatitis and filaggrin status | | | |
| *No dermatitis (FLGwt)* | 2.60 (48) | 1 (reference) | 1 (reference)** |
| *No dermatitis (FLGmut)* | 4.32 (7) | 1.69 (0.75 – 3.80) | 1.60 (0.71 – 3.64) |
| *Hand eczema (no AD) (FLGwt)* | 5.39 (23) | 2.13 (1.28 – 3.55) | 2.02 (1.21 – 3.83) |
| *Hand eczema (no AD) (FLGmut)* | 9.38 (3) | 3.88 (1.14 – 13.16) | 4.02 (1.15 – 14.11) |
| *Atopic dermatitis (±HE) (FLGwt)* | 6.43 (16) | 2.57 (1.44 – 4.60) | 2.74 (1.51 – 4.99) |
| *Atopic dermatitis (±HE) (FLGmut)* | 10.71 (6) | 4.50 (1.84 – 11.00) | 6.01 (2.37 – 15.24) |

*Adjusted for age group, sex, and atopic dermatitis ** adjusted for age group and sex

By linkage to occupational status obtained from Statistics Denmark, we found that the proportion of individuals receiving disability pension was increased among *FLG* mutation carriers (6.4%) when compared with wild type individuals (3.45%) in the general population. When stratifying by self-reported dermatitis and *FLG* genotype together, we found that *FLG* genotype increased the association between disability pension and both hand eczema and atopic dermatitis in the general population (OR = 4.02; 95% CI 1.15–14.11 and OR = 6.01; 95% CI 2.37–15.24, respectively). The grounds for awarding disability pension were unknown in this setup, which means that the primary diagnosis can be either of physical, psychological or social character, or a combination.

Among participants reporting hand eczema symptoms, around 60% of the *FLG* mutation carriers with concomitant atopic dermatitis reported symptoms of hand eczema before adulthood in both populations in contrast to 16–30% of the *FLG* mutation carriers reporting hand eczema but without atopic dermatitis. Lastly, half of the patients had a history of work in a risk occupation in contrast to one third of participants from the general population.

**Title:**

Hand eczema, atopic dermatitis, and filaggrin mutations in adult Danes: a registry-based study including risk of disability pension

**Running title:**

Hand eczema, atopic dermatitis, and filaggrin mutations in adult Danes

**Word count and illustrations in the manuscript:**

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Illustrations: 1 figure, 3 tables

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**Conflict of interest:** None declared

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**What is already known?**

- Severe AD and hand eczema are associated with sick leave and/or disability pension.
- *FLG* mutations increase the risk of AD and hand eczema in individuals with AD.
- Knowledge about occupational consequences among *FLG* mutation carriers is limited.

**What does this study add?**

- Self-reported dermatitis was associated with receiving disability pension in the general population, particularly among *FLG* mutation carriers.
- Among individuals with AD and *FLG* mutations who develop hand eczema, 60% report symptoms before adulthood.

## ABSTRACT

**Background:** Atopic dermatitis (AD) and hand eczema increase the risk of disability pension. It is currently unknown whether individuals with loss-of-function filaggrin gene (*FLG*) mutations, who often have severe and early onset of dermatitis, experience occupational consequences.

**Method:** Adult Danes from the general population (n=3,247) and patients with AD and/or hand eczema (n=496) were genotyped for three common *FLG* mutations and completed a questionnaire about skin symptoms and hand eczema. In the general population, AD was estimated using the UK criteria. Socioeconomic variables and information about previous work in risk occupations were retrieved from national registries, thus the primary diagnoses for granting disability pension in this setup was unknown. Descriptive statistics and logistic regression models were used to evaluate significance.

**Results:** Disability pension was associated with both AD and hand eczema in the general population. Moreover, the proportion of individuals receiving disability pension was increased among *FLG* mutation carriers (6.40%) when compared with non-mutation carriers (3.45%)(*P*=0.019). Notably, self-reported hand eczema and AD were associated with particularly high risk of disability pension among *FLG* mutation carriers (odds ratio (OR) = 4.02; 95% confidence interval (CI) 1.15–14.11 and OR= 6.01; 95%CI 2.37–15.34, respectively), although these interactions did not reach statistical significance.

**Conclusion:** Self-reported dermatitis, particularly in persons with a genetically determined impaired skin barrier, was significantly associated with disability pension in the general population suggesting that individuals with *FLG* mutations and dermatitis could benefit from early attention with respect to choice of occupation and optimized treatment.

## INTRODUCTION

Dermatitis is the most common skin disease and covers several disease entries, where AD and hand eczema are among the most prevalent in the general population with an estimated one-year prevalence in adults of 2–15% and 10%, respectively.[1-3] Hand eczema is further the most recognized occupational skin disease in Denmark.[4] Because individuals with AD are at particular risk of developing hand eczema later in life, early occupational counselling and preventive strategies are encouraged.[5-8]

The personal and societal consequences of dermatitis are substantial and include reduced quality of life,[9-11] increased healthcare costs,[12-14] increased sick leave, and even reduced work productivity, job change, rehabilitation, and/or disability pension.[8,15-20] Changes in the average total number of disability pensions due to skin diseases in Denmark have been reported in the last decades: 79 per year during 1970–1976, 115.7 per year during 1999–2002, and 95.8 per year during 2003–2008, the changes might reflect societal changes in terms of granting disability pensions, changes in environmental exposure, and advances in treatment.[18,19,21]

*FLG* mutations are found in approximately 8% of the general population in Northern European countries [22,23] and are associated with an increased risk of, often severe, AD.[24-26] Notably, *FLG* mutations have also been found to increase the risk of early onset and persistent hand eczema in individuals with AD.[27] It is currently unknown whether *FLG* mutation carriers experience increased occupational consequences, when compared to non-mutation carriers.

In this study we investigated the personal consequences of having both *FLG* mutations and dermatitis. We investigated socioeconomic measures, including disability pension, and history in risk-occupations to evaluate whether occupational counselling and preventive strategies should be focused not only on individuals with AD, but also on individuals with *FLG* mutations.

## METHOD

### Study populations

In this study we used data from two populations; (i) a cross-sectional study including adult dermatological patients and (ii) a cross-sectional study of the general Danish population.

*The patient population*

We included 515 patients (≥18 years) diagnosed with AD and/or hand eczema at the Dermatology Department at Gentofte Hospital during 2006–2012 (participation rate 46.5%). All patients received a written invitation and interested patients got oral information about the study and completed a questionnaire.

Genomic DNA was collected by a buccal swap (Isohelix, Harrietsham, United Kingdom) and stored at -80°C until extraction was performed with a JetQuick kit (Genomed, Löhne, Germany) according to manufactures' instructions. *FLG* genotyping was performed as previously described [28] for three of the most common *FLG* mutations: R501X, 2282del4, and R2447X, which together constitute 83% of the total risk alleles associated with AD; [24,29,30]. A positive test was defined for heterozygote, homozygote, and compound heterozygote mutation carriers.

*The general population*

We included 3,471 participants (≥18 years) from a cross-sectional study performed in the south-western part of Copenhagen (participation rate: 44.7%). The population was selected as a random sample of the general population by use of the Danish Central Personal Registry and the inclusion period started in 2006. Detailed information about the study has previously been described.[31] Participants were subjected to a general health examination, which did not include a skin examination, and completed a questionnaire regarding lifestyle factors and chronic diseases, including skin disorders and symptoms. A history of AD was estimated using the U.K. Working Party's diagnostic criteria as a history of an itchy skin condition plus a minimum of two of four minor criteria.[32] The major criterion was an itchy skin condition and the minor criteria were: (i) a history of involvement of the skin creases, (ii) a history of asthma or hay fever, (iii) a history of generally dry skin and (iv) onset before the age of 2 years.[32] *FLG* genotyping was based on blood samples and performed as described above.[28]

**Questions about history of self-reported hand eczema**

The questions about hand eczema in both populations were introduced by the following description of eczema: ''*Eczema is an itchy skin disorder showing redness, dryness, and possibly vesicles and exudation. Eczema remains on the same area of the body for some time*.'' Participants were asked: ''*Have you ever had hand eczema*?'' Participants who gave an affirmative answer were further asked '*At what age did you have hand eczema on the first occurrence?*'(<6 years, 6–14 years, 15–18 years or >18 years).

**Linkage to registry data**

Data from both populations were transferred to Statistics Denmark and linked to information from different nationwide registries on an individual level. Socioeconomic data were retrieved from the general population in 2006 and from the patient population in 2013. Logistic regression analyses were used to investigate the associations with disability pension. Occupational status, including disability pension, was obtained from the National Registry of Personal Income. The grounds for granting disability were unknown. A dichotomous variable for disability pension was created. Participants receiving disability pension formed one group, while individuals who were either 'working', 'unemployed' or 'on leave or sick leave' formed another group 'not

receiving disability pension'. Retired participants (n=429) were set to be 'missing' because they represent a group outside the work force and cannot be considered as candidates for disability pension. Because of the low number of individuals with more than one *FLG* mutation and the privacy rules of Statistics Denmark, it was not possible to investigate whether the proportion of individuals receiving disability pension differed between homozygous/compound heterozygote *FLG* mutation carriers and heterozygous *FLG* mutation carriers. A retrospective linkage of both populations during 1994–2013 investigating 'work in a risk occupation' was performed for all individuals in the work active period (16–64 years) using the *Danish Registers on Labour Market Affiliation*, which record occupational classification based on DISCO codes.[33] We use published literature to define risk occupations for dermatitis.[34-36]

**Ethics**

Written informed consent was obtained for all participants, in both populations, prior to inclusion. The patient study was approved by the Ethics committee of Copenhagen County (H-1-2013-127) and so was the general population study (KA-20060011). The study was further approved by the Danish Data Protection Agency.

