ADULTHOOD ATOPIC DERMATITIS
FOCUS ON THE SKIN BARRIER, EPIDEMIOLOGY, AND LONG-TERM OUTCOME

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ACADEMIC DISSERTATION

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"What matters in life is not what happens to you but what you remember and how you remember it.”
— Gabriel García Márquez

To Arnis
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ABBREVIATIONS AND DEFINITIONS

AD atopic dermatitis
ABC atopic blepharoconjunctivitis
AMP antimicrobial peptide
CD cluster of differentiation
CI confidence interval
CLA cutaneous lymphocyte-associated antigen
CLDN claudin
DC dendritic cell
EASI Eczema Area and Severity Index
EDC epidermal differentiation complex
FcεRI Fc fragment of IgE, the high-affinity IgE receptor
FLG filaggrin
FLG2 filaggrin 2
GWAS genome-wide association study
HSV herpes simplex virus
IDEC inflammatory dendritic epidermal cell
IGA Investigator’s Global Assessment
IgE immunoglobulin E
IL interleukin
IOP intraocular pressure
IVL involucrin
JAK Janus kinase
JAM1 junctional adhesion molecule 1 = F11 receptor
LC Langerhans cell
LELP late cornified envelope protein
LOR loricrin
OCLN occludin
OR odds ratio
SFTP S100 fused type protein
SNP single nucleotide polymorphism
SPRR small proline-rich protein
TCI topical calcineurin inhibitor
TCS topical corticosteroid
Th T helper cell
TJP1 tight junction protein 1 = Zonula occludens-1, ZO-1
TSLP thymic stromal lymphopoietin
TSLPR thymic stromal lymphopoietin receptor
ABSTRACT

Atopic dermatitis (AD, atopic eczema) is one of the most common inflammatory skin disorders. It is characterized by an impaired epidermal skin barrier and mainly—but not exclusively—a Th2 shifted immune profile. It seems plausible that even if AD is considered a single disease, it consists of a spectrum of patient subgroups differing in regard to the key factors triggering and driving the condition.

The epidermal skin barrier restricts water loss through the skin and prevents the penetration of microbes, irritants, and allergens. The main parts of this barrier are the stratum corneum with terminally differentiated keratinocytes and the tight junction zone. Filaggrin is an important protein of the skin barrier, and loss-of-function (null) mutations in the filaggrin gene (FLG) are the most significant known genetic risk factor for AD. Many other proteins involved in the skin barrier structure and function are downregulated in AD as well. However, the changes in the inflammatory milieu can secondarily lead to an impairment of the skin barrier. Furthermore, the genetic factors cannot explain the rapid increase of AD seen in the late 20th century, and gene–environment interactions seem to be important. Outcome-predicting biomarkers could help in identifying the patients in need of closer follow-up. FLG null mutations are one proposed biomarker. Serum total immunoglobulin E (IgE) is considered a general marker of Th2 shifted immunology and is elevated in most patients with AD. Higher total IgE values have been associated with worse long-term outcome in AD in previous smaller studies, making it a biomarker candidate as well.

The onset of AD often occurs in childhood, but it can take place at any age. A chronic course with complicating exacerbations (“flares”) is typical. AD seems to be more persistent than previously thought, and many patients never grow out of the disease. Patients’ phenotype changes with age. There is a relative research gap in the field of AD in adults, and AD among the elderly is only recently recognized as a separate subgroup. Adulthood AD is most often defined as AD from the age of 14 on, while AD in the elderly usually comprises patients over 70 years of age. A common symptom in adulthood AD is atopic blepharitis accompanied by atopic eye symptoms; atopic blepharoconjunctivitis. Atopic blepharoconjunctivitis can lead to problems such as inflammatory changes in the conjunctiva, scarring of the eyelid, and even vision-threatening complications of the cornea. Topical calcineurin inhibitors (TCIs) pimecrolimus and tacrolimus are commonly used for this indication, but there are
insufficient ocular long-term safety data. There is a paucity of epidemiological research on adulthood AD and AD among the elderly. Moreover, little is known about the effect of acquired factors on the risk of AD in adults.

We investigated associations of \textit{FLG} and other skin barrier gene mutations with the risk of AD in the Finnish population and tested the usefulness of these mutations and serum total IgE as outcome-predicting biomarkers in the long-term management of AD. Additionally, we explored the effect of other patient-related and treatment-associated determinants on the long-term outcome and acquired novel data on the ocular safety of the use of TCIs on the eyelid skin. We determined the prevalence of adulthood AD in Finland and explored the effect of acquired risk factors. In total, 501 patients with AD were recruited at the Helsinki Skin and Allergy Hospital for the prospective observational study on the impact of barrier genes (Study II). A chart review of patients enrolled in Study II was conducted to explore the predictive value of serum total IgE and association of acquired factors, such as environmental and lifestyle-related determinants, on the long-term outcome (Study I). To evaluate long-term safety of TCIs in atopic blepharoconjunctivitis, a chart-review of all ophthalmologist-followed patients (338 subjects) at the Helsinki Skin and Allergy Hospital between 2002 and 2011 was carried out (Study III). For the study on risk factors and epidemiology of adulthood AD (Study IV), data from a nationally representative cohort of 8,026 Finns \(\geq 30\) years of age from the “Health 2000” study was utilized.

\textit{FLG} null mutations increase the risk of AD also in the Finnish population and are associated with earlier onset of the disease. Furthermore, these mutations showed an association with several phenotypic features, namely palmar hyperlinearity, keratosis pilaris, and asthma. However, they were not associated with the outcome of patients in a real-life clinical setting. The allele frequencies of common European \textit{FLG} null mutations were also notably low in Finns. Conversely, serum total IgE showed an association with the long-term treatment results, and values \(\geq 10,000\) IU/ml predicted poor outcome. Additionally, the keratosis pilaris phenotype showed an association with worse outcome. Both TCIs demonstrated good long-term safety in the treatment of atopic blepharoconjunctivitis. No long-term ocular or skin-related adverse effects were observed. There was a favorable effect on pre-existing disease-related and corticosteroid-related changes of the eye, such as intraocular pressure, during the TCI treatment. Tacrolimus ointment seemed better tolerated and more effective than pimecrolimus cream.

\textbf{ABSTRACT}
Female sex was a risk factor for AD in adult subjects of 30–50 years of age. Ex-smokers had an increased risk for adulthood AD, while current smoking increased the risk in subjects less than 50 years of age. There were no associations of obesity, serum vitamin D, living environment, number of siblings, leisure-time physical exercise, or alcohol intake with AD. Interestingly, subjects with highly educated parents had more AD, even if their own education or income level showed no associations with it. AD is a prevalent condition in Finland with a lifetime prevalence of 21.8% and a 12-month prevalence of 10% in Finns aged ≥ 30 years.

In conclusion, FLG null mutations increase the risk of AD in Finns and are associated with a higher risk for asthma in patients with AD. Nevertheless, their value as a predictive biomarker in the follow-up of adult patients seems low. Only a fraction of Finnish patients carry the common European FLG null variants, and a possibility of an unrecognized Finnish-specific mutation remains. Instead, serum total IgE seems an inexpensive outcome-predicting biomarker in patients with adulthood AD. Atopic blepharitis is common in adult AD patients, and the safety of TCIs for this indication seems better than that of corticosteroids. The prevalence of adulthood AD in Finland is among the highest in the world, making it an important public health issue.
Atooppinen ihottuma (atooppinen ekseema) on yksi tavallisimmista inflamminatoriisista ihosairauksista. Sille on tyypillistä heikentynyt orvaskeden läpäisyeste sekä korostunut Th2 immuunivaste. Vaikuttaa siltä, että vaikka atooppinen ihottuma onkin yksi tauti, ihoatoopikot kuitenkin koostuvat toisistaan patogeneettisesti eroavista alarhymistä.


Tutkimme filaggrinigenin sekä muiden ihon läipäisyestegeenien vaikutusta atopipisen ihottuman riskiin suomalaisilla. Lisäksi selvitimme näiden sekä seerumin kokonais-IgE:n käytettävyyttä hoitovastetta ennustavina biomarkereina pitkäaikaisseurannassa. Tutkimme myös muiden fenotyyppitekijöiden ennustevaikutusta ja hankimme uutta tietoa paikallisesti käytettävien kalsineuriiniestäjäjen silmätiirakkuvuudesta. Selvitimme atoppisen ihottuman vallitsevuuuden suomalaisissa aikuisväestössä ja arvioimme ympäristötekijöiden ja elämäntapojen vaikutusta tautiriskiin.


Tulostemme mukaan FLG-mutaatiot ovat atoppisen ihottuman riskitekijä myös suomalaisilla. Ne myös lisäävät riskiä taudin varhaiseen alkuun ja suurentavat astmanriskiä atopipista ihottumaa sairastavilla. Ne ovat yhteydessä tiettyihin klinisiin piirteisiin kuten kämmenentä hyperlineariteitettiin (kämmenentä uurteisuuden korostuminen) ja talirauhasten sarveistappeihin (keratosis pilaris). FLG-mutaatioilla ei kuitenkaan seuranta-tutkimuksessa vaikuttanut olevan yhteyttä hoitovasteeseen. Eurooppalaisessa väestössä yleisimpinä mutataioiden alleelfrekvenssit olivat suomalaisilla alhaisempia ja vain 11,5% potilaista kantoi FLG-mutaatioita. Seerumin kokonais-IgE oli yhteydessä pitkäaikaisseurannukseen ja korkea taso > 10.000 IU/ml ennusti huonompaan pitkäaikaishoitovastetta, kuten myös keratosis pilaris -fenotyyppi. Paikalliset kalsineuriiniestäjät

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takrolimuussa ja pimekrolimuussa ovat turvallisia autooppsia silmäoreita sairastavien potilaiden hoidossa: pitkäaikaishaittoja ei havaittu ja aiemmat taudin ja kortikosteroidihoidon aiheuttamat muutokset sarveiskalvossa, liinissä ja silmänpaineessa korjaantuivat osin hoidon aikana. Takrolimuussin siedettävyys ja teho vaikuttivat tutkimuksessa pimekrolimuussia paremmalta.


INTRODUCTION

Atopic dermatitis (AD, atopic eczema) is a prevalent, inflammatory, and itchy skin disease with a chronic or relapsing course. It causes significant suffering and economic trouble. The prevalence increased throughout the 20th century, and this trend seems to continue still in many countries [1]. Lifetime prevalence rates up to 30% have been reported [2-4]. The understanding of the pathogenesis of AD has progressed during the past decade: it is now viewed as a heterogeneous, complex, and systemic inflammatory disease [5,6]. A defective skin barrier resulting in secondary immune system activation, further compromising the barrier, often seems essential in the pathogenesis [7-9]. Additionally, interaction of genetic and environmental factors is significant (Figure 1) [7]. The skin symptoms often pave the way to the so-called atopic march: the progression of atopic manifestations from skin symptoms to food allergies, allergic rhinitis, allergic conjunctivitis, and asthma [10].

AD was previously seen only as a childhood skin disease. The onset is often in the early years of life but an adult-onset form, representing a considerable proportion of all adult AD patients, is also known [11-13]. The view of the persistence of symptoms has evolved: AD is now understood to be a life-long process for many patients, and it can relapse after many symptom-free years [14-17]. The number of adult and elderly patients is increasing together with the aging of societies throughout the Western world, making these patients a significant subgroup of AD [17-19]. There is a vast heterogeneity in symptoms between different age groups [9]. The age of onset appears to have an impact on the phenotype as well, but the body of data is still small [11,12,19]. Atopic blepharitis, a manifestation of AD on the eyelids, is a common problem among adult AD patients: over 40% of all patients and up to 80% of patients with head and neck AD suffer from it [19,20]. It is often accompanied by symptoms of the conjunctiva and cornea, and then a term “atopic blepharoconjunctivitis” is preferred. Topical calcineurin inhibitors (TCIs) tacrolimus and pimecrolimus have been widely studied in AD with a good efficacy and safety profile [21,22]. However, there are limited published long-term safety and efficacy data on their use on the periorbital skin, and many guidelines recommending TCI use for atopic blepharitis are mostly based on expert consensus and experience only [23,24].

The crucial barrier function of the skin resides mainly in the epidermis—a healthy epidermal skin barrier provides an interface maintaining hydration and cohesion and
serves as the first line defense mechanism preventing penetration of allergens, microbes, and irritants. Filaggrin is one of the most important proteins in the skin barrier. Defects in the filaggrin gene (FLG) increase the risk for AD [25,26]. Biomarkers with prognostic (outcome, treatment response) value would present a valuable tool in the follow-up of patients with AD. The list of possible biomarkers is extensive, and loss-of-function (null) mutations in FLG are among the candidates. Long-term studies on biomarkers, such as FLG variants, in relation to outcome and treatment response are sparse, particularly in adult patients with persistent AD [27].

However, genetic factors cannot explain the rapid increase of AD. This increase points towards a significant role of environmental factors and possible gene–environment interactions. Several socioeconomic, environmental, and lifestyle-related factors, such as education level, urban environment, and smoking, have been associated with an increased risk for AD or persistence of symptoms into the adult life, but results are inconsistent [28,29].

The Finnish population is a genetically unique outcome of isolation, founder effect, and population bottlenecks that differs from other European populations [30]. Therefore, results from other populations cannot be generalized to the Finns as such. Previous smaller studies have suggested that the prevalence of AD in Finnish adults is among the highest in the world [2,31]. Epidemiological data are necessary to estimate the total burden caused by a disease. Globally the vast majority of epidemiological data still come from pediatric populations, and more data on the growing number of adult patients are needed.

This thesis focuses on several different aspects of adulthood AD. We have studied the significance of FLG null and other epidermal skin barrier gene variants on the risk of AD, and the value of FLG null mutations and total serum immunoglobulin E as outcome-predicting biomarkers in the follow-up of patients. We have also gained safety and efficacy data on the use of TCIs in atopic blepharitis and an update on the epidemiology of AD in adults and the elderly, with novel knowledge on the effect of acquired risk factors on the risk of AD.
1. ATOPIC DERMATITIS

1.1 DIAGNOSIS

The diagnosis of AD is usually clinical. It is based on the history, distribution, and morphology of dermatitis together with other associated symptoms and signs. One of the most recognized sets of diagnostic criteria is the 1980 Hanifin and Rajka criteria [32]. This extensive set of criteria is, nevertheless, not very useful in population-based research; therefore the United Kingdom Working Party criteria were developed to offer simplified criteria for epidemiological studies and non-dermatological and dermatological clinical practice [33-35]. There are many instruments for the evaluation of the severity of AD symptoms. Among these the most widely used scales are the Eczema Area and Severity Index (EASI), SCORAD (SCORing AD) index, the Rajka-Langeland scale, Investigator’s Global Assessment (IGA), of which SCORAD, EASI, and the Rajka-Langeland scale have been validated [36,37].

The clinical spectrum of AD phenotypes is wide. The clinical presentation depends significantly on the age of the patient. Four distinct clinical phenotype subgroups have frequently been defined by age: the infantile, childhood, adulthood, and elderly types of AD [9].

1.2 CLINICAL FEATURES AND DIFFERENTIAL DIAGNOSIS

1.2.1 Infantile atopic dermatitis (0–2 years)

In the infantile type of AD, the first lesions are frequently seen on the cheeks and scalp. Often edematous papules appear first and then they further form plaques with crusting and oozing. Later the neck, extremities, and trunk are involved as well, but the lesions mostly spare the diaper area. In infantile AD, the lesions on the extremities are often seen on the extensor surfaces (vs. flexural dermatitis commonly seen in older children and adults) [7,9]. The onset occurs usually very early, already during the second or third months of life. This early onset type of AD carries the highest risk of atopic comorbidities and progression to atopic march in the form of food allergy, allergic rhinitis, allergic conjunctivitis, and asthma.
The differential diagnosis of the infantile AD includes seborrheic dermatitis, irritant contact dermatitis, ichthyosis vulgaris, zinc deficiency (acrodermatitis enteropathica), and rare hyper-IgE syndrome, Netherton syndrome, Wiskott-Aldrich syndrome, and Omenn syndrome [7].