**Statistics**

We included 3,247 (93.5%) of the participants from the general population and 496 (96.3%) of the patients from the patient population in the present analyses; the remaining participants were omitted due to missing data on *FLG* genotype, AD (only in the general population) or the hand eczema questions. We analysed only complete cases to enable stratification on dermatitis and *FLG* genotype. Few people were registered as 'missing cases' in the different registries which causes small variations in the total n presented in the tables. Statistical analyses for dichotomous variables were made by use of the Chi Square Test or Fishers Exact Test. Non-parametric variables were tested for group difference using Kruskal-Wallis Test. Level of significance was set at $P<0.05$. Continuous variables were presented as median scores with $25^{th}$ and $75^{th}$ percentiles. Multiple logistic regression models were used to estimate OR and 95% CIs. All statistical analyses were performed using SAS software (SAS, Version 9.3 for Windows, SAS Institute Inc., Cary, NC, USA) and the figure was made using GraphPad Prism version 6.07 for Windows (GraphPad software, La Jolla, CA, USA).

**RESULTS**

**Dermatitis is reflected in occupational status in the general population**

Table 1 shows characteristics of the two populations stratified by dermatitis and *FLG* genotype. In the general population, significant differences were found for age, sex, occupational status and income (Table 1A). Participants with AD (± hand eczema) were younger and dominated by female sex ($P<0.001$, Table

1A). In relation to occupational status, the proportion of individuals receiving disability pension was increased among participants with a history of dermatitis; AD ± hand eczema (6.6%) and hand eczema only (5.0%) when compared with participants without dermatitis (2.3%) ($P$<0.001, Table 1A). In addition, a significant difference between occupational status and *FLG* mutations was observed. Income was also found to be lower among individuals with both dermatitis and *FLG* mutations, possibly as a result of occupational status.

In contrast, no sex difference was found among the patients with or without AD. However, the patients with AD were significantly younger and the proportion of patients under education was significantly increased when compared to patients without AD ($P$<0.001, Table 1B). Of note, the proportion of patients with a short education was highest among patients with hand eczema and no history of AD, irrespective of *FLG* genotype (hand eczema + *FLGwt* = 66.8%, hand eczema + *FLGmut* = 79.2%, AD + *FLGwt* = 48.8%, and AD + *FLGmut* = 50.9%) ($P$ < 0.001). In addition to the data presented in Table 1B, 18% of the patients were ≤ 30 years. Of these individuals, a significantly higher proportion of young patients were found among *FLG* mutation carriers when compared to non-mutation carriers which may influence the educational level (25.6% and 16.6%, respectively, $P$=0.048).

**Self-reported dermatitis in the general population was associated with receiving disability pension, particularly among filaggrin mutation carriers**

To further investigate the difference in occupational status among participants with and without dermatitis in the general population, we made logistic regression analyses with 'disability pension' as the dependent variable (Table 2). Self-reported hand eczema was found to be associated with receiving disability pension, irrespective of AD (adjusted OR=2.30; 95% CI 1.50–3.54). In addition, a history of AD was also associated with receiving disability pension, although the association became weaker when adjusting for hand eczema, sex and age group (crude OR=2.29; 95% CI 1.41–3.73 and adjusted OR=1.89; 95% CI 1.11–3.21, respectively). The proportion of individuals receiving disability pension was increased among *FLG* mutation carriers (6.40%) when compared with non-mutation carriers (3.45%) (crude OR=1.91; 95% CI (1.10–3.32). As *FLG* mutations themselves cannot predispose to disability pension but may result in symptoms leading to disability pension, we made a regression analysis taking both dermatitis and *FLG* genotype into account. Individuals without dermatitis and *FLG* wild type were used as references. Notably, self-reported hand eczema and/or AD were associated with particularly high risk of receiving disability pension among *FLG* mutation carriers, (OR= 4.02; 95%CI 1.15–14.11 and OR= 6.01; 95%CI 2.37–15.34, respectively) although these interactions did not reach statistical significance ($P$ = 0.449 and $P$ = 0.953, respectively).

**The patterns of onset of hand eczema in the general population and among patients are very similar**

Fig. 1 illustrates onset of hand eczema symptoms by AD and *FLG* genotype for the general population (Fig. 1A) and patients (Fig. 1B). The trend was very similar for the two populations and confirms that *FLG* mutations increase the risk of early onset of hand eczema symptoms in individuals with AD. In detail, 40% of the *FLG* mutations carriers with AD developed symptoms of hand eczema before the age of six and 60% before adulthood (18 years). Notably, *FLG* mutations did not predispose for early symptoms of hand eczema in individuals without a history of AD. The majority of the participants who developing hand eczema, but without a history of AD, developed hand eczema in adulthood presumably as a consequence of environmental exposure to allergens and/or irritants.

**History of work in a high-risk occupation**

We investigated employment in high-risk dermatitis occupations by retrospective linkage to Statistics Denmark during 1994–2013 (Table 3). Almost one third of the individuals in the general population had been hired in a risk occupation at some time during the period. In contrast, almost half of the patients had been occupied in a risk occupation. For both populations, the most prevalent occupations were cleaners followed by nursing assistants and nurses which together represented more than half of the participants working in a risk occupation (Table 3A).

When stratified by dermatitis and *FLG* genotype, the prevalence of having worked in a risk occupation was increased among participants who reported hand eczema and/or AD in the general population ($P<0.001$) (Table 3B). In addition to the data in table 3B, no difference in the proportion of *FLG* mutation carriers were found between risk occupations and non-risk occupations among individuals in the general population or among patients ($P=0.972$ and $P=0.121$, respectively).

**DISCUSSION**

We showed that the proportion of individuals receiving disability pension in the general population was increased among participant reporting hand eczema and/or had a history of AD. Moreover, we showed that self-reported dermatitis in the general population was significantly associated with receiving disability pension, particularly in individuals with a genetically determined impaired skin barrier, in this context manifested by presence of *FLG* mutations. *FLG* mutations themselves cannot predispose to disability pension but may result in skin symptoms leading to disability pension.

The grounds for granting disability pension was unknown in this study. However, the association between disability pension and self-reported dermatitis suggests that individuals receiving disability pension stand out in relation to reporting skin symptoms.

Albeit the number of disability pension granted due to skin diseases is low, previous publications have repeatedly shown associations between disability pension and both hand eczema and AD. [8,17,18,37] An investigation of disability pensions granted due to skin diseases in Denmark identified contact dermatitis as the dominant diagnosis in the 1970's.[18] During 2003–2008 disability pension granted due to contact dermatitis was halved, which might be an effect of regulatory interventions aiming to reduce environmental exposure to nickel.[19,38] Today, the biological interplay between contact dermatitis, *FLG* mutations and AD remains to be elucidated. Recently, *FLG* mutations were associated with contact dermatitis in construction workers, most strongly in individuals with an atopic predisposition [39]. Moreover, a Swedish study showed that 36% of disability pensions granted due to skin diseases were granted because of eczema. Of those granted disability pension, painters, cement workers, and plumbers formed the major group.[20] In our setup it was not possibly to link disability pension and occupation. In future studies, it could, however, be relevant to investigate whether *FLG* mutation carriers working in risk occupations, such as construction workers, have an increased prevalence of receiving disability pension, or rehabilitation benefits, when compared to wild type carriers.

Although the incidence of AD in Scandinavian children is now stable,[40] it is well-described that the incidence of AD has increased over the past decades.[41-43] In line with this, the number of disability pensions granted per year with AD as primary diagnosis has increased from the 1970's to the 2000's.[18,19] In our study, the prevalence of AD in the general population was found to decrease with increasing age: 13.7% (18–40 years), 10.9% (41–55 years), and 7.5% (>56 years). It could therefore be hypothesized whether more individuals are likely to apply for disability pension as a consequence of AD when the younger generations with more AD ages, which encourages effective preventive measures and early detection of skin symptoms. In addition, more individuals vulnerable to develop hand eczema will hence enter the work force in the years to come, as a history of AD is the most pronounced predictive factor for hand eczema.[6,44,45]

The finding that self-reported dermatitis in the general population was associated with receiving disability pension, primarily among *FLG* mutation carriers, was not reproduced in patients which might have several explanations. First, fewer *FLG* mutation carriers were found among patients included with hand eczema only. In total, 8.9% of the patients with hand eczema but without AD were *FLG* mutation carriers in contrast to 26.8% of the patients with AD ($P<0.001$). Moreover, disability pension is mainly granted to elder individuals. In the patient population, patients with AD (representing 71.3% of the *FLG* mutation carriers) were significantly younger that the patients with hand eczema but without a history of AD.

Furthermore, we found that the pattern of onset of symptoms of hand eczema in the general population and among patients was very similar when stratified by *FLG* genotype and AD. The trend in the patient population can thereby be interpreted as control for the trend seen in the general population. It could be

hypothesized, that because 60% of the individuals with *FLG* mutations and concomitant AD, and 40% of the non-mutation carriers with AD, who develop hand eczema, will have onset of symptoms before adulthood, individuals with AD might have 'an advantage' in terms of choosing a future career with low risk of exposure to irritants and allergens or in terms of using preventive measures. In line with this, it has been suggested that *FLG* mutation carriers, with onset of hand eczema in childhood, avoid professional exposure to irritants.[46] However, previous publications have shown that a history of AD does not influence the choice of occupation.[47-50] Nevertheless, Wei *et* al., (2016) report, that having received occupational counselling was associated with higher use of preventive measures which encourages continued occupational counselling.[47] In contrast, patients with hand eczema but without AD, was characterized by onset of hand eczema in adulthood, short education, and an increased prevalence of history in a risk occupation which suggest a different pattern in the development of hand eczema symptoms when compared with patients with AD.

The strengths of this study are the combination of cross-sectional data from two populations combined with registry data. However, there is always risk of misclassification and/or recall bias when using questionnaire data which can influence the sensitivity and specificity of both self-reported hand eczema and AD (defined by the UK criteria). Nevertheless, the greatest limitation of this study is that we had no information about the primary diagnoses leading to the local authority granting disability pension. The grounds for disability pension can therefore be of physical, psychological, or social character, or a combination.

In conclusion, we showed that self-reported dermatitis, particularly in persons with a genetically determined impaired skin barrier, was significantly associated with receiving disability pension in the general population. Although the grounds of receiving disability pension were unknown, focus on early detection of skin symptoms is suggested. Moreover, our data indicate that an economical burden on society might be expected, as the younger generations with a higher rate of AD ages, unless appropriate actions are taken to prevent this such as medical attention with respect to decisions on early change of occupation and optimized treatment.

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**Tables 1:** Demographics of the populations including: age, sex, education, family status, occupational status, and income. The general population was linked to the registries in 2006 while patients were linked in 2013. *P* value[1] = all group difference using Chi Square Test (Kruskal-Wallis Test for age and income). *P* value[2] = difference between the variable and filaggrin genotype: wild type vs. mutation carriers using Chi Square Test (Kruskal-Wallis Test for age and income). *Education* (highest Education Attained) was available from the Attainment Registry, *family status* was available from the National Danish Registry of Population Statistics, and *occupational status* and *income* were available from the National Registry of Personal Income.

| **A** | **The general adult population** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Total N= 3247** | **No dermatitis** | | **Hand eczema (no atopic dermatitis)** | | **Atopic dermatitis ± Hand eczema** | | ***P* value[1]** | ***P* value[2]** |
| | | **$FLG_{wt}$ (n=2187)** | **$FLG_{mut}$ (n=195)** | **$FLG_{wt}$ (n=493)** | **$FLG_{mut}$ (n=36)** | **$FLG_{wt}$ (n=276)** | **$FLG_{mut}$ (n=60)** | | |
| ***Age*** *(n=3247)* *(median (25-75 percentiles))* | 50 (40– 60) | 51 (41–61) | 52 (42–61) | 50 (41–60) | 47 (39–55) | 47 (37–57) | 46 (35–54) | **<0.001** | 0.744 |
| ***Sex*** *(n=3247) (%, female)* | 55.0 | 51.2 | 50.3 | 61.1 | 66.7 | 74.3 | 65.0 | **<0.001** | 0.829 |
| ***Education*** *(n=3223)(%)*[a] *Under education* *Individuals with a short education* | 5.4 71.3 | 5.3 71.1 | 3.1 75.3 | 4.9 73.8 | 5.6 72.2 | 8.8 67.3 | 3.3 66.7 | 0.501 | |
| ***Family status*** *(n=3246)* *(%, living alone w/wo children)* | 22.3 | 21.6 | 25.1 | 21.3 | 27.8 | 25.5 | 30.0 | 0.306 | 0.077 |
| ***Occupational status*** *(n=3201) (%)* *Working* *Unemployed (receiving social benefit* *Leave, maternity leave or sick leave* *Disability pension* *Retired* | 80.9 1.9 0.6 3.2 13.4 | 81.2 1.5 0.5 2.3 14.4 | | 78.4 3.3 1.2 5.0 12.2 | | Not shown[b] 2.4 Not shown[b] 6.6 8.1 | | **<0.001** | **< 0.001** |
| ***Income (gross)*** *(n=3247)* *(mean DKK)* | 316,719 | 324,522 | 298,596 | 307,955 | 296,617 | 291,096 | 293,141 | **= 0.001** | **0.044** |
| **B** | **Adult dermatitis patients** | | | | | | | | |
| | **Total** | **Hand eczema (no atopic dermatitis)** | | | | **Atopic dermatitis ± Hand eczema** | | ***P* value[1]** | ***P* value[2]** |
| | **N = 496** | **$FLG_{wt}$ (n=245)** | **$FLG_{mut}$ (n=25)** | | | **$FLG_{wt}$ (n=165)** | **$FLG_{mut}$ (n=61)** | | |
| **Age** *(n=496)* *(median (25-75 percentiles))* | 48.5 (36-58) | 50 (42 – 59) | 52 (47 – 57) | | | 42 (30 – 53) | 42 (27 – 55) | **<0.001** | 0.265 |
| **Sex** *(n=496) (%, female)* | 74.6 | 70.2 | 80.0 | | | 80.6 | 73.8 | = 0.109 | 0.741 |
| **Education** *(n=487)(%)*[a] *Under education* *Individuals with a short education* | 11.5 59.6 | 5.7 66.8 | 12.5 79.2 | | | 15.2 48.8 | 25.5 50.9 | **< 0.001** | **= 0.008** |
| **Family status** *(n=496)* *(%, living alone w/wo children)* | 28.4 | 24.5 | 28.0 | | | 30.9 | 37.7 | 0.176 | 0.144 |
| **Occupational status** *(n=487) (%)* *Working* *Unemployed (receiving social benefit* *Leave, maternity leave or sick leave* *Disability pension* *Retired* | 79.1 4.5 2.1 3.7 10.7 | 76.2 4.5 1.9 4.9 12.5 | | | | 82.4 4.5 2.2 2.3 8.6 | | 0.321 | 0.683[c] |
| **Income gross** *(n=496)* *(mean DKK)* | 353,449 | 341,439 | 312,529 | | | 389,841 | 320,019 | 0.387 | 0.177 |

[a] Highest obtained education. [b] Not shown because of privacy restrictions. [c] Fishers Exact Test

**Table 2:** Logistic regression analyses with 'disability pension' as dependent variable and filaggrin genotype, hand eczema or atopic dermatitis as explanatory variables are shown for participants in the general population. A dichotomous variable for disability pension was created. Participants who were working, unemployed or on' leave or sick leave' was considered as 'not on disability pension' while retired participants (n=429) were set to be 'missing' because they were outside the work force. Students were also considered as working. The reason for granting disability pension to the 103 individuals is unknown. The analyses for each explanatory variable were performed separately. n = 2,772

| **Explanatory variables ($n_{group}$)** | **Percent on disability pension (n=103)** *% (n)* | **Crude** *OR (95% CI)* | **Adjusted** *OR (95% CI)* |
|---|---|---|---|
| Age group (n=809, n=1,221 and n=742) | | | |
| 18 – 40 years | 0.74 (6) | Not calculated[a] | Not calculated[a] |
| 41 – 55 years | 3.52 (43) | | |
| >56 years | 7.28 (54) | | |
| Sex (n=1,245 and n=1,527) | | | |
| *Male* | 2.73 (34) | 1 (reference) | 1 (reference) |
| *Female* | 4.52 (69) | 1.69 (1.11 – 2.56) | 1.87 (1.23 – 2.86)[b] |
| Filaggrin genotype (n=2,522 and n=250) | | | |
| *Wild type* | 3.45 (87) | 1 (reference) | 1 (reference) |
| *Mutation carrier* | 6.40 (16) | 1.91 (1.10 – 3.32) | 1.79 ( 1.02 – 3.15)[c] |
| Hand eczema (n=2,157 and n=613) | | | |
| *Never* | 2.74 (59) | 1 (reference) | 1 (reference) |
| *Lifetime prevalence* | 7.18 (44) | 2.75 (1.84 – 4.11) | 2.30 (1.50 – 3.54)[c] |
| Atopic dermatitis (n=2,467 and n=305) | | | |
| *No* | 3.28 (81) | 1 (reference) | 1 (reference) |
| *Lifetime prevalence* | 7.21 (22) | 2.29 (1.41 – 3.73) | 1.89 (1.11 – 3.21)[d] |
| Dermatitis and filaggrin status (n=1,846, n=162, n=427, n=32, n=249, n=56) | | | |
| *No dermatitis (FLGwt)* | 2.60 (48) | 1 (reference) | 1 (reference)[e] |
| *No dermatitis (FLGmut)* | 4.32 (7) | 1.69 (0.75 – 3.80) | 1.60 (0.71 – 3.64) |
| *Hand eczema (no AD) (FLGwt)* | 5.39 (23) | 2.13 (1.28 – 3.55) | 2.02 (1.21 – 3.83) |
| *Hand eczema (no AD) (FLGmut)* | 9.38 (3) | 3.88 (1.14 – 13.16) | 4.02 (1.15 – 14.11) |
| *Atopic dermatitis (±HE) (FLGwt)* | 6.43 (16) | 2.57 (1.44 – 4.60) | 2.74 (1.51 – 4.99) |
| *Atopic dermatitis (±HE) (FLGmut)* | 10.71 (6) | 4.50 (1.84 – 11.00) | 6.01 (2.37 – 15.24) |

OR = Odds ratio; 95 CI = 95% confidence interval; FLGwt = filaggrin wild type; FLGmut = filaggrin mutation carrier; HE = hand eczema; AD = atopic dermatitis. [a] Hand eczema is primarily granted to individuals between aged 50–59 years. [b] adjusted for age group (18–40, 41–55, and >56 years). [c] Adjusted for age group, sex and atopic dermatitis. [d] adjusted for age group, sex and hand eczema, [e] adjusted for age group and sex.