1.2.2 Childhood atopic dermatitis (2–14 years)
In the childhood form of AD, the most distinguishable characteristic is often flexural eczema in the antecubital and popliteal flexures—hence the name “flexural dermatitis” commonly used as a synonym for AD. The distribution of dermatitis is mostly symmetrical, and other frequently affected areas include periorificial areas of the face (i.e., atopic blepharitis on the eyelids or periorbital skin and atopic cheilitis on the lips), wrists, posterior thighs and buttocks, and sometimes feet. Also hand eczema is seen at this age [7,9]. Chronic lesions with lichenification tend to become more common, and some patients present with nummular lesions as well.

The differential diagnoses of childhood AD includes nummular dermatitis, contact dermatitis, psoriasis, scabies, impetigo, and dermatophyte infection [7].

1.2.3 Adulthood atopic dermatitis (> 14 years)
Towards adolescence and adulthood, symptoms on the upper parts of the body (upper back, nape, neck, face) start to predominate while flexures often stay affected as well. Hand eczema becomes more prevalent [7]. Lesions are usually symmetrically distributed, but regarding hand eczema, the dominant hand is often more affected due to the effect of irritative factors. Atopic eye symptoms, such as atopic keratoconjunctivitis, become more common [38]. Adult-onset AD is an interesting subgroup of AD patients, representing up to 20(−40)% of all adult patients with AD [7,11-13]. Some authors have suggested a less severe clinical phenotype, more symptoms of the facial skin, lower IgE levels, female predominance, and a lesser amount of other manifestations of atopy, such as asthma, but data are few and inconsistent [9,11,12,19,29,39,40]. AD can relapse even after many symptom-free decades; hence some of the adult-onset cases may, in fact, represent cases where the childhood dermatitis was simply forgotten [41]. Over 80% of the adult patients seem to have elevated serum total IgE values [11].

The differential diagnosis includes psoriasis, seborrheic dermatitis, nummular dermatitis, irritant and allergic contact dermatitis, lichen simplex, dermatophyte infection, scabies, impetigo, and seldom ichthyosis vulgaris and cutaneous T cell lymphoma [7].
1.2.4 Atopic dermatitis in the elderly (> 70 years)
The clinical picture of AD in the elderly is similar to that of the adulthood form, but hand
eczema becomes less prevalent and xerosis even more notable [42]. Many authors
consider this type nowadays a separate subtype of AD, but it can also represent a
continuum from the adult type [13,18,43]. The significance of this subtype may have
been underestimated, and its prevalence seems to be increasing—together with the
aging of societies—in many industrialized countries, yet larger epidemiological studies
are lacking [13,18,44]. Compared with younger patients, the risk of erythroderma may
be increased, but the studies on the clinical picture of this patient group are still sparse.
[9,13,18,43,44] The diagnosis of AD can be more challenging in this age group due to
the abundance of other itchy skin conditions, age-related xerosis of the skin, co-existing
systemic diseases, and polypharmacy [18].

The differential diagnosis is more extensive and includes all those of adulthood AD and,
additionally, such as asteatotic eczema, adverse drug reactions, and blistering auto-
immune diseases in which itch often precedes the blistering [7,18].

1.2.5 Atopic blepharitis and blepharoconjunctivitis
Atopic blepharitis is a common problem among adult AD patients with up to > 40% of all
patients and a vast majority of patients with head and neck AD suffering from it [19,20].
Atopic blepharitis is the manifestation of AD on the eyelids and peri-orbital skin, and
therefore it often appears in conjunction with other AD symptoms. It is usually more
common in adults than in children, even if high prevalence rates (such as 58% in infant
AD patients in China in a study by Shi et al.) have been reported in pediatric patient
groups as well [20]. Seldom it can be seen as a separate entity and the only
manifestation of skin atopy, together with extracutaneous atopic comorbidities like
asthma. In atopic blepharitis, eyelids are eczematous: swollen, erythemic, scaly, and
sometimes even lichenification and rhagades may be present [45]. Itch is typically very
intense and troublesome. Hyperpigmentation of the peri-orbital skin is common, and so-
called “Dennie-Morgan sign” (infraorbital crease secondary to eyelid skin thickening)
can be present. In chronic, severe cases irreversible scarring and fibrosis of the lids can
be seen, and changes may lead to loss of eyelashes and ectropion or entropion of the
eyelid margins [45].

Atopic inflammation of the cornea and conjunctiva, the atopic eye disease, is called
atopic keratoconjunctivitis. When eyelid dermatitis is present as well, a preferred term is
“atopic blepharoconjunctivitis” (ABC). A male predominance has been suggested in
ABC [45]. Inflammatory activity of the eyelid margins may also interact with the ocular surface further aggravating conjunctivitis and keratitis. Tarsal conjunctivias are often hyperemic and swollen, and papillary hypertrophy and tear dysfunction are frequently present. Symptoms include itch, photophobia, and sometimes patients may complain about blurred vision. Possible corneal complications include keratoconus, infectious keratitis, and punctuate epitheliopathy. Punctuate epitheliopathy leads to a risk of corneal ulcers and vision-threatening irreversible scarring of the cornea if the condition advances [38,46]. Excess tear production together with tear dysfunction can also cause maceration and trigger blepharitis [46]. The long-term course of ABC often resembles that of AD: it is a chronic or relapsing disease with exacerbations, causing considerable discomfort as well as cosmetic trouble reducing the quality of life [45]. Both dermatologists and ophthalmologists treat patients with blepharitis, while patients with ocular symptoms often require an ophthalmological evaluation.

The most important differential diagnosis of atopic blepharitis is contact dermatitis (both irritant and allergic) of the eyelid, which is relatively common especially in women and in the elderly. Cosmetic products and ophthalmic preparations are the most common causes. Other differential diagnoses include infections, ocular rosacea, inflammation of the sebaceous glands (chalazion), and in some cases even malignant neoplasms can mimic blepharitis [45].
2. GENETICS AND PATHOGENESIS OF ATOPIC DERMATITIS

![Figure 1. A schematic presentation of the interplay of genetic and environmental factors in the pathogenesis of atopic dermatitis.](image)

2.1 STUDIES ON THE GENETICS OF ATOPIC DERMATITIS

AD used to be seen as a primarily immune-mediated disease, but genetic studies have identified an important role of epidermal barrier impairment in the pathogenesis [25]. Candidate gene approach studies, genome-wide association studies (GWAS), and comparative analysis of AD with psoriasis have all been used in the research. Additionally, exome sequencing has been utilized in studies on extreme clinical phenotypes [26]. Comparisons of transcriptomes between lesional and non-lesional and non-atopic healthy skin have been conducted as well. These were previously done by hybridization-based microarray studies, while nowadays RNA sequencing is utilized [47].

The candidate gene approach tests directly the effects of variants of a pre-specified gene in an association study. A requirement for this approach is that researchers
already have an understanding or hypothesis of the pathophysiology of the disease and potentially contributing genes. Candidate gene studies are particularly suitable for detecting underlying mechanisms in common diseases with complex pathogenesis, such as AD, where the risk increase by any single candidate gene is relatively small [47]. In GWAS, a genome-wide set of variants (usually single-nucleotide polymorphisms, SNPs) is studied in different subjects to see if they are associated with a particular disease or a specific phenotype. Data from this type of study are useful in pointing out genes that may contribute to subject’s likelihood of developing a certain outcome [26]. In exome sequencing, the entire coding DNA is sequenced (as opposed to the genotyping of SNPs in GWAS). It is particularly useful in search of rare variants causing common diseases and in diagnosing extreme phenotypes in rare diseases [47].

To date, GWAS have found almost 40 risk loci for AD [48,49]. Not surprisingly, these have primarily been associated with development and function of the epidermal skin barrier and both innate and acquired immunity [49]. The most recently identified loci include genes participating the regulation of innate host defense and T-cell function [48]. AD has been compared to psoriasis by genome-wide comparative analysis. Even if these two inflammatory skin diseases share three risk loci—such as the epidermal differentiation complex—in common, there seem to be no shared loci with effects operating in the same direction of the pathways. AD and psoriasis appear to have genetic mechanisms, which have opposing effects on pathways influencing immune response and epidermal differentiation. This is in line with the fact that these two skin disorders only rarely affect the same patient [50]. Despite the massive datasets utilized in recent GWAS, it is estimated that the susceptibility loci found to date explain less than a quarter of the hereditability of AD [49].

2.2 SKIN BARRIER DEFECT IN ATOPIC DERMATITIS

The epidermis consists of four distinct layers: stratum basale, stratum spinosum, stratum granulosum, and the outermost stratum corneum (except for thick skin on palms and soles, where an additional thin stratum lucidum is present underneath the stratum corneum). Keratinocyte differentiation is a complex process, in which keratinocytes go through a programmed sequence of differentiation from dividing cells in the stratum basale to enucleated, terminally differentiated corneocytes in the stratum corneum. The stratum corneum comprises corneocytes, held together by corneodesmosomes, and the extracellular matrix rich in lipids such as sterols, ceramides, and hydrophobic free fatty
acids. Corneodesmosomes get gradually degraded by enzymatic processing, which eventually leads to desquamation of corneocytes [51]. The epidermal lamellar body is an organelle responsible for delivering lipid precursors to interstices of the stratum corneum, where they get processed further. Lipids are essential in the hydrophobic barrier that reduces transepidermal water loss. The lamellar body secretory system and the lipid composition of the extracellular matrix have significant changes in patients with AD compared with healthy subjects [52]. Of lipid alterations, the decrease in the chain length of fatty acids related to ceramides may have the most important role [51,53]. This shift towards shorter fatty acid chain length happens not only in ceramides but also in other lipids, such as sphingomyelin [53]. Furthermore, these changes appear to be mediated by IL-4 and IL-13 in STAT6 (signal transducer and activator of transcription 6) -dependent manner. These recent findings imply another possible pathogenic effect of Th2 axis activation on the skin barrier in patients with AD [53].

A normally functioning epidermal skin barrier in the stratum corneum provides integrity and an adequate interface barrier. The skin barrier restricts transepidermal water loss maintaining hydration, and serves as the first line defense mechanism preventing penetration of microbial organisms, allergens, and irritants into the skin [52]. The key role of epidermal barrier abnormality in AD pathogenesis is indubitable: the outermost epidermis of AD patients is characterized by a notable barrier impairment, which contributes to the increased susceptibility to bacterial and viral infections, microbial colonization, and allergic sensitization [54,55]. In the acute phase of AD, the amount of Staphylococcus aureus (S. aureus) increases and its endotoxins and proteases further impair the barrier [56]. The barrier impairment is not only critical in the pathogenesis of AD, but also in progress to atopic march: food allergies, allergic rhinitis and conjunctivitis, asthma, and eosinophilic esophagitis [57,58].

It remains somewhat a matter of dispute if the skin barrier abnormality is the primary event in the pathogenesis of AD further triggering the inflammation (the so-called “outside-to-inside” model), or if the barrier defect is secondary to immune pathway alterations (the “inside-to-outside” model), or if both routes exist in a parallel manner (“outside-inside-outside”) [59]. This is because the inflammatory changes are capable of triggering secondary barrier defects as well (see also chapter "Immunology of atopic dermatitis"). Still, an increasing body of evidence implies that the barrier defect is often the primary event. A reduced transepidermal water loss at the age of two days and two months has been shown to predict clinical eczema later at the age of 12 months [60], and a regular use of emollients has been shown to prevent eczema in infants in high

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risk for AD [61,62]. On the contrary, the immune profile of major orphan ichthyosis patients appears to be IL-17 dominant, which may suggest that barrier dysfunction alone, as seen in ichthyosis, does not induce Th2 immune deviation observed in AD and additional immunological susceptibility is required [63].

2.3 Filaggrin and the epidermal differentiation complex

2.3.1 Filaggrin
Filaggrin is one of the most important proteins involved in epidermal barrier homeostasis, and it is critical for the cornification process, the final stage of keratinization (Figure 2). It is enzymatically cleaved from proproteing profilaggrin that is the main constituent of the keratohyalin granules found within the cells of the stratum granulosum [51,64]. Profilaggrin is a large histidine-rich S100 fused type protein (SFTP) encoded by the filaggrin gene (FLG). Filaggrin comprises an amino-terminal S100 domain and a long (> 3000 amino acid residues) carboxy-terminal domain that contains multiple sequence repeats [65]. At the DNA level, each FLG repeat encodes one post-translationally modified functional filaggrin monomer, and profilaggrin consist of 10, 11, or 12 of these monomers. During the cornification process, profilaggrin is proteolytically cleaved to filaggrin monomers, which then bind to keratin filaments and form keratin–filaggrin bundles. In the uppermost layers of the stratum corneum, filaggrin monomers are enzymatically cleaved from these bundles and undergo further modifications into metabolites such as trans-urocanic acid and pyrroolidone carboxylic acid [51,52]. Urocanic acid participates in maintaining the acidic pH of the skin, protects from ultraviolet (UV) radiation, and its cis-isomer mediates UV-induced immunosuppression and decreases the activity of Langerhans cells preventing the activation of Th2 axis [66,67]. Pyrroolidone carboxylic acid and other breakdown products act as osmolytes and contribute to the composition of natural moisturizing factor (NMF) of the skin that maintains water retention and hydration in the stratum corneum [66,68]. Consequently, the loss-of-function (null) mutations or the variation in the number of FLG repeats may affect the quantity of filaggrin expressed in the epidermis [69,70]. Filaggrin breakdown products appear to play a role in the abnormalities of the corneocyte morphology observed in patients with AD [71]. Moreover, a recent study suggests a link between FLG null mutations and the composition of skin microbiome in non-lesional skin of adult AD patients [72].

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Figure 2. Consequences of filaggrin deficiency and the complex interactions between them.

2.3.2 Epidermal differentiation complex

*FLG* is located within the epidermal differentiation complex (EDC) on chromosome 1q21 [65]. From the perspective of evolutionary genetics, EDC is the most rapidly evolving locus in the human genome [65]. It includes a set of genes that encode three groups of proteins participating in the complex epidermal differentiation process: SFTPs (like pro-filaggrin), S100A proteins, and cornified envelope precursor proteins.

SFTPs are mostly expressed in the stratified epithelia. They seem to be associated with terrestrial life, and they have most likely had a great importance in the establishment of the skin barrier suitable for the life in dry environment [73]. In addition to filaggrin, filaggrin 2 (coded by *FLG2*), trichohyalin (*TCHH*), trichohyalin-like protein 1 (*THHCL1*), repentin (*RPTN*), hornerin (*HRNR*), and cornulin (*CRNN*) belong to the group of SFTPs [65,74]. The genes of SFTPs have similar structures and function. Just like filaggrin, also hornerin, repentin, and cornulin are cytoplasmic matrix proteins, and they are associated with intermediate filament proteins (such as keratin) and minor components of the cornified cell envelope [65]. Trichohyalin and trichohyalin-like protein 1 are
expressed in the upper epidermis, but little is known about their function, except that both appear to have a role in hair formation [65]. FLG2 seems to be expressed to some extent similar to FLG [75]. It participates in the cornification process, and its down-regulation has been shown to decrease the expression of loricrin and claudin 1, but the exact function remains largely unknown [76,77].

The family of the cornified envelope precursor proteins includes such as loricrin (LOR), involucrin (IVL), late cornified envelope proteins (LELPs), and small proline-rich proteins (SPRRs) [65]. Loricrin is the most abundant protein component of the cornified cell envelope that is a structure of crosslinked proteins beneath the plasma membrane in terminally differentiated keratinocytes. LOR is expressed late in the stratum granulosum [65]. Its expression is reduced in the skin of the flaky tail mouse, a mouse model of AD, leading to decreased expression of occludin [78]. Involucrin contributes to the early steps of the cell envelope formation establishing the outermost layer for the attachment of ceramide lipids, and it binds to loricrin. In contrast to loricrin, involucrin does not seem crucial for the cell envelope formation, and the involucrin knockout mouse develops normally with normal epidermis and cell envelopes [79]. Topical betamethasone therapy has been shown to decrease involucrin expression (compared with TCIs) [80]. Both TCIs and betamethasone therapies seem to normalize the expression of filaggrin and loricrin [80] while ultraviolet B radiation (UVB) phototherapy seems to normalize also involucrin expression [81]. This may be at least partly explained by the effects of endogenous vitamin D3 production, which has been shown to induce expression of epidermal differentiation proteins, including filaggrin, involucrin, and loricrin [82]. SPRRs function as receivers and donors of amino-groups in cell envelope formation [65].