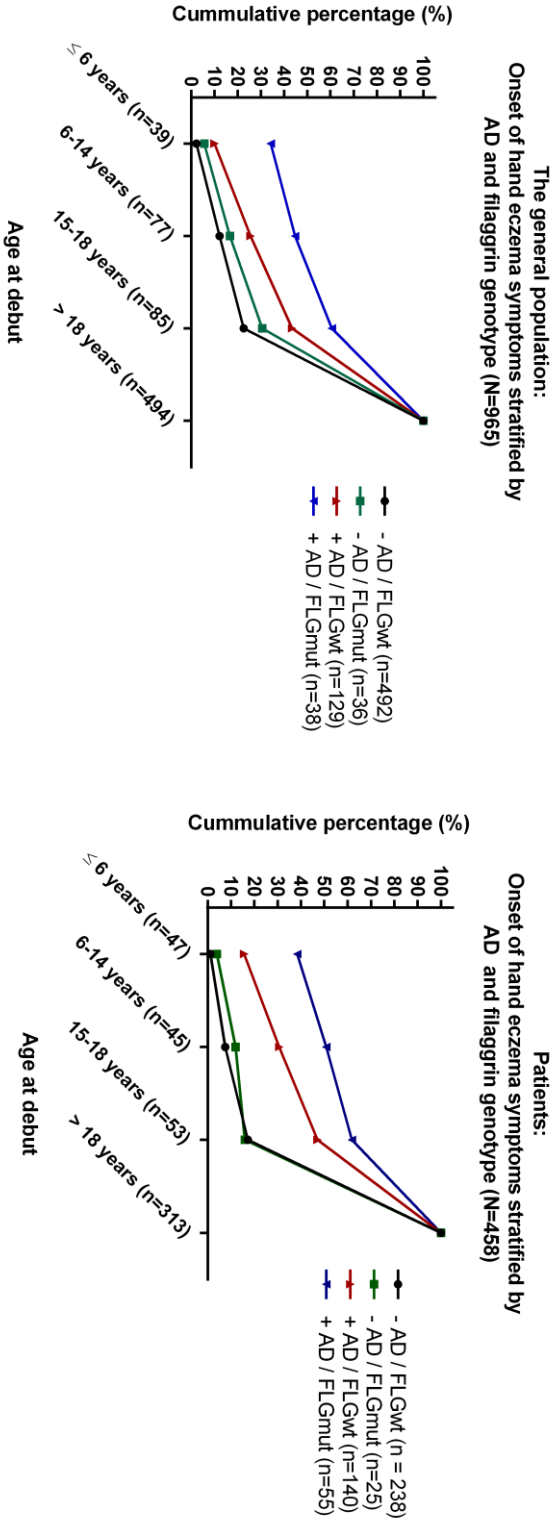
**Table 3:** Prevalence of employment in risk occupations (A). Prevalence of employment in a risk occupation stratified by dermatitis and filaggrin genotype (B). Data is obtained by linkage to the Danish Registry on Labor Market Affiliation in the period 1994-2013. Only complete cases with a filaggrin genotype, history of atopic dermatitis and hand eczema are included. Cells, with a number of participants smaller than three, are deleted because of discretion policies, causing the difference in total n. Risk occupations were selected based on the literature and represented occupations with high exposure to allergens and irritants, such as wet work.[34-36]

| **A**<br><br>***Risk occupations, %(n)*** | ***The general adult population***<br>*($n_{total}$=3245)* | ***Adult dermatitis patients***<br><br>*($n_{total}$ = 490)* |
|---|---|---|
| Hair dressers and beauticians | 0.68 (22) | 4.49 (22) |
| Bakers | 0.34 (11) | 1.02 (5) |
| Cooks | 1.85 (60) | 4.49 (22) |
| Butcher | 0.15 (5) | - |
| Painters | 0.77 (25) | 1.02 (5) |
| Mechanics | 1.97 (64) | 2.45 (12) |
| Machine workers | 1.05 (34) | - |
| Cleaners | 6.56 (213) | 8.78 (43) |
| Electricians | 1.73 (56) | 1.02 (5) |
| Plumbers | 0.92 (30) | - |
| Black Smith / Lock Smith | 1.23 (40) | 0.82 (4) |
| Nurses | 2.50 (81) | 6.94 (34) |
| Nursing assistants | 7.61 (247) | 12.65 (62) |
| Physicians | 0.31 (10) | 2.24 (11) |
| Dentists and dental surgery assistants | 0.12 (4) | 1.02 (5) |
| Laboratory technicians | 1.33 (43) | 2.04 (10) |
| History of work in a risk occupation %(n) | **29.12 (945)** | **49.18 (241)** |
| No history of work in a risk occupation %(n) | **70.88 (2300)** | **50.82 (249)** |
| **B**<br><br>***Stratification by dermatitis and filaggrin genotype, %(n)*** | ***Prevalence in risk occupation*** | ***Prevalence in risk occupation*** |
| No dermatitis (FLGwt) | 26.84 (587) | - |
| No dermatitis (FLGmut) | 28.72 (56) | - |
| Hand eczema (no AD) (FLGwt) | 35.50 (175) | 57.72 (142) |
| Hand eczema (no AD) (FLGmut) | 30.56 (11) | 44.00 (11) |
| Atopic dermatitis (±HE) (FLGwt) | 35.77 (98) | 40.24 (66) |
| Atopic dermatitis (±HE) (FLGmut) | 30.00 (18)[a] | 40.00 (22)[b] |

*FLGwt = filaggrin wild type; FLGmut = filaggrin mutation carrier; HE = hand eczema; AD = atopic dermatitis.*
[a] $P<0.001$ (overall group difference, chi square test), [b] $P = 0.002$ (overall group difference, chi square test).

## FIGURE LEGEND

**Figure 1:** Age at debut of self-reported hand eczema symptoms stratified by history of atopic dermatitis and filaggrin gene (FLG) mutations in the general population (A) and in patients (B). 'FLGwt = filaggrin wild type; FLGmut = filaggrin mutation carrier; AD = atopic dermatitis.



# 5. COMMENTS ON METHODOLOGY

In the following section the strengths and weaknesses of the thesis are considered. General comments on disease definitions used in the Health2006 cohort in Manuscript I and II are presented first, followed by general comments on genotyping, considerations regarding the patient population, and use of registry data.

## 5.1 Disease definitions in the general population

**Atopic dermatitis:** When reviewing the definition of atopic dermatitis in population-based studies with no clinical confirmation, several disease definitions exist. In 1980, Hanifin and Rajka formulated a guideline for the clinical diagnoses of atopic dermatitis based on a set of three or more basic features plus three or more minor features.[123] However, the guidelines were intended to be used in hospital settings and in clinical trials, so in the 1990s the UK working party (consisting of 13 dermatologists) refined the Hanifin and Rajka criteria and developed a new guideline for use in epidemiological studies. The UK diagnostic criteria were published in 1994 and consist of one major criterion of a pruritic skin condition plus three or more of the following minor criteria: history of flexural involvement, personal history of asthma or hay fever, history of a generally dry skin, onset of symptoms under the age of 2 years, and visible flexural dermatitis.[124] The UK criteria have since been widely used to define atopic dermatitis in epidemiological studies with a sensitivity of 69–85% and a specificity of 96%, depending on the validation design.[125] A limitation is that the validation of the criteria in population settings has been mainly in children[126] and their usefulness in adults is questionable, leading to several other diagnostic criteria for atopic dermatitis being suggested. In Denmark, other definitions include the Schultz Larsen criteria, which have been validated in epidemiological studies,[24] and the Nordic Occupational Skin Questionnaire including the question "*Have you ever had eczema on the front of the elbows or behind your knees?*" has also been used to estimate atopic dermatitis in adult work-place populations.[127,128] Jepsen and Flyvholm (2007) conclude that the UK question '*Have you ever had an itchy rash that has been coming and going for at least 6 months, and at some time has affected skin creases? (by skin creases we mean folds of elbows, behind the knees, fronts of ankles, under buttocks, around the neck, ears, or eyes)*' led to an over-reporting of atopic dermatitis when compared with the question from the Nordic Occupational Skin Questionnaire.[127] However, they included only the major criterion of the UK criteria in their analyses,[127] which is why an overrepresentation must be expected, and stress the importance of using both the major and minor criteria.

We used a slightly modified version of the UK criteria to estimate the prevalence of atopic dermatitis in the Health2006 population. Our version include a major criterion 'history of an itchy skin condition' plus a minimum of 2 of 4 minor criteria: (1) a history of involvement of the skin creases, (2) a history of asthma or

hay fever, (3) a history of generally dry skin, and (4) onset before the age of 2 years. We have no possibility to validate the UK criteria in the Health2006 cohort, but we can look into the criteria in the patient population because this population was asked the same questions. In the patient population we have both a clinically diagnosed lifetime prevalence of atopic dermatitis reported in the MOAHLFA-index and the UK criteria. Table 4 illustrates the frequencies of patients diagnosed with atopic dermatitis based on the MOAHLFA-index and the self-reported UK criteria.

**Table 4:** Comparison of atopic dermatitis diagnoses by a dermatologist and by using the UK criteria for questionnaire studies.

| Frequency<br>*Col. Per cent.* | | **Clinical diagnoses of atopic dermatitis from the MOAHLFA-index** | | Total |
|---|---|---|---|---|
| | | NO | YES | |
| **UK diagnostic criteria of atopic dermatitis** | NO | 176<br>*65.9* | 39<br>*17.0* | 215 |
| | YES | 91<br>*34.1* | 190<br>*83.0* | 281 |
| Total | | 267 | 229 | 496 |

Of the patients, 83% diagnosed with atopic dermatitis at Gentofte Hospital also had a history of atopic dermatitis according to the UK criteria. This percentage could be compared to a "measure of sensitivity" if we consider the clinical diagnoses as the true estimate. This "sensitivity measure" is comparable to the previous report on sensitivity of the UK criteria.[129] However, the "specificity measure" of 65.9% is very low because 91 patients fulfil the UK criteria for atopic dermatitis but have no clinical diagnoses. Nevertheless, it would be wrong to view the proportion of 65.9% as a "specificity measure". First, because all patients included in this population had been in contact with Gentofte Hospital between 2006 and 2012 and they completed the questionnaire throughout 2014, which means that patients could have developed atopic dermatitis in the meantime. Moreover, even though a clinical diagnosis is considered as the gold standard, all clinical databases rely on the validity of the data entered, which could introduce misclassification. If it was the "sensitivity measure" that was in question, we would have had the possibility to retrieve the patient's file and confirm the atopic dermatitis diagnosis; however, this is not possible with the "specificity measure". Last, we removed the minor criterion regarding visible eczema because we were interested in estimating the lifetime prevalence of atopic dermatitis, which might compromise the validity of the UK criteria. Nonetheless, there is no evidence that the abovementioned should vary between subgroups e.g. *FLG* mutation carriers and non-carriers. Table 4 is an attempt to address the challenges of using the UK criteria to estimate atopic dermatitis in the general population and we do not use the UK criteria to access

atopic dermatitis in the patient population (in Manuscript III and IV). Lastly, a systemic review from 2008 concluded that no diagnostics criteria for atopic dermatitis have 100% validity, but the UK criteria are the most extensive validated which is why they were chosen when the Health2006 population was established.[129]

**Hand eczema:** Several epidemiological studies have aimed to validate self-reported hand eczema in adults from various populations. Meding and colleagues (1990) were among the first to investigate the 1-year prevalence of hand eczema in the general Swedish population,[130] and many later studies have also focused on reporting 1-year prevalence.[70] Meding and Barregård (2001) validated the following question regarding 1-year prevalence: "*Have you had hand eczema on any occasion during the past 12 months*" in a population of car mechanics, dentists and office workers, and found the sensitivity to be 53–59% and the specificity to be 96–99% in the three groups.[131] Results from questionnaire studies depend on the definitions in the analyses, which is why questionnaires should be as reliable and valid as possible. Moreover, prevalence estimates may differ because of methodological decisions such as using a "symptom-based diagnosis" or a "self-reported diagnosis".[128,132] In line with this, reports have shown that it can be difficult for the individuals to identify skin symptoms compatible with the clinical diagnoses and this is why self-reported diagnoses asking "*Do you have hand eczema?*" are preferable.[133]

Many studies have used the question "*Have you ever had hand eczema?*" to estimate lifetime prevalence of hand eczema in the population in question. In 2011, Bregnhøj and colleagues validated hand eczema questions from the Nordic Occupational Skin Questionnaire designed to provide standardized data that could be compared between countries;[128] good agreement was found between self-reported point prevalence of hand eczema and clinical examination with a sensitivity of 70.3% and a specificity of 99.8%.[134] Self-reported data on hand eczema were used throughout this thesis including lifetime-, 1-year- and point prevalence. Because of the follow-up design in the Health2006 cohort, participants answered questions about hand eczema twice, separated by a 5-year interval.

Figure 5 illustrates self-reported hand eczema at baseline and follow-up in the Health2006 population and presents the hand eczema groups used in Manuscripts I, II, and IV. The lifetime prevalence at baseline and follow-up was very similar being 21.8% and 20.7%, respectively. When looking more closely into the data, the lifetime prevalence at follow-up is not a confirmation of the lifetime prevalence reported by the participants at baseline. At follow-up, 138 participants (29.0%) did not confirm their lifetime prevalence five years later. Thus, the lifetime prevalence of hand eczema at follow-up is close to that at baseline because of the 133 incident cases.

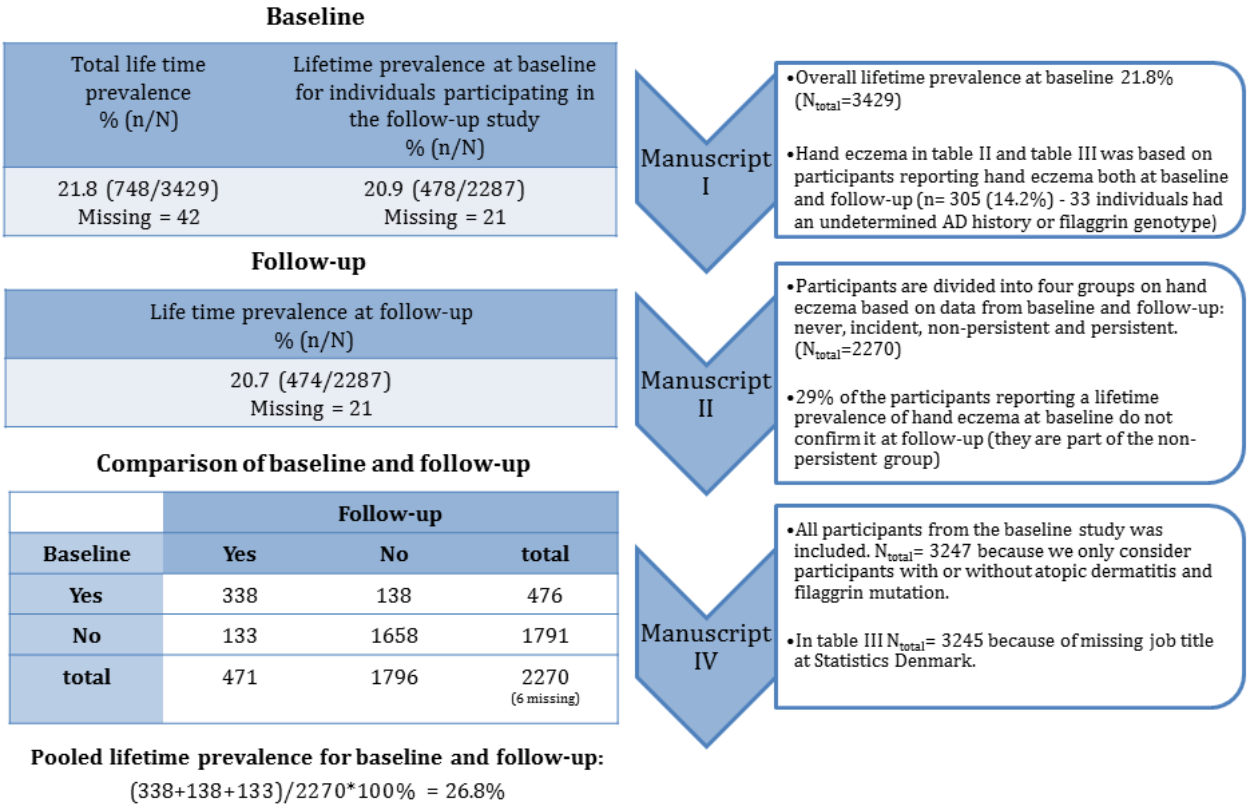



**Figure 5** Illustrates reports of hand eczema at baseline and follow-up in the Health2006 cohort.

In Manuscript II, we investigate predictive factors of hand eczema. Here we define four groups of hand eczema and report the 1-year prevalence of hand eczema. If we had included the lifetime prevalence in this manuscript, one could argue that the "true lifetime prevalence" for the Health2006 cohort should have included all participants who had ever reported hand eczema, which would have given a lifetime prevalence of 26% ((338+138+133/2270)*100%). A lifetime prevalence of hand eczema of 26% is high when compared with the overall lifetime prevalence at 15% reviewed by Thyssen and colleagues (2010).[70] A cross-sectional study from 1998, focusing on the association between contact allergy and hand eczema, conducted in the same catchment area as the Health2006 population, reported a lifetime prevalence of hand eczema of 26.6%.[135] Even though there are methodological issues when comparing the "pooled lifetime prevalence" in the Health2006 population to the lifetime prevalence from the cross-sectional study in 1998, the similarity is striking and indicates consistency. In line with this, reports have suggested that the self-reported prevalence is underestimated when compared with the true prevalence.[131] It would have been relevant to contact participants who reported "yes" to having had hand eczema in the baseline study and "no" in the follow-up study to estimate whether they had actually had hand eczema. Unfortunately, this was not possible. The cumulative incidence rate of hand eczema during the 5-year period was 14.8 per 1000 years. This is also high when compared with previous incidence rates of 8.8 per 1000 person-years,

which have been found in two studies with Danes from the general population.[66,136] However, the incident cases in Manuscript II were defined by comparing two lifetime prevalence. As discussed in the manuscript, this method has not been validated and might increase recall bias. Last, the following definition of dermatitis*: "Dermatitis is an itchy skin disorder showing redness, dryness, and possibly bladders and exudation. Dermatitis remains on the same area of the body for some time",* was introduced prior to the questions on dermatitis. It was intended to help participants define dermatitis and thereby reduce misclassification. It is unknown whether the definition of dermatitis works as intended.