2.3.3 Genes of the epidermal differentiation complex in atopic dermatitis

The first genetic evidence that a primary epidermal barrier defect may be involved in the etiopathogenesis of AD came from the findings that the FLG null mutations are an important risk factor for AD in European and Asian populations [25,83-87]. Already before these findings, homozygous or compound heterozygous FLG null mutations were known to cause ichthyosis vulgaris, the most common hereditary disorder of keratinization characterized by xerosis of the skin [88]. FLG null mutations seem to be related to earlier onset of AD, and it has been suggested that they would increase the risk for the early-onset form of AD only [89,90]. These mutations have been shown to increase the risk of other atopic manifestations as well. They show an association with allergic rhinitis and food allergies in childhood and adolescence [57,91,92]. FLG null mutations increase the risk for asthma in patients with AD, but in patients without signs
of skin atopy results are conflicting [58,83,84,92,93]. They show an association with some phenotypic features of AD patients, such as palmar hyperlinearity, keratosis pilaris, hand eczema, xerosis, and a longer duration of the disease [94-99]. Regarding susceptibility to irritant contact dermatitis, results are inconsistent [100,101]. Including the two common null mutations (R501X and 2282del4) reported first, almost fifty mutations of the filaggrin gene have been described [102]. Intragenic copy number variation is present in the filaggrin gene, and at least three variants (10, 11, and 12 repeat alleles) are recognized [64]. R501X mutation occurs on the 11-repeat allele and 2282del4 mutation on the 10-repeat allele [69,102]. This copy number variation could have a dose-dependent effect on disease severity, as suggested by data from the Irish population [69]. Interestingly, there seems to be another independent, but additive effect of FLG null variants: having a mother with FLG null genotype. Esparza-Gordillo et al. showed recently that FLG null genotype of the mother predisposes offspring to AD independently of the FLG status of the offspring. This effect is probably mediated by the immune responses of the mother because it was only seen if the mother had allergic sensitizations as well [103]. In the African-origin populations, however, FLG mutations do not appear to be important in AD pathogenesis. A recent exome sequencing study by Margolis et al. did not yield significant results on susceptibility genes in African American AD patients [104,105].

Expression of FLG2 and hornerin are decreased in patients with AD, even more so in the lesional skin, following the expression of FLG [106,107]. FLG2 mutations are associated with more persistent AD in African American patients, but this association has not been noted in other populations [108,109]. Cornulin has been reported to be downregulated in patients with AD [110]. LELP1 and SPRR3 appear to be significantly downregulated and SPRR1 upregulated in the skin of AD patients, when compared with healthy controls, and an association between the LELP1 polymorphism and high IgE values has been published [106,111]. Both involucrin and loricrin have been identified as AD-associated genes, and their expression is downregulated by Th2 cytokines, mainly by IL-4, in both lesional and non-lesional skin of patients with AD [112-114]. More extended genotyping in a German AD cohort did not reveal any additional risk variants in other genes located in the EDC [115].
2.4 TIGHT JUNCTIONS IN ATOPIC DERMATITIS

2.4.1 Structure and function
In addition to the stratum corneum, the other essential component of the epidermal skin barrier is the tight junction zone. Tight junctions not only seal the paracellular space between keratinocytes but also regulate paracellular permeability, the movement of solutes and ions between the cells [116]. They are also essential for maintaining the integrity of the skin barrier. Tight junctions are large, apically located protein complexes constituting of peripheral (such as tight junction protein 1, TJP1, also known as zonula occludens ZO-1) and transmembrane proteins (such as claudins and occludin). Tight junctions form a sort of a second line of defense under the stratum corneum. Since the stratum corneum is missing in sweat glands and hair follicles, tight junction barrier presumably works as the primary barrier in these appendages [51,117]. Claudins are mainly responsible for the specificity of tight junction permeability. Claudin 1 (CLDN1) and claudin 23 (CLDN23) expressions appear to be reduced in AD. Th2 cytokines seem to downregulate claudin 1, and this reduction has been shown to diminish the integrity of tight junctions [118]. Occludin and scaffold proteins, such as TJP1, interact with claudins [119]. In a comparison of the effects of clobetasol and tacrolimus on tight junction protein expression on mouse skin, clobetasol decreased claudin 1, claudin 4 and occludin, while tacrolimus did not affect claudin 1 and claudin 4 but also downregulated occludin to a lesser extent [120]. Histamine has been shown to impair the skin barrier function by suppressing the expression of TJP1, occludin, claudin 1, and claudin 4 [121]. Yokouchi et al. showed in a study conducted in the FLG knockout mice that FLG mutation did not directly affect the paracellular tight junction barrier but, instead, the subsequent inflammation did [122].

2.4.2 Tight junction genes in atopic dermatitis
Certain SNPs of CLDN1 are associated with increased risk of AD in North American populations [117,123]. Furthermore, it has been shown that CLDN1 defects may be involved in the higher susceptibility to herpes simplex virus (HSV) infections seen in patients with AD [123]. In addition, molluscum contagiosum infections are seen more in patients with AD, and the virus is known to enter through hair follicles where stratum corneum is lacking and tight junction barrier is important.
2.5 IMMUNOLOGY OF ATOPIC DERMATITIS

2.5.1 Inflammation

Another distinctive feature of AD, additionally to the defective skin barrier, is cutaneous inflammation. AD is considered a primarily T cell-driven disorder in which infiltrating T cells, particularly Th2 cells, are essential. They express many adhesion molecules enabling homing and recruitment into the skin [124], and these skin-homing T cells show a higher state of activation in patients with AD compared with healthy controls [124]. T cells also persist as peripheral resident effector memory cells and are capable of deploying fast recall responses to cutaneous factors, such as microbes and allergens [124]. The number of type 2 innate lymphoid cells (ILC2s) is increased in AD lesions, and they are an important enhancer of Th2 responses [125]. Even the non-lesional skin of AD patients shows subclinical low-grade inflammation [126]. The Th2 axis deviation is the hallmark of inflammation in both acute and chronic AD, in both lesional and non-lesional skin. The importance of the Th22 axis in lesional skin has gained attention lately. Both Th2 and Th22 responses are progressive in the course of the disease towards chronicity [127]. Th1 responses play a bigger role in chronic AD, and the Th17 axis seems to be more significant in AD in the Asian populations, as well as in pediatric AD [127,128].

In acute AD lesions, the levels of Th2 cytokines, such as IL-4, IL-5, IL-10, IL-13, and IL-31, are remarkably increased, and the same applies to Th22 cytokines, particularly IL-22 and S100A proteins (Figure 3) [127,129,130]. In addition to the Th2 cells, Th2 cytokines can be secreted by mast cells, eosinophils, basophils, and ILC2s [131]. Mast cells have also recently been identified as a major source for IL-22 in patients with AD [132]. These inflammatory mediators, in turn, compromise the skin barrier function by downregulating tight junction proteins and expression of genes participating in terminal differentiation of keratinocytes. Particularly IL-4 and IL-13 reduce the expression of filaggrin, loricrin, and involucrin. This downregulation of filaggrin is also seen in patients without FLG null mutations and is further enhanced by IL-22, IL-17, and IL-33 [133-135]. Moreover, IL-4 and IL-13 are essential in the induction and initiation of IgE production by activated B cells. Together with IL-33, IL-4 is also an important upregulator of IL-31, one of the key mediators of itch in AD (Figure 3) [126]. IL-33 works as an alarm signal (alarmin) and alerts the immune system. Physical damage, like scratching, increases its expression. It activates Th2 pathway, but may work as a proinflammatory cytokine in various immune responses [136]. IL-5 acts on eosinophils accelerating their chemotaxis and eosinophilopoiesis [127].
The JAK-STAT (Janus kinase-signal transducer and activator of transcription) pathway is a signal transduction pathway for many cytokines. JAK1, JAK3, and STAT6 are IL-4 signaling components and important for Th2 differentiation [137]. Additionally, the JAK-STAT pathway enhances the maturation of B cells, activates eosinophils, upregulates pro-inflammatory cytokines, downregulates antimicrobial peptides (AMPs), and suppresses regulatory T cells [137].

In the acute phase, the effector memory T cells are also attracted, and the Th2 and Th22 responses increase. Cytokines from Th1 and Th17 responses further impair the
integrity of epidermal barrier, which instead leads to a release of pruritogenic and proinflammatory mediators (such as thymic stromal lymphopoietin, TSLP; and IL-31) by keratinocytes. The expression of many AMPs reduces, among them beta-defensins and cathelicidins (Figure 3). In patients with AD this downregulation of AMPs by inflammatory response seems to exceed the upregulation caused by the mechanical damage via scratching [138]. The reduction of AMPs further promotes the S. aureus colonization and the increased risk of viral infections [138].

Dendritic cells (DCs) are leucocytes that by antigen presentation participate in the transmission of information between the environment and the immune system, and they are important messengers also in the complex pathogenesis of AD. However, in AD they also have an independent activating role [139]. Epidermal DCs in the skin of patients with AD express the high-affinity receptor for IgE (Fc fragment of IgE FcεRI), and several different DC subtypes have been detected in both lesional and non-lesional skin of AD patients [139]. Langerhans cells (LCs) are a subgroup of DCs that reside in the epidermis, mostly in the upper part, and are capable of evoking both immunogenic and tolerogenic T cell responses [69]. Cis-urolan acid, a filaggrin breakdown product, seems to reduce LC activation. Therefore a relative cis-urolan acid deficit caused by filaggrin deficiency may lead to the enhanced Th2 immune response in AD patients by activation of LCs [67]. Inflammatory dendritic cells (IDEC) are the second subset of DCs found in the epidermis of the lesional—but not in the non-lesional—skin of patients with AD. They further intensify the Th2 response in the acute phase of AD [139,140]. In chronic AD lesions the infiltrations of IDECs are even more prominent [139].

Continued activation of the Th2 and Th22 axes and upregulation of Th1 and Th17 pathways further impair the skin barrier structure and function and promote remodeling leading to progressive epidermal hyperplasia, altered terminal keratinocyte differentiation, and fibrosis manifesting as lichenification [127]. TSLP and type 2 innate lymphoid cells appear to be important in the fibrosis of chronic AD as well [125]. TSLP triggers Th2 and to lesser extent Th22 cytokine response. It is strongly expressed in AD lesions, but it is not expressed at all in the normal skin. The key target for TSLP seems to be the DCs where its receptor, TSLPR, is strongly expressed in lesional AD skin [141]. TSLP promotes IL-4, IL-5, and IL-13 production via activation of DCs, but it can also directly induce proliferation of Th2 cells, and it seems to promote Th2 cytokine responses through its actions on type 2 innate lymphoid cells, mast cells, epithelial cells, and basophils as well. Similar to that of IL-33, TSLP expression can be enhanced
through physical trauma like scratching and exposure to pathogens. Additionally, TSLP is an important inducer of itch [28,125,141].

2.5.2 Immunoglobulin E

Immunoglobulin E (IgE) is the least abundant of serum immunoglobulins. Its main original function is defense against helminths, venoms, and toxins via facilitating degranulation of basophils and mast cells, and promotion of the Th2 axis. It triggers immediate allergic reactions in people with type I hypersensitivities by allergen recognition, leading to fast immune responses [142,143]. IgE is a mammal-specific immunoglobulin that is always present in a monomeric form, and this small size allows easy diffusion. The half-life of free IgE is only few days [143]. Activated B cells (plasma cells) produce IgE with the help of Th2 cytokines: IL-13 induces the IgE production, while IL-4 is significant in the initiation of production via activation of STAT6. IgE signaling is transmitted through two types of receptors: the high-affinity receptor FcεRI and the low-affinity receptor CD23 (also known as FccRII). IL-4 upregulates CD23, and it is considered a natural regulator of IgE synthesis [143]. CD23 may play a role in antigen presentation and regulation of immune responses because IgE–antigen complexes bound to it can be taken up by endocytosis [144]. In addition to its role as a membrane-bound low-affinity receptor for IgE, it can also be cleaved from the cell surface to yield a range of soluble CD23s, which also bind IgE and have different cytokine-type activities. CD23 has many different ligands, such as CD21 and some integrins, but the primary ligand is still IgE [144].

Several subtypes of FcεRI-bearing DCs have been detected in the epidermis and dermis of patients with AD [139]. They can take up antigens and induce T-cell-mediated delayed type reactions, contributing to the increase of inflammation [145]. The skin barrier dysfunction and the increased S. aureus colonization contribute to increased IgE production as well [139]. Compared with healthy controls, patients with AD have also more specific IgE antibodies against S. aureus antigens, but their correlation with disease severity is uncertain [146].

Elevated serum total IgE levels are seen in about 80% of the AD patients. In patients with severe disease, the percentage can be higher [9,11,147]. Higher total IgE values have been noted also in AD patients with asthma [148]. Specific IgE levels tell about the sensitization profile of an individual patient, but serum total IgE levels do not always correlate with them and reflect largely the general activation of the Th2 axis. Patients with normal total IgE can have many clinically relevant allergies, while patients with
elevated total IgE values may not have any significant IgE sensitizations [9]. Higher total IgE values at the age of 6-18 months in subjects without AD has been shown to predict subsequent dermatitis in later childhood [149].

A correlation between total IgE values and clinical severity has been shown in many studies, and some have suggested an association between clinically decreased disease activity and a decrease in total IgE levels during follow up as well [22,147,150-153]. There are only a few small studies, such as the 10-year follow-up study by Mandelin and colleagues, in which total IgE has been assessed as a prognostic marker in adult patients with AD. In them, higher baseline values were associated with worse long-term outcome [147,152].

The view of two types of AD as the intrinsic form (normal total IgE levels) and extrinsic form (elevated total IgE) is widely recognized by many [9,154]. This dichotomy is mostly based only on measuring total IgE level and a limited set of specific IgE levels and remains consequently somewhat debatable. It seems that the Th2 deviation is similar in both forms, but there may be an increased additional relative activation of the Th17 and Th22 axes in the intrinsic form [155]. Long-term outcome data supporting this division of AD patients in two distinct groups are sparse. The intrinsic type has been associated with female predominance and later onset, but data are inconsistent [9,155]. It is also proposed that these so-called intrinsic and extrinsic forms of AD likely represent the opposite parts of one disease spectrum, caused by variations in the cytokine profile of patients [9]. This view is further supported by the similar efficacy of dupilumab, a fully human monoclonal antibody against IL-4 receptor alpha that inhibits signaling of IL-4 and IL-13, in both of these subtypes of AD independently of total IgE levels [156].

More recently, the importance of IgE in autoimmunity has been gaining interest. However, the increased total IgE levels seen in some autoimmune diseases, such as systemic lupus erythematosus, are not associated with increased prevalence of atopy in these patient groups [142]. IgE autoreactivity has been suggested in AD as well: some of the T cell responses might also be directed towards self-antigens, at least in subgroups of patients [145]. Many IgE-binding keratinocyte-derived possible autoantigens have been described. It has been proposed that autoreactivity could play a role in up to a third of the AD patients, which could then be considered as autoimmune subgroup of AD. The body of evidence supporting this is currently small and more studies are needed [145].
Omalizumab, recombinant monoclonal anti-IgE antibody, sequesters free IgE, binds to FcεRI blocking the binding of IgE, accelerates the dissociation of IgE bound to basophils and mast cells, and decreases the expression of IgE receptors. Despite the high proportion of patients with elevated total IgE values, omalizumab has shown no or very limited efficacy in the treatment of AD. This further implies that clinically significant direct effects of IgE on the course of AD are unlikely in majority of patients [157].

2.5.3 Immune-related genes

Remarkable and replicated findings in genes participating in immune reactions include associations for variants in the genes encoding essential pathways in Th2 response: IL-4, IL4-R, IL-13, and STAT6 [158-161]. An IL-13 variant also seems to be connected to high serum total IgE [158]. The most recently identified susceptibility loci from multi-ancestry GWAS include genes participating in the regulation of autoimmunity-related pathways, such as the IL-7 receptor gene (IL7R) [48]. IL7R also codes the heterodimeric TSLP receptor together with TSLPR. Polymorphism of IL7R seems to be a risk factor for AD, while the TSLPR variants show a modest association as well [162]. IL7R and TSLP variants seem to be related to extensive HSV eruptions as well [162]. Thromboxane TXA2 receptor polymorphism has demonstrated an association with AD and higher IgE values [163]. A functional mutation in the gene coding IL-6 receptor (IL-6R) appears to be linked with more persistent AD [164]. A recent exome sequencing study showed an association between a cytochrome P450 enzyme 27A1 (CYP27A1) variant and AD with high total IgE in Japanese subjects [109]. CYP27A1 is an essential enzyme for vitamin D3 25-hydroxylation, and vitamin D is important in modulating immune function.