## 5.2 Considerations about filaggrin genotyping

We aimed to investigate the patterns of dermatitis and the personal consequences of a genetically impaired skin barrier, which we defined by the presence of at least one mutation in the gene coding for the filaggrin protein. To date, more than 50 mutations have been identified throughout the profilaggrin molecule, of which 23 are variants found in European populations and 8 are believed to be recurrent.[13,113] In our analyses we genotyped all participants for three of the most common loss-of-function mutations constituting 83% of the total risk alleles associated with atopic dermatitis; R501X, 2282del4, and R2447X.[1,13,113] If we had examined two additional mutations S3247X[113] and 3702delA[137] we could have increased the coverage to include 96% of the identified risk alleles, 10% and 3%, respectively. The *FLG* tests for both populations were performed at the Department of Clinical Biochemistry, Copenhagen University Hospital Herlev-Gentofte. In this project we use a slightly modified version of the method which has previously been described in detail.[138] At the Department of Clinical Biochemistry, efforts have been made to include additional mutations in the routine analyses for *FLG* mutations. The allele-specific PCR prior to the microbead-specific hybridization in the *FLG* genotype is complex and primers for the specific mutations have different optima regarding temperature and salt concentrations. Thus inclusion of an additional primer, representing another mutation, is complex and was not possible at the time of genotyping. Other strategies for full sequencing have been reported[113] but were not chosen because of time and financial aspects. Nevertheless, we believe that the three mutations included are representative of the Northern European population, but we cannot determine whether some individuals might be carriers of some of the rarer *FLG* mutations.

## 5.3 Establishment of the patient population

**Defining atopic dermatitis and hand eczema in the patient population:** Clinical diagnoses of atopic dermatitis and hand eczema were scored by the attending dermatologist and were registered in the patient's file using the internationally recognized MOAHLFA-index[115] (Male, Occupational dermatitis, history of Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, and Age above 40 years), which

is registered routinely for all patients who are patch tested. It should be highlighted that while the diagnosis of atopic dermatitis reflects a lifetime prevalence, the hand eczema diagnosis reflects a point prevalence at the time of patch testing. Thus many patients in the group with atopic dermatitis may have had hand eczema at some point in life, especially since atopic dermatitis is known to be a major risk factor of hand eczema.[66,74,75] This was confirmed in the questions about hand eczema included in the questionnaire completed by the patients showing that 68.3% of the 102 patients included with atopic dermatitis only reported a lifetime prevalence of hand eczema (Table II, Manuscript III). To address this, we divided patients into a "skin barrier variable" (Manuscripts III and IV) based on history of atopic dermatitis and on results from *FLG* genotyping. By doing this, the group with atopic dermatitis only and the group with both atopic dermatitis and hand eczema were pooled. Notably, one third of the participants with atopic dermatitis and hand eczema in this cohort were *FLG* mutation carriers, which highlights the importance of the interplay between *FLG* mutations, hand eczema and atopic dermatitis.

**Expected prevalence of filaggrin mutation carriers:** When we made power calculations for the patient population prior to project initiation, we estimated that the prevalence of *FLG* mutations in hand eczema patients would be around 20%.[139] Moreover, reports showed that the prevalence of *FLG* mutations in patients with atopic dermatitis is around 25%.[140] In adult patients with severe and persistent atopic dermatitis, the prevalence of *FLG* mutations was found to be around 40%.[34] Because we did not consider severity when including patients with atopic dermatitis, we expected a prevalence of *FLG* mutations of 25%.

When considering the results from Manuscript III, the following prevalence of *FLG* mutations was found: patients included with atopic dermatitis (18.8%), patients included with hand eczema (8.9%) and patients included with both atopic dermatitis and hand eczema (33.1%). In retrospect, we overestimated the prevalence of *FLG* mutation carriers among patients with hand eczema and no history of atopic dermatitis, which showed to be similar to the prevalence found in the general population.[15] Because of this, we had only 25 patients in the group with no atopic dermatitis and *FLG* wild type (patients with hand eczema), which represented 4.8% of the study population.

**Participation rate:** The low number of participants in this group is also a result of the 46.5% participation rate. It is generally considered that participation rates are a measure of representativeness and should be as high as possible to minimize the risk of selection bias.[141] Nevertheless, it is also known that there is a declining response rate for participation in health surveys and that change in the non-respondents group exists.[142] Table 5 presents socioeconomic variables for the 1119 invited patients and reveals that there are several significant differences between the two groups. Non-participants differed significantly from

participants with respect to sociodemographic characteristics. Non-participants were younger and a significantly larger proportion were receiving social benefits, which may have bearing on the lower income observed in the non-participant group when compared with the participant group ($P \leq 0.025$). Because the non-participants were significantly younger, it could be speculated whether the lower income is a result of a larger proportion of students in group. In total, 4.9% of the patients included were students, and although a higher prevalence of students was found among non-participants (5.5%) when compared with participants (4.2%), the difference was non-significant.

**Table 5:** Socio-demographic characteristics of participants and non-participants in the patient population. *****Kruskal-Wallis test.

| | **Participants (n = 520)** | **Non-participants (n = 599)** | ***P* value Chi square test** |
|---|---|---|---|
| **Sex** *(Male, %)* | 26.2 | 31.2 | = 0.062 |
| **Age** *(median in years, min – max)* | 49 (18-83) | 40 (18-86) | < 0.001* |
| **Family status** *(%, living alone w/wo children)* | 28.3% | 32.1% | 0.098 |
| **Occupational status** *Working (%)* *Unemployed (%, receiving social benefits)* *Leave, sick leave or early retirement (%)* *Retired (%)* | 79.1 4.3 5.7 11.0 | 76.0 8.7 6.3 9.1 | 0.025 |
| **Income (gross)** ***(mean in dkk, (n))*** | 352,619 | 268,444 | <0.001* |

In 2013, Thuesen and colleagues published a cohort profile of the Health2006 cohort showing the same tendency. Here, non-respondents to the baseline examination were younger, had lower educational level and a lower personal income than did respondents.[114] In the patient cohort, it was however not only a questionnaire that should be completed and send back. Because of the *FLG* genotyping, all interested patient should actively take contact to the National Allergy Research Centre where they got oral information about the study prior to inclusion. The results from Table 5 could point to participants having larger resources than non-participants. However, we have no reason to believe than the non-participants should differ from participants in relation to prevalence of *FLG* mutation carriers. Concluding, Rothman and colleagues (2013) state that "*It is not representativeness of the study subjects that enhances the generalization, it is knowledge of specific conditions and an understanding of mechanism that makes for a proper generalization*".[143] Parts of the work with the patient population however have a descriptive aim which is why one could argue that a high participation rate would, of cause, had been preferable.[143]

**Usefulness of the DLQI questionnaire:** In Manuscript III, we evaluated HRQoL using the DLQI questionnaire. The DLQI was developed to assess skin-specific HRQoL.[104] In general, HRQoL can be defined as the

subjective perception of the impact of health status, including disease, treatment, and both physical and psychological wellbeing.[144] The DLQI has been widely used since its introduction; however, the clinical validity of the instruments has been debated. Twiss and colleagues (2012) questioned whether the DLQI could be used to measure the impact of psoriasis and atopic dermatitis in patients,[145] and later Nijsten (2012) concluded that the DLQI had limitations and that instruments assessing quality of life had evolved since 1994.[146] Despite the critique, the DLQI instrument has repeatedly been found to be reliable, valid, and easy to use.[147] Disease-specific questionnaires to measure HRQoL continue to be developed. One of the newer hand eczema questionnaires 'The Quality of Life in Hand Eczema Questionnaire' was introduced in 2014 by Ofenloch and colleagues and the authors concluded that it showed strong properties in terms of validity, reliability and responsiveness to change.[148] In general, disease-specific questionnaire are preferable to catch the relevant symptoms, but because we had different diagnoses in the patient population, hand eczema and/or atopic dermatitis, we needed a skin-specific questionnaire rather than a disease-specific questionnaire, which is why we believe that the DLQI questionnaire was the best choice for this study population.

## 5.4 Using retrospective registry data

In Manuscript IV we investigated the personal consequences of having a genetically impaired skin barrier using self-reported questionnaire data and registry data from Statistics Denmark. We chose to present data from both patients and the general population in the same manuscript but did not compare the two populations directly because there are differences in time of inclusion (2014 vs. 2006), sex and age. Because of the high number of participants in the Health2006 population, we could have chosen to match participants in the Health2006 population, on sex and age, with the patients. However, if we had chosen this method, we would have discarded data from many *FLG* mutation carriers and would then have compromised power in the analyses. Therefore, we chose to include all participants from both populations and made descriptive analyses, well-knowing that the populations were representative of the adult general population and adult dermatitis patients but could not be considered as "controls and cases", respectively.

When we investigated the association between disability pension and dermatitis/*FLG* genotype in Table 2, Manuscript IV, we made a dichotomous variable for disability pension to use in the regression analyses. In this 'disability pension variable' participants receiving disability pension formed one group, while individuals who were either 'working', 'unemployed' or 'on leave or sick leave' formed another group 'not receiving disability pension'. Retired participants (n=429) were set to be 'missing' because they represent a group outside the work force and cannot be considered as candidates for disability pension. The grounds for granting disability pension were unknown in this setup, but we can conclude that the proportion of

individuals receiving disability pension in the general population was increased among individuals reporting dermatitis. To account for time differences, we repeated the association between *FLG* mutations and disability pension in three additional years, 2008, 2010 and 2013, and found significant associations ($P \leq 0.025$). The relevance of this control can be discussed since *FLG* genotype is permanent and most cases of disability pensions are granted as lifelong benefits. However, societal changes in social benefits, including retirement age and terms for granting social benefits, are introduced on a regular basis, which could influence the terms of granting disability pension in the period and introduce bias.