2.6 OTHER SUSCEPTIBILITY GENES

A mutation of the KIF3A gene (kinesin family protein 3A) coding a subunit of the kinesin 2 complex, a motor protein participating in ciliary transport, showed an association with AD in an Italian study [165]. Desmosomes are intercellular junctions that provide mechanical strength to the skin and are degraded in a controlled way during differentiation of the epidermis. The syndrome of Severe dermatitis, multiple Allergies and Metabolic wasting (SAM) is an extreme phenotype of atopic skin disease that is proposed to be caused by mutations in two genes coding desmosome/corneodesmosome proteins (DSG1, desmoglein 1; DSP, desmoplakin) [166,167]. This association implies that normally functioning desmosomes and corneodesmosomes may be needed for the
inhibition of atopic inflammation. This is further supported by the finding that desmoglein 1 expression seems to be reduced in the proteomic analyses of the lesional AD skin [107]. Among other genes identified to be associated with AD are LAMA3 (laminin subunit alpha-3, coding a subunit of laminin 5 protein) and TMEM79 (transmembrane protein 79). Laminin 5 is vital for the normal structure and function of the basal membrane and TMEM79 codes matrin, a protein that regulates the assembly of lamellar body secretion [168,169]. Netherton syndrome is an autosomal recessive condition caused by mutations in SPINK5 (serine protease inhibitor Kazal-type 5), encoding a serine protease inhibitor. The features of Netherton syndrome include an ichthyotic and eczematous skin phenotype and markedly increased IgE levels resembling a severe phenotype of AD. An association between SPINK5 variants and AD has been demonstrated [170].

2.7 GENE–ENVIRONMENT INTERPLAY AND EPIGENETICS

The increase of AD during the latter half of the 20th century happened too rapidly to be explained by genetic factors only. Apparently, environmental factors and gene–environment interactions are of great importance. There are increasing data that epigenetic mechanisms can mediate these effects [26]. Still, not all the studies support the concept of gene–environment interactions. For instance, a twin study from Kahr et al. did not find any evidence of genetic effect modification by environmental factors [171]. Epigenetic mechanisms include such as DNA methylation, histone methylation or acetylation, non-coding microRNAs, and genomic imprinting. The definition of “epigenetics” varies, and strictly defined it includes the concept of heritability, while some use the term widely to describe mediators of gene–environment interactions and phenotypic plasticity. Indeed some of the mentioned mechanisms may represent heritability without an alteration of the DNA sequence if they happen in germline cells. However, the concept of epigenetic inheritance is still partly controversial in mammals [172].

Epigenetic mechanisms seem to play a role in the pathogenesis of AD as well. Transcriptome studies have revealed altered methylation and expression of genes necessary for keratinocyte proliferation, differentiation, and immune response in the lesional epidermis of patients with AD compared with healthy controls [173]. Furthermore, methylation of the TSLP gene has been proposed to be the mediator of the increased risk of AD caused by prenatal tobacco exposure seen in the offspring of
mothers who smoked during pregnancy [174]. MicroRNAs influence gene regulation via inhibition of translation or degradation of messenger RNAs. They can be involved in the AD pathogenesis by their impact on immune responses or by transmission of environmental effects [175,176]. There is also some evidence of an interaction between genotype and environmental factors in AD, such as a suggested modifying effect of FLG null genotype on the effect of breastfeeding on AD risk [177]. Genomic imprinting is an epigenetic process that causes genes to be expressed in a parent-of-origin-specific manner. The significance of genomic imprinting in AD has been studied in only a few studies, and there is some evidence of imprinting on chromosomes 6, 11, 14, and 13 [178].

2.8 SKIN MICROBIOME IN ATOPIC DERMATITIS

Microbial dysbiosis is typical in AD [56]. However, the causality between the microbiome composition and skin symptoms has not been conclusively shown, as pointed out in a recent systematic review by Bjerre et al., and it is possible that observed differences are secondary to the dysfunctional skin barrier and altered immune responses. The microbiome of patients with AD has low diversity; this is the case especially in the lesional skin, but the same has been noted to a lesser extent also in the non-lesional skin. The relative amounts of S. aureus and S. epidermidis are often elevated at the expense of other microbes, such as Propionibacterium [56]. A recent study showed that colonization of the skin with commensal Staphylococcae at the age of two months had a negative association with the risk for AD at the age of one year [179]. However, the skin barrier genetics were not assessed, and mutations in the filaggrin gene appear to affect the microbiome in AD [72]. A causal relationship between microbial dysbiosis and inflammation has been suggested in a mouse model, but more studies are needed to confirm this in humans [180].

3. EPIDEMIOLOGY OF ATOPIC DERMATITIS

The prevalence of AD increased considerably during the latter half of the 20th century, especially in the industrialized countries [1]. Based on large international studies, there appears to be a substantial variation in the prevalence of AD in children [1,181]. It seems to be increasing still in many countries, while in some—such as Denmark or
Sweden—the prevalence growth has ceased and a plateau presumably reached. [1,181,182] According to the ISAAC survey, the 12-month prevalence of AD in childhood (up to 14 years of age) varied between 0.2% and 24.6% (China and Columbia, respectively) [1].

The vast majority of prevalence studies have been conducted in children and to a lesser extent in adolescents. Even if its onset often occurs in the early life, AD is no longer considered only a childhood disease. Both data from longer follow-up studies and clinical remarks of many experienced dermatologists tell the same story: AD appears to be more persisting than previously thought and the number of adult and elderly patients is increasing [9,13-15,18,183,184]. It seems plausible that the increase of AD in children during the late 20th century leads to an increase in adulthood AD with a delay. Additionally, it has been estimated that up to one-fifth of adult patients may, in fact, have adult-onset AD [11,39,40]. Even higher percentages have been reported, but the aforementioned possibility of recall bias has to be kept in mind in interpretation [12,41,185]. Nevertheless, large epidemiological studies on the prevalence of AD in the adult and senior populations are limited.

Among adults aged 18 to 85 years, the 12-month prevalence of eczema was 11.5% in Sweden and 10.2% in the United States, while among 28-30-year-old Germans the self-reported 12-month prevalence was 17.1% [3,15,17]. In a previous multicenter survey of 27-56-year-old adults in Europe and the United States, the 12-month prevalence varied between 2.2% (Switzerland) and 17.6% (Estonia) [29]. A recent international survey conducted in the United States, Canada, Japan, and European Union (represented by United Kingdom, Spain, France, Germany, Italy) showed a 12-month prevalence up to 8.1% (Italy) and a lifetime prevalence of physician-diagnosed AD up to 17.1% (Spain) [186].

In Finland, there are little population-based data on the prevalence of AD in adults. In 25-54-year-old subjects in North Karelia region in 1998, the lifetime prevalence was 28.1% and the 12-month prevalence 14.7% [31]. In a study by Lampi et al. among subjects of 31 years of age in 1997 (Northern Finland 1966 birth cohort) the lifetime prevalence of AD was as high as 38%, but the 12-month prevalence was not assessed [2]. Sinikumpu et al. conducted a study in the same Northern Finland 1966 birth cohort when the subjects were 46 years of age and found a point prevalence of 4.6% for AD [187].
4. RISK FACTORS AND COMORBIDITIES OF ATOPIC DERMATITIS

4.1 RISK FACTORS

In children, an association between high socioeconomic status of parents and the risk of AD in the offspring has been reported in several studies [188-190]. However, there are only few studies in adults with similar results [191]. Lower socioeconomic status seems to lead to worse adherence to treatment in adult AD patients [192].

A higher number of older siblings has been linked to a lower risk of AD in children [189,193,194], but results in adult populations are inconsistent [29]. Research on the association of rural living environments with the risk of AD shows diverging results, and there are very little data from adult subjects. It is also possible that early-life exposures do not predict AD in adult life [16]. A Finnish study among university freshmen detected no correlation between a childhood farming environment and risk for AD, even though a rural environment seemed to protect from allergic rhinoconjunctivitis [195]. Contrary to these results, rural living environments increased the odds for remission of AD in Swedish children, and countryside environment was associated with a lower risk of AD in Swedish young adults [196,197]. In the United States, higher levels of household income and education were associated with eczema in adults aged 18–85 years in a national health interview survey [17]. Known risk factors for persistent AD are more severe disease with early onset, other atopic conditions, and a family history of AD [198]. In addition to the rural living, other environmental or acquired factors, such as high socioeconomic status, may have an impact on the persistence of AD over puberty or the onset of AD later in life [16].

There is some evidence, that smoking (both active and passive) is associated with an increased risk of AD, as shown for example in a recent meta-analysis [199-201]. Furthermore, increased expression of Th2 cytokines has been reported in the offspring of smokers. This could be mediated by epigenetic mechanisms, comparable to those mediating an increased risk caused by prenatal tobacco smoke exposure [174,202]. Furthermore, additionally to the lifetime tobacco smoke exposure, early exposure may increase the risk of adult-onset AD as well [203]. This implies that some effects of early-life exposures can, in fact, be life-long. In a multicenter survey by Harrop et al. on adult AD patients, eczema was more common in ex-smokers than in lifetime non-smokers or current smokers [29].
Alcohol intake in pregnancy seems to be a significant risk factor for AD of the offspring, and a higher level of alcohol abuse in AD patients has also been reported [200,204]. Sweating is a known aggravating factor for AD symptoms, but studies on the effect of vigorous exercising on AD have shown conflicting results [205]. Furthermore, it seems that AD may sometimes lead to avoidance of sports and exercising, but results in regard to this are inconsistent [206,207]. Interestingly, AD appears to be connected to obesity in the Asian and American populations, but not in Europeans [208-210]. This could be related to the findings that in the Asian populations AD seems to be immunologically closer to psoriasis, in which the association to obesity is more straightforward [9]. Results from the Chinese population by Luo et al. suggest that the relationship of obesity and AD is stronger in non-smokers than smokers, so a cumulative effect of these two factors seems unlikely [210].

4.2 COMORBIDITIES

AD has been linked to a higher risk of many psychiatric disorders. Patients with AD seem to be at an increased risk for ADHD, depression, autism spectrum disorders, schizophrenia, and anxiety in both pediatric and adult populations, and a possible dose-dependent relationship has been suggested [211-213]. This can be due to interruption of the regular sleep-wake cycle, but direct effects of altered immune responses are possible as well. Perceived stress has a clear association with AD—a factor that is apparent for all physicians treating AD patients [214]. Barrier deficiency induced by stress and anxiety-induced Th2 shifted immunology have been proposed to be the likely explanations for this link [215,216].

Next to staphylococcal, molluscum contagiosum, and HSV infections often seen in patients with AD, the risk for many other infections (such as common warts, high-risk cervical HPV infections, H1N1 influenza) is elevated as well [217-219]. There appears to be an increased risk for rheumatoid arthritis, inflammatory bowel disease, alopecia areata (also linked to FLG-null mutations) and non-melanoma skin cancer, while the risk for melanoma, type I diabetes and SLE seems to be decreased [5,220-223]. An inverse relationship between Type 2 diabetes and AD has been reported—even if the use of corticosteroids in treatment of AD seemed to increase the type 2 risk instead [224]. An increase in cardiovascular risk has also been proposed [6]. More studies are needed, but it seems plausible that the inflammatory effects of AD are not limited to the skin.
5. BIOMARKERS IN ATOPIC DERMATITIS

In a broad sense, any objectively measurable or evaluable substance, process, or structure in the body or in body products that has diagnostic or prognostic value can be considered a biomarker. They can be indicators of a normal biological process, a pathological process, or a pharmacologic intervention [225]. Biomarkers with prognostic (outcome, treatment response) value are a valuable instrument in the follow-up of chronic diseases. In a complex and heterogenic disease like AD, ideal biomarkers would offer information additionally to the clinical picture and help in the stratification of patients into subgroups. This kind of stratification could then make tailoring of treatment interventions possible and assist in choosing the optimal treatment regimen. Biomarkers can also help in the prediction of treatment response, give information on the disease severity and progression, and offer help in the targeting of (often limited) healthcare resources. They are also useful in the development of new treatment modalities [27].

The list of proposed biomarkers in AD is extensive reflecting the wide spectrum of patients. There are proposed biomarkers for the assessment of severity, screening, diagnostic purposes, predicting treatment response, and for assessing comorbidities. Most of the suggested biomarkers are linked to the disease severity or otherwise used for the follow-up of patients [9,27]. These include such as soluble CD30, interleukins 16, 18, 31, and 33, the soluble IL-2 receptor, Thymus and activation-regulated chemokine (TARC, also known as C-C motif chemokine ligand 17, CCL17), macrophage-derived chemokine (MDC, also known as CCL22), cutaneous T-cell attracting chemokine CCL27, serum eosinophilic protein ECP, brain-derived neurotrophic factor BDNF, FLG and FLG2 mutations, cutaneous lymphocyte antigen positive T cell subsets, squamous cell carcinoma antigen 1 and 2, and serum total IgE [9,22,149,153,226-233]. Of these, TARC seems to correlate best with the clinical severity [234].

The era of targeted immunotherapies has increased the interest in biomarkers [27]. Further studies on biologics may also offer help in the immunological stratification of patients. However, due to the high cost, these treatment modalities will presumably not be available for the majority of patients with AD. There are no predictive biomarkers for standard topical therapy. Long-term follow-up studies on feasible biomarkers with predictive value are also limited and inconclusive [234].
6. TREATMENT OF ATOPIC DERMATITIS

6.1 TOPICAL TREATMENT

Topical therapies are still the cornerstone of the treatment of AD. Topical corticosteroids (TCS) with a broad anti-inflammatory activity have been used since the 1950s. They are efficacious for both active inflammation in the acute phase or flaring of the disease and exacerbation prophylaxis in maintenance therapy. However, there are no data to support a choice of a specific agent among different TCS. Available products differ a lot in respect of formulations, potency, and concentrations. There are limited data on the optimal dosing frequency and quantity of TCS as well. Once-daily application seems to be as effective as application twice daily, at least for the more potent TCS, and twice weekly maintenance therapy has been shown to reduce flares [235,236]. Despite their use for decades, long-term vehicle-controlled studies with TCSs are scant and relatively short in duration. Possible local adverse effects of TCS include acne- or rosacea-like eruptions, perioral dermatitis, glaucoma, focal hypertrichosis, hypopigmentation, and prolonged use can lead to atrophy-related adverse effects like striae, telangiectasia, and purpura. Systemic adverse effects are extremely rare but include a risk of growth suppression and suppression of the hypothalamus-pituitary-adrenal axis, cataracts, and an increased risk for type 2 diabetes in adult patients [224]. The fear of adverse effects of TCS is widespread to the extent that a term "corticosteroid phobia" can be used [237]. Pediatric patients are at a higher risk of systemic adverse effects other than diabetes [236].

Topical calcineurin inhibitors (TCIs), tacrolimus and pimecrolimus, are topical anti-inflammatory agents that have been shown to be efficacious in AD in both acute flares and in maintenance therapy [21,147,238-240]. TCIs act mainly on T cells inhibiting the activation of both Th1 and Th2 cells via a decrease in the activation of T cell nuclear receptor NFATc, leading to decreased calcineurin activity. This, in turn, reduces the amount of many inflammatory cytokines, such as IL-2, IL-3 IL-4, IL-5, interferon gamma, IL-31, and TNF-α [241]. Several guidelines agree that use of TCIs at sites of sensitive or thin skin offers an advantage over the use of stronger than low-potency TCSs, but this is mostly based on expert consensus instead of published long-term data [23,24]. TCIs can be used as an alternative for TCS as a first-line treatment option, but are often used as second-line therapy for AD in patients with inadequate response to other topical treatments or difficulties in the maintenance therapy. Twice-daily application of TCIs is efficacious in treating inflamed AD lesions, and twice-weekly application has been
shown to be efficacious in preventing relapses as maintenance-type of therapy [239,242]. Tacrolimus 0.03–0.1% ointment is generally more potent in the treatment of AD than pimecrolimus 1% cream [243]. The most common adverse effect of TCIs is a localized sensation of burning, stinging, and pruritus, commonly occurring during the initiation of treatment. Oral acetylsalicylic acid has been described to reduce these adverse effects, but a placebo-controlled study would be needed to confirm this finding [244]. Additionally, TCIs can increase the prevalence of localized viral infections at the beginning of treatment [245]. A recent meta-analysis of clinical trials showed that the frequency of adverse effects is higher in TCIs compared with TCS and that the cost of TCI treatment is higher with a comparable efficacy [245], but another systemic review found the treatment of AD with TCIs cost-effective [246].

Crisabororole, a topical phosphodiesterase 4 (PDE4) inhibitor has been available in the United States since 2016 for mild to moderate AD [247]. Trials of other topically applied PDE4 inhibitors and JAK inhibitors are currently ongoing [248].

6.2 OTHER TREATMENT MODALITIES

Phototherapy can be used in AD as a second- or third-line treatment option. Narrow-band UVB utilizing 311nm wavelength of UV-radiation is probably the safest and the most effective phototherapy option for AD, considering its relative efficacy and low associated risks. There is a possibility of several cutaneous adverse reactions: actinic damage with elastosis, local erythema, and altered pigmentation. An increased risk for skin cancer has been proposed, yet not extensively studied [23].

Systemic, non-specific immunosuppressants used in the treatment of AD are cyclosporine A, azathioprine, methotrexate, and mycophenolate mofetil. Cyclosporine A is a calcineurin inhibitor that suppresses T cells and IL-2. Common adverse effects include hypertension and gastrointestinal issues. Azathioprine is a purine analog affecting rapidly dividing cells, such as B and T cells in inflammatory situations. Adverse effects include gastrointestinal problems, liver abnormalities, and myelosuppression. Methotrexate functions as a folic acid antagonist impairing the production of nucleotides. Nausea is the most frequent adverse effect. Mycophenolate mofetil impairs purine synthesis, selectively affecting B and T cells. The most common side effects include gastrointestinal and urinary symptoms [23].
Dupilumab is a fully human monoclonal antibody against IL-4 receptor alpha that inhibits signaling of IL-4 and IL-13. It is the first licensed biologic medication for treatment of moderate to severe AD. Dupilumab seems highly efficacious with a rapid improvement of symptoms and pruritus in both adults and children. It shows a good safety profile, and the most common adverse effect is conjunctivitis [156,249,250]. Other biologics inhibiting TSLP, IL-4, IL-5, IL-13, IL-31 and their receptors) are currently in clinical trials [251]. Oral anti-inflammatory medications inhibiting targets such as histamine 4 receptor, PDE4, and JAK are presently studied as well [248].

Additionally to anti-inflammatory treatment, the regular use of emollients is an important adjunctive therapy in AD and encouraged by treatment guidelines. Emollients improve skin barrier function and reduce transepidermal water loss, which leads to increased skin hydration [61]. The threshold for irritation is lower in the atopic skin. Therefore the avoidance of skin barrier damaging factors, such as drying and irritating soaps with alkaline pH, is important as well [23].

6.3 TREATMENT OF ATOPIC BLEPHAROCONJUNCTIVITIS

Both components of atopic blepharoconjunctivitis (ABC), keratoconjunctivitis and blepharitis, should be treated appropriately. For conjunctivitis, ophthalmic antihistamines or mast cell stabilizers are frequently used, and sometimes the addition of ophthalmic corticosteroids or cyclosporine is needed [45]. There are some data on the use of tacrolimus eye drops with good efficacy on symptoms of the conjunctiva as well [252]. Traditionally, the treatment of choice for blepharitis has been topical corticosteroids, which are a good choice if only occasional therapy with a low-potency TCS preparation is sufficient [45]. However, the prolonged use of corticosteroids on this sensitive area has a well-established risk of adverse effects, such as an increase of intraocular pressure (IOP) or corticoid-induced glaucoma, subcapsular cataract, and even atrophy of the eyelid skin [253-256]. This limits the use of corticosteroids to short courses, which is often problematic due to the need of long-term maintenance therapy in this chronic problem.

As stated before, the safety and efficacy of TCI s (tacrolimus and pimecrolimus) have been well demonstrated in the long-term treatment of AD [21,147,238-240]. Previous smaller studies on the use of tacrolimus ointment in atopic blepharitis have shown good safety and efficacy; also when compared with mid-potent topical corticosteroids
[257,258]. An improvement of conjunctivitis and conjunctival cytology, as well as a neutral effect on IOP, have been reported [258,259]. Safety, efficacy, and tolerability of tacrolimus ointment have been studied in allergic conjunctivitis with application directly into the conjunctival sac in a small 8-week study by Attas-Fox and colleagues [260]. The study showed relatively good efficacy and tolerability with no short-term safety concerns. Recently, Liendo et al. reported good efficacy and safety of tacrolimus 0.03% ointment in atopic keratoconjunctivitis in children with the application on the eyelid skin [261].

There are no placebo-controlled randomized studies on tacrolimus ointment in atopic blepharitis or blepharoconjunctivitis. Pimecrolimus has also shown a good efficacy compared with emollient in treatment of corticosteroid-dependent head and neck dermatitis, including blepharitis, in the only placebo-controlled study on the use of TCIs on the eyelids by Murrell et al. [262]. A reversal of the eyelid thinning caused by earlier prolonged TCS treatments was also reported in this study. There are no previous long-term follow-up studies on the periorbital use of TCIs.
AIMS OF THE STUDY

This thesis aimed to reduce gaps in the research on adulthood AD. To achieve this, we utilized large cohorts of patients with atopic dermatitis and atopic blepharoconjunctivitis from the Helsinki Skin and Allergy Hospital and a nationally representative health examination study cohort of 8,028 adult Finns.

The specific objectives of this study were:

1. To determine the usefulness of FLG and other skin barrier gene variants and serum total IgE as outcome-predicting biomarkers in the long-term management of AD (Studies I and II).
2. To study the significance of FLG and other skin barrier gene variants for the risk of AD in the Finnish population (Study II).
3. To study the effect of patient- and treatment-related factors on long-term outcome of AD (Studies I and II).
4. To gain novel long-term safety data on the use of topical calcineurin inhibitors on the eyelid skin and their efficacy in atopic blepharoconjunctivitis (Study III).
5. To get an update on the epidemiology of atopic dermatitis in Finnish adults including the elderly, and to study the effect of environmental and lifestyle-related factors on the risk of adulthood AD (Study IV).
## MATERIALS AND METHODS

### Table 1. The setting and design of Studies I – IV with basic demographics of study subjects.

<table>
<thead>
<tr>
<th>Study I: IgE and long-term outcome</th>
<th>Study II: FLG and other barrier genes in AD</th>
<th>Study III: TCIs in ABC</th>
<th>Study IV: Epidemiology &amp; risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>169&lt;sup&gt;a&lt;/sup&gt;</td>
<td>501 cases, 1,710 controls</td>
<td>338</td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>33.0 ± 15.0</td>
<td>32.3 ± 14.9, 50.6 ± 10.7</td>
<td>33.0 ± 15.0</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>41/59%</td>
<td>37/63%, 52/48%</td>
<td>35/65%</td>
</tr>
<tr>
<td>Follow-up, years (mean ± SD)</td>
<td>4.2 ± 2.8</td>
<td>1.4 ± 0.6, 1.5 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.7 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Setting</td>
<td>University hospital</td>
<td>University hospital</td>
<td>University hospital</td>
</tr>
<tr>
<td>Design</td>
<td>Retrospective chart review</td>
<td>Prospective observational study</td>
<td>Retrospective chart review</td>
</tr>
<tr>
<td>Exclusion criteria</td>
<td>Age &lt; 14 years</td>
<td>Non-Finnish origin, 1st degree relatives in the study</td>
<td>Age &lt; 14 years, immuno-suppressive medication, other facial skin disorders</td>
</tr>
</tbody>
</table>

<sup>a</sup> A subgroup of subjects from Study II  
<sup>b</sup> Chart review for malignancies  
<sup>c</sup> Clinical follow-up  
IgE, immunoglobulin E; FLG, flaggin; AD, atopic dermatitis; TCI, topical calcineurin inhibitor; ABC, atopic blepharoconjunctivitis; SD, standard deviation
1. STUDY SUBJECTS AND DATA COLLECTION

1.1 STUDIES I AND II

1.1.1 Study design, subjects, and controls
The Study I on serum total IgE as a predictive biomarker of the long-term outcome in adulthood AD was a retrospective extension of Study II on barrier genes. In Study I, a chart review of adult and adolescent AD patients from Study II was performed (Figure 4). Baseline was the start of the follow-up at the Helsinki Skin and Allergy Hospital, a tertiary referral center. Baseline data were collected from patient records from 2002 onwards and included IgE levels (CAP system-specific IgE fluorometric enzyme immunocapture assay), assessment of AD severity (investigator’s global assessment score, IGA), treatment modalities, previous or concomitant medications, and information on patch-test-diagnosed contact allergies. The endpoint of Study I was the enrollment for Study II, and the baseline data collection for Study II was, therefore, the source of endpoint data for Study I. Patients aged <14 years and with less than one year of follow-up were excluded (Table 1 and Figure 4).

![Flowchart showing the filtering and exclusion criteria in Studies I and II.](image)

**Figure 4.** A flowchart showing the filtering and exclusion criteria in Studies I and II.
In Study II, which was a prospective observational study on skin barrier gene mutations in AD, a total of 501 cases with a dermatologist-confirmed diagnosis of AD were recruited consecutively for the planned ≥ 12-month follow-up at the Helsinki Skin and Allergy Hospital between June 2011 and December 2012 (Table 1 and Figure 4). Data on medical history, the course of AD, therapies, clinical features (keratosis pilaris, palmar hyperlinearity, hand eczema, dermographism), other manifestations of atopy (asthma, allergic rhinitis, allergic conjunctivitis), and history of HSV symptoms were collected. The baseline data were confirmed from medical records when necessary. The symptoms of AD were scored by dermatologists using the validated Eczema Area and Severity Index (EASI) [263], IGA, and Rajka-Langeland scale [264]. The scoring of AD and related symptoms, and serum total IgE measurements were carried out at baseline and during follow-up visits. DNA samples were collected at baseline but genotyped after the completion of follow-up. Physicians involved in the study were therefore unaware of patients’ genotype, and treatment interventions were made solely on a clinical basis. Treatment response was defined as a decrease in the clinical scoring of symptoms. The local ethics committee approved the study, and all participants gave a written informed consent.

1.1.2 Control samples for Study II
A total of 1,710 control DNA samples representing the general Finnish population were obtained from the H2000 GenMets Study (Table 1). H2000 was a combination of health examination and health interview survey from autumn 2000 to spring 2001 to obtain comprehensive data on important public health issues and the general functional and working capacities of the Finnish population. The H2000 study cohort comprised 8,028 subjects representative of the Finnish population aged ≥ 30 years. The GenMets subset included those fulfilling the metabolic syndrome criteria, and a matched control for each [265]. Subsets of asthma positive H2000 controls (n = 137) and AD negative H2000 controls (n = 1,243) were used in the association analysis.

1.1.3 Allele frequencies, variant selection, and genotyping
For determining the FLG allele frequencies in the Finnish population and non-Finnish European population, data from the Sequencing Initiative Suomi (SISu) project (http://www.sisuproject.fi) and the Exome Aggregation Consortium (ExAC) dataset (http://exac.broadinstitute.org/about) were utilized. The SISu dataset comprised 1,941 whole-genome sequenced individuals from the FINRISK (a large Finnish population
survey on risk factors on chronic, noncommunicable diseases) and H2000 study cohorts, and 400 healthy individuals from Kuusamo region.

Samples were screened for the *FLG* null mutations R501X, 2282del4, R2447X, S3247X, S1020X, and V603M, and the 12-repeat allele (rs12730241). R501X, 2282del4, R2447X, and S3247X are the four most prevalent *FLG* null mutations in the European population, and S1020X and V603M are variants enriched in the Finnish population. Additionally, 59 functional variants within 10 other barrier genes (claudin 1, *CLDN1*; claudin 4, *CLDN4*; claudin 20, *CLDN20*; claudin 23, *CLDN23*; occludin, *OCLN*; involucrin, *IVL*; filaggrin 2, *FLG2*; loricrin, *LOR*; junctional adhesion molecule A, *JAM-1*, tight junction protein 1, *TJP1*) predicted to be deleterious were genotyped (see chapter “Original publications” for the complete list of genotyped variants). Seven variants within genes *FLG*, *FLG2*, and *CLDN20* were drawn from a systematic survey of loss-of-function variants in the Finnish founder population [266].

Genotyping was conducted using the Sequenom MassARRAY system and the iPLEX Gold assays (Sequenom Inc., San Diego, CA, USA) based on primer extension with single mass-modified nucleotides, followed by matrix-assisted laser desorption ionization-time of flight mass spectrometry for allele discrimination. Genotyping reactions were performed on 20 ng dried genomic DNA in 384-well plates, using manufacturer’s recommendations and their reagents. MassARRAY Assay Design software was used for designing of PCR and extension primers. The data were collected using the MassARRAY Compact System, and the genotypes were called using TyperAnalyzer software (Sequenom Inc., San Diego, CA, USA). The genotype calls were checked manually for quality control. Genotyping quality was ensured by a detailed quality control procedure that comprised success rate checks, duplicated samples, water controls and Hardy-Weinberg principle testing. Genotypes with a significant association were validated by capillary sequencing. Altogether 27 of 59 variants were either monomorphic, had unreliable clustering, or failed Hardy-Weinberg principle testing and were therefore excluded from the association analysis.

### 1.2 Study III

Study III on the long-term safety of TCIs in ABC was a retrospective chart review of patients treated for ABC with individually scheduled ophthalmological examinations between January 1, 2001, and December 31, 2011, at the Helsinki University Skin and...
Allergy Hospital. The final number of included subjects was 338 (Table 1 and Figure 5). For all patients, data were collected on topical treatments of blepharitis (TCS, pimecrolimus, or tacrolimus), ophthalmic treatments for conjunctivitis, treatment frequency, previous treatments and treatment attempts, and follow-up time. A full ophthalmological examination was performed at every control visit. Disease characteristics were the severity of symptoms of blepharitis and conjunctivitis, which were assessed separately on a modified IGA scale 0-3 (no symptoms, marginal/mild, moderate or severe, respectively). Positive treatment response was defined as reduced grading of severity at the end of follow-up. Significant prior use of topical corticosteroids was defined as a cumulative use of at least a month per year. Treatment with ophthalmic antihistamines, mast cell stabilizers (cromones), or cyclosporine, and the use of topical or ophthalmic corticosteroids concomitantly with TCI treatment was defined as regular if used on average at least weekly during follow-up. Additionally, data on the use of inhaled/nasal corticosteroids and the use of immunosuppressive medications for other indications but AD were collected.

Safety parameters included visual acuity, intraocular pressure (IOP), the status of the cornea and lens, and data on adverse events. IOP was measured from both eyes with Goldmann applanation tonometer (Haag-Stet Ag. Bern, Switzerland) and values over 21 mmHg were considered elevated. A significant increase in IOP was defined as an increase of ≥ 6 mmHg if IOP at the end was ≥ 16 mmHg or a ≥ 20% rise from the baseline if IOP at the end was elevated.

Baseline was the induction of the primary topical therapy used, and the endpoint of the clinical follow-up was the last ophthalmological examination during this therapy. For the chart review of malignancies, the endpoint was January 2012 for all the study subjects. Patients with significant periods of systemic immunosuppressive medication (defined as a history of oral immunosuppressive medication ≥ 1 year before the baseline or use of it ≥ 10% of the follow-up time), concurrent other causes of blepharitis (such as contact allergy), or concurrent other facial skin disorders were excluded. A lack of a full ophthalmological control examination was a reason for exclusion as well. The local ethics committee approved the study.
Figure 5. A flowchart showing filtering and exclusion criteria in Study III.

1.3 STUDY IV

Study IV on the epidemiology and risk factors of adulthood AD was a cross-sectional population-based study that utilized data collected through the H2000 survey. The study cohort comprised 8,028 subjects representative of the Finnish population aged 30 years and over (Table 1). A two-stage stratified cluster sampling procedure assured representativeness. The H2000 study consisted of extensive in-home interviews, questionnaires, measurements, laboratory and functional capacity testing, and clinical examinations by medical doctors and dentists. Of the study sample, 6,986 subjects (87%) were interviewed in their homes or an institution, 6,354 subjects (79%) participated in a comprehensive health examination, and 416 subjects (5%) were examined at home. Physical examinations that were performed at home did not include an examination of the skin. National Institute for Health and Welfare carried out the survey in co-operation with experts and researchers from other organizations [267,268].