In Manuscript IV we also investigated the differences in former and present work in a risk occupation. Statistics Denmark made a retrospective analysis where they investigated whether the participants in question had a history in risk occupations during 1994–2013 (as long as possible using the Danish Register on Labour Market Affiliation). In Table 3, Manuscript IV, we defined 18 risk occupations based on the existing literature.[83,149-151] By using this approach we were able to retrieve prevalence estimates about work in a risk occupation and years in the most frequent risk occupation. Because of the form in which the tables were delivered, if was not possible to account for risk time in the occupations.

Moreover, Statistics Denmark has some strict rules regarding privacy to ensure data protection. This means that if one cell contains less than three observations, another cell must be left blank to ensure that it is impossible to trace the individual. Because of this we were not able to make separate analyses on homozygous and heterozygous *FLG* mutation carriers but had to focus our analyses on participants with or without a *FLG* mutation. Lastly, not all the registers are 100% complete, which is why there are small variations in $N_{total}$ in the different tables. That being said, the Danish registers represent a unique data source that is very accurate because of the linkages using the personal identification numbers.

# 6. DISCUSSION

This section includes a condensed discussion additional to the discussion sections presented in the manuscripts.

**Epidemiology of dermatitis in the general population:**

One third of the adult Danish population reported dermatitis at some point in their lives on some point of their bodies. Hand eczema was the most prevalent site of dermatitis with a lifetime prevalence at baseline of 20.9%; of this percentage, 14.2% of the individuals confirmed having had hand eczema at any point in their lives at follow-up. In relation to genetic predisposition of dermatitis, we showed that patients with atopic dermatitis had a distinct pattern of dermatitis and that *FLG* mutations increase the lifetime prevalence of persistent hand eczema and dermatitis on the feet.[Manuscript I]

Our finding that *FLG* mutations increased the prevalence of self-reported dermatitis only on the extremities is possibly influenced by the daily environmental and/or physical challenges, such as irritants, allergens, sweat and/or pressure, to which the hands and feet are subjected. Accordingly, a clinical report evaluating contact allergy in children with atopic dermatitis concludes that children with dermatitis on the hands and/or feet should always be patch-tested.[152] In line with our findings, Isaksson and colleagues (2015) indicate that patients with atopic dermatitis and hand or foot dermatitis stand out, but no genotyping for *FLG* mutations was performed in this study.[152] Notably, body regional variations in epidermal thickness exist.[153,154] Further, regional variations for drug penetration have been shown with decreased absorption in the regions of the skin having a thickened stratum corneum, such as the feet.[155] These notions could be contradictive with a hypothesis of increased penetration in the foot and hand region, where the epidermis is thick: sole (636.5 µm), dorsum of foot (177.5 µm), palm (602.4 µm) and dorsum of hand (189.5 µm) and where fewer hair follicles are present.[153] Nevertheless, we also showed that *FLG* mutations were significantly associated with skin fissures on the hand and the heels, which may increase penetration of exogenous agents. [Manuscript III]

In agreement with this, research in filaggrin-deficient mice (often flaky tail (ft/ft) mice)[156] has shown that filaggrin deficiency predisposes to enhanced epicutaneous sensitization with less resistance to mechanical stress in the deeper layers of stratum corneum.[157-159] This supports a hypothesis of increased penetration in the foot and hand region, regions which are exposed to mechanical stress. However, it must be highlighted that the majority of the *FLG* mutation carriers in the Health2006 population were heterozygous carriers and that atopic skin itself is known to affect the skin barrier, leading to, for example, defects in lipid metabolism, altered protease activity and tight junction defects. [Reviewed in 46,160]

**Several factors predispose to hand eczema:**

Several factors predispose to hand eczema. In Manuscript II we confirmed previous studies showing a relationship between persistent hand eczema and contact sensitization.[161] Notably, we also found the association between contact allergy and persistent hand eczema to be significant in individuals without atopic dermatitis (OR = 2.84; 95% CI 1.24–5.60).[Manuscript II]

While positive associations between *FLG* mutations and intolerance to nickel have previously been reported,[15,162,163] other studies in patients have rejected an association between *FLG* mutations and contact sensitization.[164] A direct association, independent of atopic dermatitis, between *FLG* mutations and hand eczema has been identified in few studies.[165,166] An increased risk of developing hand eczema, both irritant contact dermatitis[167] and combined irritant and allergic contact dermatitis,[166] has been found for *FLG* mutations carriers, particularly in individuals with a history of atopic dermatitis.[48] Recently, *FLG* mutations were found to be associated with contact dermatitis in construction workers, particularly in individuals with an atopic predisposition, stressing the need for prevention among individuals with an impaired skin barrier.[88]

Of the 108 individuals with persistent hand eczema in Manuscript II, 19.6 % (n=20) were *FLG* mutation carriers compared with 7.5% of those with incident and non-persistent hand eczema (*P*<0.001). *FLG* mutations were found to predict persistent hand eczema, but no difference was found in the prevalence of contact sensitization between *FLG* mutation carriers and non-carriers (data not shown). Other predictive factors for hand eczema were also found including self-reported food allergy and 'redness, rash or itch related to use of cosmetic products', which confirms that environmental factors in daily life have a major impact on the disease.[Manuscript II] As patients with atopic dermatitis have repeatedly been shown to have a compromised skin barrier function, resulting in an increased reactivity to skin irritants[168-170] and food allergy,[43] we stratified for atopic dermatitis and found both food allergy and 'redness, rash or itch related to use of cosmetic products' to be predictors of hand eczema independently of atopic dermatitis. Again, the finding stresses the need for continuous skin care and knowledge about irritants and allergens in the population. Notably, a recent European multicentre study found no increased overall risk of contact sensitization in individuals with atopic dermatitis from the general population.[171]

Thus the interplay between contact dermatitis, *FLG* mutations and dermatitis remains to be elucidated to identify whether *FLG* mutations are contributing factors or causal components for contact sensitization.

A clear tendency towards debut of hand eczema depending on history of atopic dermatitis and *FLG* genotype was illustrated in Manuscript IV, and the patterns were very similar for patients and individuals

from the general population. We report that almost 40% of participants with both atopic dermatitis and *FLG* mutations report a debut of hand eczema before the age of 6 years, and around 60% will have developed symptoms of hand eczema before reaching adulthood (> 18 years).[Manuscript IV] The fact that the majority of individuals with *FLG* mutations and atopic dermatitis will develop signs of hand eczema before adulthood shows the importance of early detection of skin symptoms and frequent use of emollients, which improves the skin barrier[172] and has been found to be useful in the therapy of hand eczema.[173,174]

**Do filaggrin mutations exert their function early in life?**

In Manuscript II we showed that *FLG* mutations are not associated with incident hand eczema in adults and introduced the hypothesis that *FLG* mutations exert their function early in life. This notion is supported by a few experimental studies.[175;176]

Today, we know that a delicate balance between genetic and acquired filaggrin deficiency exists.[55-61] However, we do not know whether this balance changes in relation to time. In this thesis we did not look into the underlying biology but aimed to investigate the complexity using epidemiological analyses. In both Manuscripts I and II, we stratified for atopic dermatitis and found that without co-existence with atopic dermatitis, the effect of having impaired skin barrier was neutralized. In other words, there was a tendency to regard *FLG* mutations as an "add on effect" in individuals with a history of atopic dermatitis.

**The role of filaggrin on comorbidity:**

Research investigating cutaneous and non-cutaneous comorbidity associated with *FLG* mutations has advanced in recent years. In our cohort of patients, we investigated self-reported comorbidity using the question: '*has a doctor ever told you that you have/have had…'* Here we found, that the highest prevalence of atopic comorbidities (asthma, rhinitis and food allergy) was reported by patients with both atopic dermatitis and *FLG* mutations.[Manuscript III] However, the association between *FLG* mutations and both asthma and rhinitis was lost when adjusting for age group, sex and a history of atopic dermatitis, which is in line with the existing literature.[30,31,42]

We also identified an association between *FLG* mutations and self-reported actinic keratosis, with the highest prevalence reported by homozygous carriers, followed by heterozygous carriers and wild type individuals ($P$ = 0.003).[Manuscript III] Degradation of the filaggrin protein in the stratum corneum results in *trans*-urocanic acid,[177] an important absorber of UV radiation in the stratum corneum.[178,179] Accordingly, it could be suggested that the association between self-reported actinic keratosis and *FLG* genotype could be a result of the reduced photoprotective capacity of filaggrin deficient skin. However, it is also possible that the association is an artefact of UV therapy to treat patients' dermatitis. Because of the small number of cases and lack of prior power calculations, the association between *FLG* mutations and actinic keratosis

needs to be confirmed. A consensus report from 2016 calls for action in recognizing non-melanoma skin cancer, including actinic keratosis, as an occupational disease[180] because outdoor workers were found to have a higher risk of actinic keratosis when compared with indoor workers (OR=1.55; 95% CI 1.55–2.54). If the association between *FLG* mutations and actinic keratosis is confirmed, use of UV therapy in *FLG* mutation carriers should be reconsidered. Finally, *FLG* mutation carriers, especially homozygous individuals, should be advised to avoid excessive sun exposure, and workers in an outdoor occupation should have extensive information about primary protection.

**Personal consequences of dermatitis:**

Research addressing the personal consequences of having *FLG* mutations is limited. This was the reasoning behind looking into the personal consequences of having *FLG* mutations in adult dermatitis patients.