Data on medical history, socioeconomic factors (education level, income level, number of siblings, current and childhood living environment, education level, and the highest
parental education) were collected in an extensive in-home interview, together with
detailed data on lifestyle-related factors, such as exercise habits, alcohol use, and
smoking. Serum cotinine, a metabolite of nicotine and a biomarker of cigarette smoke
exposure, was measured to assess a possible dose-dependent effect of smoking
among smokers [269]. Exercise level was defined as ‘none’, ‘on average less than 3
hours weekly’, ‘on average ≥ 3 hours weekly’, or ‘doing competitive sports’. Alcohol use
was assessed both as the absolute amount of ethanol (grams per week converted from
questionnaires) and the frequency of use. A history of smoking was defined as ‘never’,
‘ex-smoker’, or ‘daily or occasionally’.

Subjects were defined as having a positive history of AD if they had signs of AD upon
clinical examination, a confirmed diagnosis of AD in their medical records, or they
answered ‘yes’ to the validated [34,35,270] diagnostic question “Have you ever had
itchy dermatitis called atopic eczema, atopic dermatitis, or flexural dermatitis?” The
symptomatic age was defined by a question “When did you have this type of eczema?”
(At the age of < 2 years, 2-7 years, 7-18 years, or ≥ 18 years, within the past 12
months, or currently). Because of the fluctuation of AD symptoms, symptomatic disease
in the past 12 months was considered as active AD. Serum vitamin D (25-
hydroxycholecalciferol) levels were determined by radioimmunoassay (RIA; DiaSorin,
Stillwater, MN, USA) from frozen fasting serum samples taken between September and
March when UV exposure was low. The Ethics Committee for Epidemiology and Public
Health approved the H2000 study. Written informed consent was obtained from all
participants.

2. STATISTICAL METHODS

2.1 STUDY I

Univariate and adjusted odds ratios (OR) with 95% confidence intervals (CI) were
calculated using logistic regression models with “treatment response” and “complete
remission” as the dependent variables. Variables with a p-value ≤ 0.10 in the univariate
analysis were included in the multivariate analysis for adjusted ORs. Variables eligible
for the multivariate logistic regression model were tested for significant interactions. The
p-values for the changes in serum total IgE and clinical severity of AD between the
baseline and the end of the follow-up were calculated using nonparametric Wilcoxon
signed-rank test. IgE values were analyzed on a logarithmic scale [271]. Statistical analyses were conducted using SPSS 21 and SPSS 22 software (IBM, Armonk, NY, USA).

2.2 STUDY II

Power calculations for the primary discovery analysis were conducted with an estimated 10% prevalence of AD [272,273]. We predicted that with an individual FLG null mutation allele frequency of 0.01, recruitment of 500 AD cases and 1500 population controls would give a 97% power at a p = 0.001 to detect an allelic OR of 3. We compared allele frequencies in cases, controls, and different phenotypic groups by using Fisher’s exact test and logistic regression for the binary traits, and a linear model for the quantitative traits with the null hypothesis that there is no association with genotype. Statistical analyses were performed using PLINK whole genome association analysis toolset version 1.07 and R software version 3.1.2 [274,275]. Subjects of non-Finnish origin (defined as having at least one non-Finnish parent) were excluded from the analysis. Bonferroni correction was applied to adjust for multiple testing; the p-value cut-off was set to p = 0.0016 for the initial association analysis and p = 0.001 for the final association analysis of variants and clinical features.

The association analysis for keratosis pilaris and palmar hyperlinearity were performed within the clinical sample set (n = 445), and association with early-onset AD was tested in H2000 supercontrol group (subjects without any atopic diseases, n = 1,243) and early-onset AD cases. Asthma negative H2000 subjects (n = 1,527) were included as controls and asthma positive (n = 137) as cases in the analysis for association with asthma. Association analysis using the combined FLG null genotype included carriers of FLG mutations R501, 2282del2, and R2447X associated with AD in the primary analysis.

2.3 STUDY III

Means of factors were compared using Pearson's chi-squared test for categorical variables and Student's t-test or Fisher's exact test for continuous variables, as appropriate. We used nonparametric Wilcoxon signed-rank test to calculate p-values for the changes of IOP and visual acuity at the baseline and the end of follow-up. A logistic
regression model was used to calculate univariate and adjusted ORs with 95% CI with “total clearance of blepharitis”, “total clearance of conjunctivitis”, “treatment response in blepharitis”, and “treatment response in conjunctivitis” as the dependent outcome variables. Multivariate analysis for adjusted ORs included variables with a p-value ≤ 0.10 in the univariate analysis. There were no significant interactions between variables eligible for the multivariate analysis. The corticosteroid treatment group was excluded from the logistic regression analysis due to the small (n = 8) and selected group of patients. All statistical analyses were performed using SPSS 19.0 software (IBM, Armonk, NY, USA).

2.4 STUDY IV

For the analyses on associations of AD with environmental, socioeconomic, and lifestyle-related variables, ORs for active AD with 95% CIs were calculated. First, ORs were calculated adjusting for age and sex, and then a multivariate logistic regression model with all variables and “asthma” as confounding factors was used for the final adjusted ORs. Wald’s test was used to test the statistical significance of associations. Analysis weights correcting for the oversampling and general population structure were provided with the data by the National Institute for Health and Welfare. All analyses were conducted with SPSS 24.0 (IBM, Armonk, NY, USA).
RESULTS AND DISCUSSION

1. SERUM TOTAL IMMUNOGLOBULIN E IN THE FOLLOW-UP OF PATIENTS WITH ATOPIC DERMATITIS (STUDY I)

1.1 TOTAL IMMUNOGLOBULIN E AND OUTCOME

Study I was a retrospective chart review of 169 patients aged ≥ 14 years with a mean follow-up of 4.2 years (range 1.0–10.4 years). The mean serum total IgE values were 1,501 IU/ml at baseline and 1,130 IU/ml at the end of follow-up (p < 0.001). Associations for positive treatment response and complete remission were assessed separately.

In the adjusted analysis, high baseline total IgE ≥ 10,000 IU/ml was the most significant risk factor for a poor long-term outcome, with an OR of 0.062 (95% CI 0.03 – 0.18, p = 0.002) for positive treatment response and OR 0.16 (95% CI 0.03 – 0.84, p = 0.031) for complete remission. There was no correlation between baseline total IgE and IGA. In patients with baseline total IgE ≥ 10,000 IU/ml, only 9% achieved complete remission and 14% positive treatment response, compared with 52% and 80% in patients with total IgE < 1,000 IU/ml (Figure 6). Both analyses were adjusted for asthma, baseline total IgE, confirmed contact allergies, age, and sex, and the analysis for treatment response also for baseline severity.

After completion of genotyping of Study II samples, Study I data were re-analyzed with the data on FLG status included. However, results remained unchanged, and there was no association between total IgE values and FLG status, or association of FLG null mutations with treatment response (unpublished data).
Figure 6. The association between baseline serum total immunoglobulin E and treatment outcome in Study I with a mean follow-up of 4.2 years.

1.2 DISCUSSION AND CONCLUSIONS

In Study I the most important factor predicting poor long-term outcome in patients, independently of treatment regimen, was serum total IgE. This is in line with previous smaller studies suggesting a predictive value of total IgE in follow-up of patients with adulthood AD [22,152]. Considering the lack of efficacy of IgE antibodies in AD treatment, IgE is presumably an epiphenomenon instead of being an actual driving factor in AD. It mediates atopic comorbidities and is a marker of general Th2 deviation, but direct effects on the inflammation in AD seem unlikely. Furthermore, other factors such as the vitamin D pathways seem to be important. This is demonstrated by the observed association of AD and higher total IgE values with polymorphism of CY27A1 that is involved in vitamin D3 metabolism and participates in the modulation of immune function [109]. Total IgE has been shown to correlate with increased *S. aureus* colonization as well [276]. Unlike in some previous, mostly treatment intervention studies [22,150,151,153], no clear correlation between the severity of symptoms and total IgE was seen in the current study. However, due to the flaring nature of AD, a point evaluation of symptoms may be inadequate in the assessment of disease severity. Most of the patients had also received treatment before baseline due to the tertiary center
setting of the study. A recent meta-analysis did not find a strong association between total IgE values and severity of skin symptoms either, but the predictive value was not analyzed [234]. Retrospective setting and the selected tertiary referral center study population were shortcomings of Study I. The strengths included a long follow-up duration and a relatively large number of patients. Furthermore, the control examinations were conducted mostly by a single dermatologist, which ensured a low inter-observer variation.

Results suggest that measuring serum total IgE offers an inexpensive way to predict the long-term outcome of patients independent of the treatment regimen and can be helpful in selecting those patients who need closer follow-up and particular attention.

2. EFFECT OF FILAGGRIN NULL VARIANTS (STUDIES I and II)

2.1 RISK FOR ATOPIC DERMATITIS

In Study II, the effects of skin barrier genes on the risk of AD and outcome were investigated in a prospective, observational setting with a focus on adult patients. The mean follow-up was 1.4 years. After applying exclusion and filtering criteria (Figure 4), screening of variants provided results for 445 cases (aged 32.3 ± 14.9 years), and 1,664 controls (aged 50.6 ± 10.7 years). There was a female predominance in both groups (62.8% and 51.5%, respectively). A majority (96%) of study subjects with AD were ≥ 14 years of age representing adulthood AD.

In total, 51 (11.5%) AD patients had a FLG null mutation (50 heterozygotes and one compound heterozygote). Individual FLG null variants R501X (1.8% of patients) and 2282del4 (6.7% of patients) were associated with AD, and there was a suggestive association for R2447X (2.7% of patients). These variants were included in the combined FLG genotype analysis. In the combined analysis, having any of the associated FLG null mutations (R501X, 2282del4, R2447X) was highly significantly associated with AD (OR 3.22, p = 3.16 x 10⁻⁸; Table 2).
2.2 PHENOTYPE AND TREATMENT OUTCOME

The combined FLG null genotype was highly significantly associated with asthma (OR = 2.46, p = 8.24 x 10^{-6}), early onset AD (OR = 4.05, p = 1.51 x 10^{-10}), palmar hyperlinearity (OR = 4.67, p = 1.46 x 10^{-5}), and suggestively associated with keratosis pilaris (OR = 3.1, p = 0.0021). Variant R501X showed an association with early onset AD, asthma, and suggestively with keratosis pilaris. Variant 2822del4 was associated with early-onset AD and showed a suggestive association with asthma. R2447X showed a suggestive association with early-onset AD (Table 2). The number of mutation-positive cases was small for each variant.

Baseline IgE values were higher in patients with FLG null mutations (median 1,184 vs. 511 IU/ml), but the association was not significant (p = 0.1274). FLG null variants were not associated with hand eczema, skin-prick test positivity, peanut allergy, dermographism, allergic rhinitis, allergic conjunctivitis, or a history of HSV infections (Figure 7). Excluding patients < 14 years of age (18 patients) did not change the results.

There were no significant differences in the baseline AD severity between FLG null carriers and non-carriers (FLG wild-type). Topical therapy was for 12% monotherapy with TCS, for 57% monotherapy with TCIs, and for 26% a combination of TCIs and TCS. Only 2% of the patients received systemic immunosuppressants during follow-up, combined with topical TCS or TCIs. Subjects did not receive UV therapy during follow-up. There were no significant differences in the use of different topical treatment modalities, oral antibiotics, or oral antihistamines during follow-up between FLG null carriers and non-carriers. There were no differences in treatment outcome: treatment response rates were similar in FLG null carriers and non-carriers (71% and 72%, respectively) and 18% achieved complete remission of symptoms in both groups. FLG null mutations did not seem to increase the lifetime risk of hospitalization due to AD either (36% in FLG null carriers vs. 33% in non-carriers, p = 0.64). The numbers of FLG null patients on different treatment regimens were too small for comparing differences in treatment response, and the number of treatment-naive patients was small.

To examine the possible effect of the lack of treatment-naive patients on results of Study II, we re-analyzed the longer Study I data including the FLG status of patients. However, no association between FLG null mutations and treatment response was detected in this analysis either (unpublished data).
Table 2. Odds ratios (OR) and p-values for individual and combined FLG null mutations associated with atopic dermatitis and phenotypic features.

<table>
<thead>
<tr>
<th></th>
<th>R501X OR p-value</th>
<th>2282del OR p-value</th>
<th>R2447X OR p-value</th>
<th>Combined OR p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
<td>11.29 p = 0.0022</td>
<td>2.66 p = 0.00016</td>
<td>3.10 p = 0.0072</td>
<td>3.22 p = 3.16×10⁻⁹</td>
</tr>
<tr>
<td>Early onset (&lt; 2 years of age)</td>
<td>16.09 p = 0.00055</td>
<td>3.26 p = 1.007×10⁻⁵</td>
<td>3.86 p = 0.0018</td>
<td>4.05 p = 1.51×10⁻⁹</td>
</tr>
<tr>
<td>Palmar hyperlinearity</td>
<td>NA</td>
<td>3.05 p = 0.0062</td>
<td>6.13 p = 0.023</td>
<td>4.67 p = 1.46×10⁻⁵</td>
</tr>
<tr>
<td>Keratosis pilaris</td>
<td>13.09 p = 0.0035</td>
<td>2.44 p = 0.060</td>
<td>1.53 p = 0.60</td>
<td>3.1 p = 0.0021</td>
</tr>
<tr>
<td>Asthma</td>
<td>10.22 p = 0.00022</td>
<td>2.10 p = 0.0030</td>
<td>2.04 p = 0.11</td>
<td>2.46 p = 8.24×10⁻⁶</td>
</tr>
</tbody>
</table>

a) carriers of R501X, 2282del, or R2447X mutations

Figure 7. Prevalence (%) of different phenotypic features in FLG null mutation carriers and non-carriers (wild-type) in patients with atopic dermatitis in Study II.
2.3 ALLELE FREQUENCIES AND COPY NUMBER VARIATION

Individual allele frequencies of R501X, 2282del4, and S3247X were low in Finns compared to most other European populations. The combined minor allele frequency was only 5.62%. To our surprise, having 24 repeats (two 12-repeat alleles) was more common in AD patients than in subjects without AD (3.6% and 2.8%, respectively). It was associated with an increased risk for AD (OR = 1.96, p = 0.00056) and suggestively associated with the risk for early-onset AD (OR = 1.47, p = 0.0076) compared with subjects with < 24 repeats. Having 24 repeats did not show associations with any other phenotypic features or treatment response.

2.4 OTHER STUDIED SKIN BARRIER GENE VARIANTS

Association analysis of additional 24 variants in genes involved in the skin barrier function and structure (CLDN1, CLDN4, CLDN20, CLDN23, OCLN, IVL, FLG2, JAM-1, and TJP1) including Finnish-enriched variants did not bring up any significant associations with AD in Finnish patients (see chapter “Original publications” for the complete results).

2.5 DISCUSSION AND CONCLUSIONS

Study II represents the first study on the effect of FLG null and other epidermal skin barrier gene variants on AD in the Finnish population. The individual frequencies of FLG null mutations 2282del4 and R501X were significantly higher in patients with AD compared with controls. Findings are in line with many previous studies that have reported an association between FLG null mutations and AD [25,85,86,277,278]. In most of the studied European populations, the proportion of FLG null carriers among AD patients has varied between 14–42%, while in current study only 11.5% of patients were FLG null carriers.

Asthma is associated with FLG null mutations also in the Finnish patients with AD. This is in concordance with the reports from other populations [85,92,93]. However, in the current study, there were no associations between FLG null mutations and allergic rhinitis and conjunctivitis, of which especially allergic rhinitis has been previously repeatedly associated with FLG null mutations in patients with AD [58,85,93].
Furthermore, *FLG* null mutations seem to predispose to early onset of AD also in Finns, as reported in studies on other populations [89,90,97].

Although asthma as a significant comorbidity of AD can be viewed as an indicator of more severe disease, regarding the degree of skin symptoms (assessed by EASI, Rajka-Langeland scale, and IGA), total IgE, or hospitalization due to AD, there were no associations between *FLG* mutations and disease severity. In previous studies, this correlation has been noted mostly in pediatric populations [279]. The number of treatment-naive patients in the current study was low, which may dilute the associations. It is also possible that the primary barrier defects are not that important in chronic adulthood AD, even if they are important in the trigger phase of the disease.

To our surprise, population carrier frequencies for R501X, 2282del4 and S3247X were remarkably lower in Finns compared with other European populations, despite the high prevalence of AD in Finland [277,278]. Allelic frequency of R501X was similar to that reported from Volga-Ural region of Russia (0.90%) [280]. We expected to observe higher allele frequencies due to the previous findings that *FLG* null mutations increase the persistence of AD symptoms [96,97], and the majority of the study subjects were adults with persistent AD. ExAC data search did not reveal any further *FLG* null mutations that would be specifically prevalent in Finns, and R501X, 2282del4 and R2447X were the most common mutations in the Finnish population, just like in other European populations. Therefore it seems unlikely that additional loss-of-function variants in the filaggrin gene would have a major impact on AD in Finland. Many studies have reported the enrichment of rare variants in isolated populations. We wanted to test the effect of Finnish-enriched variants by drawing potential liability variants of barrier genes from a systematic survey of distribution and medical impact of loss-of-function variants identified as likely deleterious, rare, and noticeably enriched in the Finnish population [266]. However, the Finnish-enriched variants did not provide significant associations with AD.

Contrary to some previous findings [69,281], AD was more common in patients with 24 repeats (two 12-repeat alleles) of *FLG*, and there was a suggestive association with early onset of the disease as well. Considering this unexpected result and the notably low *FLG* null prevalence in Finnish AD patients, it is possible that other skin barrier or immune-related genes have a significant impact on the risk of AD in *FLG*-independent manner. Furthermore, this observed association could be caused by a linkage of the 12-repeat allele to an unrecognized Finnish-specific *FLG* null mutation, which could then
also explain the observed association of keratosis pilaris with worse outcome independently of the studied FLG null mutations (see chapter “Risk factors for adulthood atopic dermatitis and its poor long-term outcome”). It is also noteworthy that the tested allele (rs12730241) is known to be in linkage with the 12-repeat allele in the Irish population. Only FLG mutation screening by sequencing would confirm the same linkage in the Finnish population, and uncover this potential previously unrecognized FLG null mutation. Furthermore, additional copy number alleles can exist in the Finnish population.

Strengths of Study II included a prospective setting, a large number of adulthood AD subjects, and a relatively long follow-up. Most of the patients were treated with topical treatments, which made this population suitable for studying effects of barrier defects. The data on healthy controls were also collected from clinical examinations, not only from questionnaires. Adherence to treatment was not measured in the current study. The number of patients on each treatment regimen was too low for evaluating possible differences between the responses to different treatment modalities, and the setting was observational.

Regardless of the apparent association of FLG null mutations with AD in the Finnish population, they still explain only a fraction of the total risk. The pathogenesis of AD is multifactorial, and the effect of acquired or environmental factors is undeniable considering the steep increase in the prevalence throughout the late 20th century. Numerous immunological factors can downregulate the expression of barrier proteins and cause secondary barrier impairment [282]. Whether the primary etiology is barrier deficiency (“outside-to-inside”), immunological (“inside-to-outside”), or combination of both (“inside-outside-inside”), it still remains to be clarified how and to what extent external triggers affect this, and what are the key primary players additionally to filaggrin.

The current study extends the knowledge of the effect of barrier genes in adult patients with AD. Our findings confirm FLG null mutations as risk factors for AD in the Finnish population, and further support the complexity of the pathogenesis of AD. Moreover, based on our data, the feasibility of FLG null mutations as outcome-predicting biomarkers in the follow-up of patients with adulthood AD seems low.
3. TOPICAL CALCINEURIN INHIBITORS IN THE LONG-TERM MANAGEMENT OF ATOPIC BLEPHAROCONJUNCTIVITIS (STUDY III)

3.1 SAFETY AND TOLERABILITY

In Study III we conducted a retrospective chart review of 338 patients with ABC. For 33 patients the treatment of blepharitis was pimecrolimus cream, for 297 tacrolimus ointment (for 94% 0.03% ointment and for 6% 0.1% ointment) and for eight patients topical corticosteroids (Figure 5). There were no significant baseline differences between tacrolimus and pimecrolimus groups. A majority of patients used ophthalmic mast cell stabilizers (cromones) for conjunctivitis during follow-up.

The mean clinical follow-up with ophthalmological examinations was 1.5 years and the mean chart follow-up for malignancies was 5.7 years (a total of 1,945 patient years). There were no observed malignancies of the eye and periorcular skin, or other severe or long-term adverse effects. There were no significant changes in visual acuity and no treatment-related changes in the lens and cornea. In turn, there was a reduction of minor pre-existing disease-related corneal changes and corticosteroid-related changes in the lens. The mean IOP decreased in both groups in a similar manner, reaching statistical significance in the tacrolimus group (p = 0.042). None of the TCI-treated patients were diagnosed with glaucoma during follow-up. Four patients (12%) in the pimecrolimus group and 18 patients (6%) in the tacrolimus group had symptoms of ocular or eyelid HSV infection during follow-up, but a vast majority of these (4/4 and 14/18 in the pimecrolimus and tacrolimus groups, respectively) were confirmed reactivations. Eight (3%) patients in the tacrolimus group had other infection of the eye or eyelid skin. There were no observed infections in the small 0.1% tacrolimus ointment subgroup (Table 3).

Total discontinuation rates were 56% in the pimecrolimus group and 11% in the tacrolimus group. For pimecrolimus cream as the first initiated TCI treatment, the discontinuation rate was 60%. For tacrolimus ointment as the first initiated TCI, the discontinuation rate was 11%. A third of pimecrolimus treatments and 9% of tacrolimus treatments were discontinued due to patient-reported side effects: burning, stinging, or itching. Lack of treatment response led to discontinuation for 23% in the pimecrolimus group and 1.6% in the tacrolimus group (Table 3). In the small tacrolimus 0.1% subgroup, one (6%) patient discontinued due to patient-reported adverse effects.
Table 3. Adverse effects, reasons for treatment discontinuation, and the relative efficacy of pimecrolimus cream and tacrolimus ointment in symptoms of atopic blepharoconjunctivitis.

<table>
<thead>
<tr>
<th>Pimecrolimus</th>
<th>Tacrolimus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Used as the primary treatment for blepharitis</strong></td>
<td>33</td>
</tr>
<tr>
<td><strong>Total number of treatment periods</strong></td>
<td>57</td>
</tr>
<tr>
<td><strong>Discontinued treatment periods</strong></td>
<td></td>
</tr>
<tr>
<td>Insufficient response</td>
<td>32 (56%)</td>
</tr>
<tr>
<td>Adverse effects/tolerability</td>
<td>13 (23%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>19 (33%)</td>
</tr>
<tr>
<td><strong>HSV symptoms during follow-up</strong></td>
<td></td>
</tr>
<tr>
<td>History of HSV symptoms</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>No prior HSV symptoms</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>Other infections during follow-up</td>
<td>0</td>
</tr>
<tr>
<td>Malignancies of the eye or periorbital skin</td>
<td>0</td>
</tr>
<tr>
<td><strong>New glaucoma diagnoses during follow-up</strong></td>
<td></td>
</tr>
<tr>
<td>Significant rise of IOP during follow up</td>
<td>0</td>
</tr>
<tr>
<td>Elevated IOP at baseline</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>Elevated IOP at the end of follow-up</td>
<td>1 (3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of patients</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OR</strong></td>
<td><strong>OR (CI)</strong></td>
</tr>
<tr>
<td>Treatment response in blepharitis</td>
<td>79%</td>
</tr>
<tr>
<td>Complete remission of blepharitis</td>
<td>58%</td>
</tr>
<tr>
<td>Treatment response in conjunctivitis</td>
<td>48%</td>
</tr>
<tr>
<td>Complete remission of conjunctivitis</td>
<td>33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>OR (CI)</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>2.37 (0.90 - 6.22)</td>
</tr>
<tr>
<td>1.00</td>
<td>1.61 (0.73 - 3.55)</td>
</tr>
<tr>
<td>1.00</td>
<td>2.34 (1.02 - 5.40)</td>
</tr>
<tr>
<td>1.00</td>
<td>1.56 (0.71 - 3.45)</td>
</tr>
</tbody>
</table>

a) 5 patients with bacterial conjunctivitis, 2 patients with sporadic eyelid molluscum contagiosum, and 1 patient with bacterial blepharitis
b) None of these patients had elevated IOP at the end of follow-up
c) Compared with pimecrolimus

3.2 RELATIVE EFFICACY

Efficacy in blepharitis was assessed with “positive treatment response” and “total remission” as separate endpoints, and the analysis was adjusted for age and additional use of corticosteroids. There were no statistically significant differences between the pimecrolimus and tacrolimus groups: ORs for tacrolimus were 2.37 (95% CI 0.90 – 6.22) for positive treatment response and 1.61 (95% CI 0.73 – 3.55) for complete remission when compared with pimecrolimus. Complete remission of blepharitis was achieved among 58% and 70% (p = 0.22 for the difference) of patients in the pimecrolimus and tacrolimus groups, respectively (Table 3).
Similarly, the efficacy in conjunctivitis was assessed with “positive treatment response” and “complete remission” as separate endpoints. The analysis was adjusted for age, additional use of corticosteroids, and continuous use of nasal corticosteroids. Tacrolimus was more efficacious than pimecrolimus with an OR of 2.34 (95% CI 1.02 – 5.40) for positive treatment response, but in regard to complete remission of conjunctivitis, there was no significant difference (OR 1.57, 95% CI 0.71 – 3.45). Treatment outcome was worse in patients aged > 40 years, compared with younger subjects. The use of nasal corticosteroids was associated with a better treatment outcome of conjunctivitis, while additional use of topical/ophthalmic corticosteroids was linked to a worse outcome. Complete remission of conjunctivitis was achieved more often in the tacrolimus group (55%) than in the pimecrolimus group (33%) (Table 3).

3.4 DISCUSSION AND CONCLUSIONS

In previous studies on TCIs for ABC, the number of patients has been small and follow-up time limited [257-259,262]. The proximity of the eye adds additional safety parameters to the use of topical treatments on the delicate eyelid skin, and therefore a longer study with ophthamological data was needed. There were no observed malignancies. Additionally, there was a favorable effect on the mean IOP in study subjects, and a decrease in the proportion of patients with elevated IOP. The reduction in IOP likely reflects the normalization of corticosteroid-induced elevation in IOP since calcineurin inhibitors are not known to have a direct effect on IOP homeostasis [254-256,259]. This hypothesis is further supported by the finding that the mean IOP reduction was larger in patients with a history of frequent topical corticosteroid use than in those without it (0.73 mmHg and 0.19 mmHg, respectively). There were no ocular adverse events additionally to the observed HSV infections. Patients with AD are known to have an elevated risk for HSV infections due to a defective skin barrier and the Th2 response predisposition, and they are also at a higher risk for herpetic eye disease [283,284]. Reactivations occurred mostly during the first weeks following the treatment initiation and decreased after the first months of treatment, presumably reflecting the improvement in ABC symptoms and the skin barrier. Similar increase-decrease tendency has been reported before in a study with topical tacrolimus in the treatment of AD [285]. Contrary to some previous reports [45], the majority (65%) of patients with ABC were women in the current study.
Many patients, who were allowed to choose a product they prefer after the first application of both products upon the first appointment, chose tacrolimus ointment. These study results are in concordance with this experience, and tacrolimus showed better tolerability. Tacrolimus also showed significantly better efficacy in the treatment of conjunctivitis compared to pimecrolimus, and there was a trend towards better efficacy in blepharitis as well. The ointment vehicle and the faster relief of symptoms can explain the better tolerability of tacrolimus ointment on the eyelid. The vehicle in tacrolimus 0.03% and 0.1% ointments consists mainly of liquid paraffin and petrolatum jelly, which are often used in ophthalmic preparations as well. Petrolatum jelly has been shown to increase the expression of filaggrin and AMPs, which means that the vehicle may not be completely inert and can offer some minor additional therapeutic effect [286]. The tacrolimus 0.1% subgroup was too small for subgroup analysis, but results were similar to those in the 0.03% group without any increase of adverse effects. Considering our results, the sensitivity of eyelids, and the good absorption of medications through this thin skin area, 0.03% tacrolimus ointment seems to provide a sufficient treatment result in most of the cases, possibly with minimized risks. This study was designed to study the long-term safety. Retrospective setting and a significantly smaller number of patients in the pimecrolimus group were shortcomings of this study. Strengths included a long follow-up and a large total number of patients. A randomized controlled trial would be the optimal setting for assessment of efficacy.

Atopic blepharitis is common in adult patients with AD. Corticosteroid-related risks are problematic due to the frequent need for long maintenance therapy. We conclude that both pimecrolimus and tacrolimus show a favorable long-term safety profile and efficacy in this indication. Tacrolimus ointment seems better tolerated on the eyelid than pimecrolimus cream. TCIs do not increase IOP or cause glaucoma, cataract, or skin atrophy, and no malignant neoplasms were detected. Therefore they can safely be prescribed for this indication by dermatologists and ophthalmologists. We recommend prophylactic oral antiviral therapy upon initiation of eyelid TCI treatment for patients with a history of ocular HSV symptoms or a recent active HSV on the facial skin. Based on our data, topical tacrolimus and pimecrolimus are safe treatment options in the long-term management of ABC and tacrolimus ointment is a possible first-line treatment option.
4. RISK FACTORS FOR ADULTHOOD ATOPIC DERMATITIS AND ITS POOR LONG-TERM OUTCOME (STUDIES I, II, AND IV)

4.1 RISK FACTORS FOR POOR OUTCOME (STUDIES I AND II)

In Study I, a history of confirmed contact allergies showed a negative association with positive treatment response (OR 0.162, p = 0.007) and with complete remission of symptoms (OR 0.29, p = 0.048). Only 16.5% of patients with confirmed contact allergies achieved complete remission of symptoms, while in patients without contact allergies the proportion was 45.4%. Allergic rhinitis and conjunctivitis, food allergy, hand eczema, any positive reactions in skin-prick testing, prick-test positivity for peanuts, a history of HSV infections, systemic immunosuppressive medication for indications other than AD, smoking, age of onset, main topical therapy used, and regular use of oral antihistamines or montelukast showed no associations with treatment outcome (assessed by IGA).

In Study II, keratosis pilaris (OR 0.30, 95% CI 0.14 – 0.66, p = 0.003) and a history of HSV infections (OR 0.51, 95% CI 0.29 – 0.88, p = 0.015) showed negative associations with treatment response (assessed by EASI) independently of FLG null mutations (analysis adjusted for age, sex, FLG status). Other phenotypic features or lifestyle-related factors (including palmar hyperlinearity, allergic conjunctivitis, allergic rhinitis, the age of onset, asthma, food allergies, skin-prick test positivity, hand eczema, dermographism, body mass index, smoking) did not show associations with the outcome (unpublished data).

4.2 RISK FACTORS FOR ADULTHOOD ATOPIC DERMATITIS (STUDY IV)

4.2.1 Socioeconomic status, living environment, and lifestyle-related factors

Age was negatively associated with AD in this study population of subjects ≥ 30 years (OR per year 0.98, 95% CI 0.97 – 0.99). Parental university-level education was significantly associated with active AD (OR 1.58, 95% CI 1.05 – 2.38), but there were no associations between AD and the education or income levels of subjects themselves. Female sex was linked to active AD only in subjects aged 30–49 years (OR 1.33, 95% CI 1.01 – 1.75) but not in older age groups. Childhood or adult life living environments and the number of siblings showed no associations with AD (Table 4).
Being an ex-smoker was associated with an increased risk of active AD with an OR of 1.45 (95% CI 1.13 – 1.85). Current daily smoking was associated with AD in subjects less than 50 years of age, but not in older subjects. Serum cotinine did not show an association with AD, and there was no dose–response effect among smokers either. Alcohol consumption, body mass index, and leisure-time exercise showed no associations with AD. Regarding serum vitamin D levels, there was a borderline significance of the variation in AD risk (p for heterogeneity 0.057). This was mostly due to the higher prevalence in the third quintile than in the second, fourth, and fifth ones, and there was no dose–response effect of serum vitamin D levels on the risk of active AD (OR per unit 0.99, 95% CI 0.97 – 1.00) (Table 4).