In Manuscript III, we showed that patients with concomitant atopic dermatitis and *FLG* mutations also stood out in relation to HRQoL. We investigated quality of life using the DLQI questionnaire and found that *FLG* mutations significantly reduced HRQoL. When stratifying for a history of atopic dermatitis, *FLG* mutations reduced HRQoL only in patients with a history of atopic dermatitis and not in patients with only hand eczema. This finding is in line with our previous hypothesis, based on results from the general population, indicating that *FLG* mutations mainly have a function in coexistence with atopic dermatitis. HRQoL is an important validated estimation of patients' wellbeing and our data suggest that *FLG* mutation carriers with atopic dermatitis can experience an additional challenge in their everyday lives that can have clinical implications.

We also investigated the personal consequences of having a genetically impaired skin barrier by linkage to socioeconomic variables from Statistics Denmark in Manuscript IV. In general, we found that among participants receiving disability pension, 21% had a lifetime prevalence of atopic dermatitis, 40% reported a lifetime prevalence of hand eczema, and 15.7% were filaggrin mutation carriers. Using a logistic regression model we found that disability pension was associated with both atopic dermatitis and hand eczema in the general population. Notably, self-reported hand eczema and atopic dermatitis were associated with a particularly high risk of disability in *FLG* mutation carriers, although these interactions did not reach statistical significance. From a causal point to view, *FLG* mutations themselves cannot predispose to disability pension but may result in symptoms leading to disability pension.

According to the Social Appeals Board in Denmark, 8198 individuals applied for disability pension in 2014, of these applications 75% were recognized.[181] The majority of cases were due to psychological diagnoses, and the second most prevalent diagnosis was related to diseases in the musculoskeletal system. Notably, dermatitis was not included as a separate diagnosis but is assumed to be incorporated in the third most

prevalent category 'other or missing diagnoses'.[181] Moreover, disability pension was reported to be most frequently recognized among individuals aged 50–59 years.[181]

In our setup we had no information about the diagnoses leading to the local authority granting disability pension. The grounds for disability pension can therefore be of physical, psychological, or social character, or a combination. In general, Ibler and Jemec (2011) concluded that the number of permanent disability pensions granted due to skin diseases in Denmark was low when compared with the high prevalence of dermatitis and suggested that skin diseases might have less impact on the individual or are not considered as debilitating as other prevalent diseases by the authorities.[182] Thus several diagnoses can be grounds for granting disability pension, but only one disease will be registered.

In Denmark, the number of disability pensions granted because of atopic dermatitis increased from 3.6 per year in the 1970s to 14.2 per year in the 2000s.[182,183] Although the incidence rate of atopic dermatitis in children has stabilized,[184] it is well-described that the incidence of atopic dermatitis has increased over the past decades, which may affect the observed trend.[185] The prevalence of atopic dermatitis in the Health2006 population was found to decrease with increasing age: 13.7% (18–40 years), 10.9% (41–55 years), and 7.5% (> 56 years). It can therefore be speculated whether the number of disability pensions granted because of atopic dermatitis will increase when the younger generations ages. If this is the case, this also increases the incentive to promote awareness of skin diseases from a societal point of view.

In contrast, contact dermatitis was found to be the dominant diagnosis of disability pensions due to skin diseases in Denmark in the 1970s.[183] During 2003–2008 disability pensions granted due to contact dermatitis were halved, which has been proposed to be a consequence of the National and European nickel legislation, aimed at reducing environmental exposure to nickel.[182,186]

A recent paper from Sweden found eczema to be the cause of 36% of all disability pensions granted due to skin diseases. Of those granted disability pension, painters, cement workers and plumbers formed the major group.[187] Moreover, a Danish study from 2009 suggests potential for prevention of work-related disabilities, especially among job groups exposed to cleaning agents.[188] In our setup we could not link disability pension and job title. We did, however, see that 6.6% of the general population had been working in a cleaning occupation at some point. Moreover, the majority of patients included with hand eczema (and no atopic dermatitis) had a short education, irrespective of filaggrin genotype (hand eczema + FLGwt 66.8% and hand eczema + FLGmut = 79.2%).[Manuscript IV] In addition to having a short education, patients with hand eczema but without atopic dermatitis were characterized by onset of hand eczema in adulthood and an increased prevalence of history in a risk occupation which indicate a different disease pattern when compared with individuals with hand eczema but with a history of atopic dermatitis.[Manuscript IV]

Although several studies have found that having childhood atopic dermatitis does not prevent future work in risk occupations,[77,189,190] Wei and colleagues (2016) found that having received job counselling was associated with higher use of preventive measures,[190] which encourages occupational guidance from an early time and knowledge about preventive measures in risk occupations.

In addition, a healthy-worker effect has previously been suggested based on data from the Health2006 cohort where Bandier and colleagues (2013) reported that *FLG* mutation carriers with early onset of hand eczema in childhood avoid occupational exposure to irritants later in life.[87] In our analyses we found that individuals with a history in a risk occupation reported more dermatitis, but we did not see any self-exclusion from risk occupations among individuals with atopic dermatitis or *FLG* mutations. Manuscript IV
However, our data build on retrospective linkage, thereby showing a lifetime prevalence and we cannot know whether the dermatitis preceded the work in a risk occupation or was a consequence. Thus new studies, preferably prospective cohort studies, are needed to address the relationship between risk occupations, dermatitis and *FLG* mutations and they should include duration of the work. When considering risk occupations, new studies must also consider that the use of job title as a proxy for water exposure has been shown to underestimate skin exposure to water[150] and that high water exposure outside work is frequent.[191]

Taken together, our results indicate that *FLG* mutation carriers with atopic dermatitis are a subgroup of patients who stand out on several parameters. The parameters are biologically manifested by increased prevalence of foot dermatitis and increased persistence of hand eczema; psychologically manifested by reduced HRQoL; and socially manifested by the finding that self-reported dermatitis was associated with disability pension, particularly in individuals with *FLG* mutations. This points towards *FLG* mutations predisposing to increased severity, stressing the need to further promote skin awareness in this subgroup.

# 7. CONCLUSIONS

This thesis concerns the epidemiology of dermatitis and focuses on genetic predisposition, defined by *FLG* mutations, and personal consequences of dermatitis. The main findings from the four manuscripts included are listed below:

- More than 1/3 of the Danish population reports dermatitis
- *FLG* mutations are associated with dermatitis on the hands and feet in individuals with atopic dermatitis
- A history of atopic dermatitis predicts both incident and persistent hand eczema in adults
- *FLG* mutations strongly predict persistent hand eczema, but the association with incident hand eczema in adults is nonsignificant
- Contact sensitization is significantly associated with persistent hand eczema, also in participants without atopic dermatitis
- Patients with atopic dermatitis and *FLG* mutations report reduced HRQoL of life. However, no association between HRQoL and *FLG* mutations was found in patients without atopic dermatitis
- An association between self-reported actinic keratosis and *FLG* mutations is suggested
- Self-reported dermatitis is associated with receiving disability pension in the general population, particularly in individuals with *FLG* mutations
- Of the individuals with atopic dermatitis and *FLG* mutations who develop hand eczema, more than 50% are likely to develop symptoms before adulthood

# 8. FUTURE RESEARCH

Although research within skin diseases and skin barrier function is increasing, many aspects need further investigation. In this thesis, estimated genetic predisposition to dermatitis was defined by measuring the genotype of a single gene. However, the effects of *FLG* mutations, and potentially other susceptibility genes, are difficult to assess quantitatively because of the complex interplay between *FLG* genotype, dermatitis and, specially, occupational exposure.

It has previously been suggested that surveillance programmes in high-risk occupations can contribute to better identification of susceptible individuals and to personalized prevention.[77] This is supported by the findings of this thesis. New studies are needed to build upon the results from this thesis and to investigate whether *FLG* mutation carriers are overrepresented among individuals receiving rehabilitation benefits and/or disability pension granted as a consequence of dermatitis. If this is the case, the need for medical attention with respect to decisions on early change of occupation is further highlighted.
Moreover, future research should aim to elucidate the biological mechanisms controlling filaggrin expression in the skin and investigate changes in expression according to different body sites, difference in age groups, and the effect of environmental down-regulation.[192]

It remains a mystery why only some *FLG* mutation carriers develop dermatitis and some do not, assuming that all heterozygote carriers have the same risk of sensitization. It is evident that environmental exposure and consumer habits can play a key role. However, inter-individual variations in the level of compensatory mechanisms also exist. One of the other genes encoded within the epidermal differentiation complex on human chromosome 1q21.3 is the *filaggrin-2* gene.[193] Filaggrin-2 has been suggested to share many properties with filaggrin including maintenance of skin barrier integrity. In line with this, studies have shown that filaggrin-2 levels are down-regulated in skin affected by atopic dermatitis and hand eczema.[56,63]
It has also been proposed that filaggrin-2 might be able to compensate in diseases characterized by an abnormal or lost function of filaggrin.[Reviewed in 194]

Thus research investigating gene-environment interactions and individual susceptibility driving different forms of dermatitis is needed. New studies should focus on identifying disease patterns and should use bioinformatics tools to combine differences in transcriptome activity, immune profile and even epigenetics to understand the molecular pathophysiology of atopic dermatitis and irritant and/or allergic contact dermatitis.

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Regarding Figure 1 (page 2) from Irvine *et al*., 2011.[13]

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# REVISION SHEET

**Regarding manuscripts**

The publication status of two of the included manuscripts has changed since submission of the thesis:

- Manuscript III has been accepted for publication in Contact Dermatitis.
- Manuscript IV has been submitted for publication in Contact Dermatitis.

**Public defence**

Defence of the thesis: 5 December 2016

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