4.2.2 Asthma and allergic rhinoconjunctivitis
Both allergic rhinoconjunctivitis and asthma showed an association with active AD with respective ORs of 2.72 (95% CI 2.24 – 3.30) and 2.16 (95% CI 1.62 – 2.87). Of all the subjects with a history of AD, 14% had asthma and 51% allergic rhinoconjunctivitis. The lifetime prevalence of allergic rhinoconjunctivitis was 35.2% and the lifetime prevalence of asthma 9.1% (Figure 8).

![Diagram showing the lifetime prevalences of atopic dermatitis, asthma, and allergic rhinoconjunctivitis and their overlapping in the adult Finnish population ≥ 30 years of age.](image)

**Figure 8.** The lifetime prevalences of atopic dermatitis, asthma, and allergic rhinoconjunctivitis and their overlapping in the adult Finnish population ≥ 30 years of age.
Table 4. Demographic, socioeconomic, and lifestyle characteristics of study subjects and odds ratios (OR) with 95% confidence intervals (CI) for active atopic dermatitis (AD). Analysis adjusted for all variables in the table (significant associations in bold).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>n (AD)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<td>40–49</td>
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<td>122</td>
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<td><em>(0.45–0.76)</em></td>
</tr>
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<td>129</td>
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<td><em>(0.55–0.96)</em></td>
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<td>72</td>
<td><strong>0.58</strong></td>
<td><em>(0.41–0.82)</em></td>
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<td>43</td>
<td><strong>0.33</strong></td>
<td><em>(0.21–0.50)</em></td>
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<td>260</td>
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<tr>
<td>female</td>
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<td>1.19</td>
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<td>semi-urban</td>
<td>798</td>
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<td><em>(0.62–1.13)</em></td>
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<td>1.01</td>
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<tr>
<td>low</td>
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<td>0</td>
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<td>99</td>
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<td>2</td>
<td>1,030</td>
<td>116</td>
<td>0.87</td>
<td><em>(0.56–1.34)</em></td>
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<td>4</td>
<td>602</td>
<td>53</td>
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<td>ex-smoker</td>
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<td>142</td>
<td><strong>1.45</strong></td>
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<td>daily/occasionally</td>
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<td>172</td>
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<td>0</td>
<td>1,713</td>
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<td>25–100</td>
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<td></td>
<td></td>
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<tr>
<td><strong>Body mass index, kg/m²</strong></td>
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<td></td>
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<tr>
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<td>176</td>
<td>26</td>
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<tr>
<td>20–25</td>
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<td>≥ 30</td>
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<td>0.68</td>
<td><em>(0.41–1.14)</em></td>
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<td><strong>Exercise at leisure</strong></td>
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<td></td>
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<tr>
<td>never</td>
<td>1,494</td>
<td>161</td>
<td></td>
<td></td>
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<tr>
<td>&lt; 3 hours a week</td>
<td>2,940</td>
<td>295</td>
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<td>≥ 3 hours a week</td>
<td>845</td>
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<td>10</td>
<td>1.00</td>
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<td><strong>Vitamin D quintile, mmol/l</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0–30</td>
<td>1,039</td>
<td>106</td>
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<tr>
<td>31–38</td>
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<td>0.85</td>
<td><em>(0.62–1.17)</em></td>
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<td>39–46</td>
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<td><em>(0.96–1.72)</em></td>
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</tr>
<tr>
<td>≥ 58</td>
<td>1,089</td>
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<td>0.89</td>
<td><em>(0.65–1.23)</em></td>
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<tr>
<td>mean/median values</td>
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**Asthma**

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<tr>
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<th>N</th>
<th>n (AD)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<td>475</td>
<td></td>
<td></td>
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<tr>
<td>yes</td>
<td>475</td>
<td>77</td>
<td><strong>2.16</strong></td>
<td><em>(1.62–2.87)</em></td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
4.3 DISCUSSION AND CONCLUSIONS

In our data, delayed contact hypersensitivity predicted worse long-term outcome in patients with adulthood AD. The analysis was adjusted for the baseline severity. It is therefore unlikely that more extensive patch testing of patients with more severe symptoms would explain this observed association. A recent comprehensive meta-analysis did not find an overall association between AD and contact allergies [287]. FLG null mutations may predispose to contact allergies when eczema is present [288]. Contact allergies have also been associated with more persistent AD [15]. The immune profile of delayed contact sensitization is more Th1 polarized in contrast to the general Th2 deviation seen in AD. However, there is increased exposure to allergens in AD via altered skin barrier function and frequent use of emollients and topical medications. The mean total IgE values were slightly lower in patients with contact allergies in a non-significant manner, but no other differences in phenotype or demographic factors were observed. It is possible that these patients represent a subtype of AD patients with additional activation of other but Th2 axis, such as increased Th1 responses associated with more chronic AD [127].

A history of HSV infections predicted worse outcome (assessed by EASI) in the prospective study, but this association was not seen in the smaller retrospective study (assessed by IGA). Additionally, keratosis pilaris phenotype was linked to poor outcome. These associations were independent of the studied FLG mutations. Susceptibility to HSV infections has been associated with a more severe skin barrier deficiency and a decreased production of AMPs, and it seems likely that this susceptibility represents a stronger activation of Th2 axis [289]. All of these factors can mediate the observed association between a history of HSV symptoms and worse long-term outcome seen in Study II. Furthermore, increased exposure to staphylococcal alpha-toxin can also promote viral skin infections, and susceptibility to HSV can be secondary to extensive colonization with S. aureus [290]. As stated before, the observed association of keratosis pilaris with worse treatment outcome could suggest a possibility of a novel FLG null mutation in the Finnish population. However, FLG defects explain only about a third of this common phenotypic feature, and the rest of the factors in the pathogenesis of keratosis pilaris are mostly unknown [291]. Other shared genetic or pathogenic mechanisms between keratosis pilaris and AD are possible considering that FLG null mutations showed no association with the outcome in this population.
As shown in Study II, the FLG null frequency in Finland seems low, which may indicate a significant contribution of acquired or environmental factors. Low vitamin D levels have shown association with AD in adult populations in some previous studies, but the results are conflicting [292,293]. Considering the lack of a dose–response effect, the observed borderline significance in our study was likely due to chance alone. The vitamin D values were overall suboptimal. There was no association between the childhood or adulthood living environment and AD, which is in line with previous results from Finland and Sweden [195,294] but differ from those reported from the German, US, and Italian populations [17,185,272]. Interestingly, subjects with highly educated parents had more AD even in this study population of adults. However, there was no effect of higher education in subjects themselves making information bias an unlikely explanation. This observed association could be connected to the social class and hygienic status during childhood—particularly considering the lack of association of adult-onset AD with parental education.

Past smoking was linked to adulthood AD and also to adult-onset AD, while current smoking was associated with AD only in subjects < 50 years of age. A direct effect of smoking on the inflammation appears unlikely because there was no association between serum cotinine levels and AD. In a subgroup analysis of smoking status, a recent (during the past year) cessation of smoking showed even stronger association with AD with an OR of 3.03 (95% CI 1.85 – 4.98). Stress-mediated factors could be one possible explanation but there are no studies on this matter. Stress has been shown to be able to impair the skin barrier and enhance the Th2 shift in immunology in AD [215,216]. Epigenetic mechanisms could also explain this association. Previous studies have suggested a link between smoking and AD [199,200], and a similar curious association between past smoking—but not current smoking—and adulthood AD was also been reported from an international multicenter study by Harrop et al. [29]. We did not detect an association between obesity and AD, which is in accordance with a recent meta-analysis suggesting that obesity is linked to AD only in the North American and Asian populations [208].
5. EPIDEMIOLOGY OF ADULTHOOD ATOPIC DERMATITIS (STUDY IV)

5.1 PREVALENCE

In Finnish adults ≥ 30 years of age, the 12-month prevalence of AD was 10.1%, and it decreased with age from 15.4% (30-39-year-old subjects) to 4.8% (subjects ≥ 70 years). The prevalence of AD in adulthood was 15.1% and the lifetime prevalence was 21.9%. The highest lifetime prevalence (31.0%) was seen in 30-39-year-old women, and the lifetime prevalence decreased with age as well. In subjects ≥ 70 years of age, active AD was more common in men (5.8%) than in women (4.2%), but the difference was nonsignificant (p = 0.28; Figure 9).

5.2 AGE OF ONSET AND PERSISTENCE OF SYMPTOMS

Of all the subjects with a history of AD, only 41% reported onset in childhood before the age of 7 years, 17% between the ages of 7 and 18, and 42% in the adult life. Of the subjects reporting the onset of AD before the age of 7, 37% had symptoms in the adult life, and 26% had active disease. Women reported onset in childhood significantly more often than men (p < 0.001), while men reported onset in adulthood more frequently (p = 0.013). Subjects reporting onset in childhood were significantly younger than subjects reporting onset later in life (the mean age 48 vs. 56 years, p < 0.001).

5.3 ADULT-ONSET ATOPIC DERMATITIS

When data were analyzed in regard to active adult-onset AD, age and parental education level showed no more associations with active disease. There were also no associations with sex, or any socioeconomic or environmental factor. The only lifestyle factor that showed an association with active AD in the adult-onset group was past smoking with an OR of 1.49 (95% CI 1.08 – 2.05, p = 0.012). There was no association with asthma either, but the significant association with allergic rhinoconjunctivitis remained (OR 2.03, 95% CI 1.57 – 2.63).
Figure 9. Prevalence of atopic dermatitis in Finnish adult population aged ≥ 30 years.
5.4 DISCUSSION AND CONCLUSIONS

The current study presents the first population-based data on the prevalence of AD in Finnish adults and the elderly. Over 15% of Finns of over 30 years of age had a history of adulthood AD. Both the 12-month prevalence of 10.1% and the lifetime prevalence of 21.9% are high in comparison to most reports from other industrialized countries. In Italy, the lifetime prevalence of AD was 8.1% in 20-44-year-old subjects [185], while in Germany a lifetime prevalence of 4.3% among subjects aged 50–75 years was reported from a population-based study [272]. Yet our results are in line with the previously published reported lifetime prevalence numbers from Finland (28.1% among 25-54-year-old subjects in the North Karelia district) and Denmark (34.1% among young adults of 28–30 years of age) [15,31] and the 12-month prevalence rates of 6.9–11.6% in Japanese, American, and Swedish adults [3,17,270]. High numbers are not surprising considering that the climate factors (such as temperature, latitude, low UV exposure, and precipitation) prevalent in Finland have been linked to an increased risk of AD [295].

Several studies have shown that in a selected AD population, such as in a university hospital setting, the persistence of AD varies between 40–90% depending on the severity of the disease [14,15,296]. In our study, 37% of subjects reporting onset in childhood were still symptomatic in adulthood and 26% had active disease. Considering the unselected population including also all the subjects with mild symptoms of AD, these percentages are high. In previous studies, the proportion of patients with adult-onset AD has mostly been 20% or less [39,40,297]. In the current study, 42% of subjects reported onset of symptoms in adult life, and this was more common in men. Women, in turn, reported onset in childhood more frequently, which corresponds with reports showing an association of female sex with more persistent AD [298,299]. Asthma was not associated with adult-onset AD, and the association with allergic rhinoconjunctivitis was weaker compared to subjects with the persistent AD with early onset. This is in concordance with studies on other populations and emphasizes the significance of AD in development of atopic comorbidities—the atopic march [12]. Early childhood AD may have been forgotten, which could have caused recall bias and overestimation of the number of adult-onset cases [41]. Subjects reporting onset in childhood were younger than the ones reporting onset in adulthood, which can reflect this bias. In the case of this recall bias, the lifetime prevalence could then be underestimated. Our results are, however, in parallel to those by Pesce et al. from Italy [185] and Silverberg et al. from
the United States [12], where about 40% of study subjects reported onset of symptoms in adult life.

The Health 2000 study provided a nationally representative, large cohort of the Finnish adult population aged ≥ 30 years minimizing selection bias. The data on AD were not based solely on self-reported data. Shortcomings of the study were that subjects in poor health (5% of subjects) were examined at home or an institution without a skin examination and the severity of AD was not assessed. Data on lifestyle factors were mostly based on questionnaires, which may have lead to some reporting bias in habits considered unhealthy, such as smoking. We did not have data on supplements or sun-bathing during the study period, which may have an impact on data on vitamin D, even if samples were collected during the time of low UV exposure in Finland.

The number of adult patients with AD is growing in conjunction with the aging of the society. The prevalence of adulthood AD in Finland is among the highest reported, making it an important public health issue.
FUTURE PROSPECTS

The research on AD has progressed greatly during the past decade, but many questions remain open. The impaired epidermal barrier is a characteristic component of AD. Yet its overall importance as a driving component in AD pathogenesis needs further clarification. Trials have shown a preventive effect of moisturizer use on AD in high-risk infants emphasizing the significance of barrier defects in early-onset AD [61,62]. This supports the findings from the filaggrin studies, including our results. FLG null mutations are a consistent risk factor for AD, but the majority of patients do not carry them. Furthermore, biologics, such as dupilumab, seem to work similarly in FLG null carriers and non-carriers. Based on our data, these primary defects do not appear to have an impact on the outcome of topical therapy either. The research gaps in adulthood AD—and especially in AD of the elderly—are still considerable. In the Finnish AD population, there was an association between keratosis pilaris and poor long-term outcome independent of the studied FLG null mutations. Additionally, we observed a higher risk of AD in subjects carrying two 12-repeat alleles of FLG. FLG mutation screening by sequencing could potentially uncover an unrecognized FLG null mutation in the Finnish population, although only a third of keratosis pilaris is explained by mutations in the filaggrin gene. A little is known of other factors of the pathogenesis of keratosis pilaris, which could then mediate and explain the observed effect on AD outcome [291].

Our results support the view that the effect of the primary barrier defect is limited to the trigger phase of AD. The complicated interactions between the impaired skin barrier and immunological milieu remain to be further clarified. Future studies on biologics will presumably help in pointing out the key immunological players in different patient subgroups. However, due to the high costs, these therapies will be available only for a fraction of patients, those with severe AD. Randomized controlled studies on the efficacy of different topical treatments in treatment-naive patients stratified based on immunological and barrier-related factors would provide more insight into precision medicine in patients with mild or moderate AD. This could expand the field of targeted therapies, making individually designed interventions possible also for these patients. However, this kind of studies seem unlikely due to the current focus of commercial interests on biologics.

The relationship of atopie blepharoconjunctivitis and AD is clear but little is know about the shared genetic or acquired risk factors behind these two. Many epidermal
differentiation complex proteins, such as S100A proteins, filaggrin, and hornerin, are normally expressed on the ocular surface. Based on a small preliminary study, filaggrin expression appears to be reduced in the cornea of patients with atopic keratoconjunctivitis, but only secondary to inflammation [300,301]. It is currently unknown if primary genetic defects are important in this atopic eye disease and it would be interesting to study the links between this atopic comorbidity and AD. Maybe the conjunctivitis observed in some dupilumab users [249] will eventually provide us the missing links between the atopic skin and the atopic eye?

Xerotic dermatitis of the senior population is often classified as "asteatotic eczema" (eczema craquelé), but this entity characterized by itchy skin dryness is poorly defined and even more poorly studied. Inflammation is often present, aggravating factors are mostly the same, and even the changes in the stratum corneum lipids resemble those seen in AD [302]. Many of these patients can, in fact, represent elderly patients with AD. The growing number of the elderly in Western societies will hopefully draw more research attention on the inflammatory conditions of the aged skin.

Considering the increase in the prevalence of AD, the significance of acquired factors in the pathogenesis of AD is undisputable. Future studies are needed on these determinants and the relationship between acquired and genetic factors and their significance in the atopic march. Epigenetic mechanisms mediate many of the observed effects of acquired factors, such as smoking. Research on epigenetics is still in its infancy and will undoubtedly provide us exciting findings in the years to come.

A growing body of research, including our results, shows that AD is more persisting than previously thought, and that adult-onset form is a true entity. For that reason AD can often have a negative impact on the lives of patients and their family members throughout the life, affecting both social and occupational life. Financial burden consists of direct costs, such as medical expenses, and indirect costs like decreased productivity. In the United States, the costs of AD were estimated to be 5.3 billion dollars in 2015 [303], which would indicate roughly a total annual cost of 50 million euros in Finland.

The number of adult and elderly patients is larger than expected and possibly still growing. The spectrum of AD phenotypes is wide and the burden of AD is not limited to childhood.
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