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CHILDHOOD ATOPIC DERMATITIS

ALLERGIC SENSITISATION, LONG-TERM TREATMENT, AND GENETICS

Miia Perälä

DOCTORAL DISSERTATION

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Errata

Page 3: Missing one parenthesis.

Page 14: “To collect” two times

Page 16: “Kolmannella”, correction: “Kolmanneksella”

Page 17: “Suptstusherkkyyttä”, correction: “Supistumisherkkyyttä”

Pages 18-20, 22: Extra “%” after some sentences

Page 45: Figure 4 “Children with AD n=125”, correction: “n=152”

Page 60: “Baseline” two times

Page 61: Table 6 “p-value” two times

Page 65: “We”, correction: “were”

Page 69: “Whit”, correction: “with”

“It is only with the heart that one can see rightly,
what is essential is invisible to the eye.”

-Antoine de Saint-Exupéry, The Little Prince

To Matti, Juho, Matilda, and my parents

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LIST OF ORIGINAL PUBLICATIONS

This doctoral thesis is based on the following publications:

I. Young children with moderate-to-severe atopic dermatitis can be treated safely and effectively with either topical tacrolimus or mild corticosteroids. **Perälä M**, Ahola M, Mikkola T, Pelkonen AS, Remitz A, Mäkelä MJ. *Acta Paediatrica* 2020; 109(3):550-556.

II. Safety of tacrolimus 0.03% and 0.1% ointments in young children with atopic dermatitis: a 36-month follow-up study. Salava A, **Perälä M**, Pelkonen A, Mäkelä M, Remitz A. *Clinical and Experimental Dermatology*. 2022; 47(5):889-902.

III. Topical tacrolimus and corticosteroids in childhood moderate-to-severe atopic dermatitis with impact on airways: A long-term randomized open-label study. **Perälä M**, Salava A, Malmberg P, Pelkonen AS, Mäkelä MJ, Remitz A. Clinical *and Experimental Dermatology*. 2023; 48(6):660-666.

IV. Relevance of coding variation in *Filaggrin* and *DOCK8* in Finnish pediatric patients with early-onset moderate-to severe atopic dermatitis. **Perälä M**, Kaustio M, Salava A, Jakkula E, Pelkonen AS, Saarela J, Remitz A, Mäkelä MJ. *JID Innovations*. 2023; 3(4):100203

The publications are referred to in the text by their roman numerals.

ABBREVIATIONS

AD	Atopic dermatitis
AMP	Antimicrobial peptide
AR	Allergic rhinitis
BSA	Body surface area
BHR	Bronchial hyperresponsiviness
CI	Confidence interval
CE	Cornified envelope
CLA	Cutaneous lymphocyte antigen
CLDN1	Claudin 1
CNV	Copy number variation
CRTH2	Chemoattractant receptor-homologous molecule
DC	Dendritic cell
DLQI	Dermatology Life Quality Index
DOCK8	Dedicator of cytokinesis protein 8
EASI	Eczema Area and Severity Index
ECD	Epidermal differentiation complex
EIB	Exercise-induced bronchoconstriction
FA	Food allergy
FcεRI	High-affinity receptor for IgE type I
FeNO	Fraction exhaled nitric oxide
FFA	Free fatty acid
FLG	Filaggrin
FLG2	Filaggrin 2
GWAS	Genome-wide association study
HRNR	Hornerin
HSV	*Herpes simplex virus*
H2000	Health 2000 (Terveys 2000) Survey
IDEC	Inflammatory denditic epidemal cell
IDQoL	Infant´s daily quality of life
IFN	Interferon
IGA	Investigator´s Global Assessment
IgE	Immunoglobulin E
IL	Interleukin
ILC2	Group 2 innate lymphoid cell
IQ	Interquartile
IQR	Interquartile range
ITT	Intention to treat
JAK	Janus kinase
K	Keratin

LC	Langerhans cell
LCEP	Late cornified envelope protein
LOF	Loss-of-function
LOR	Loricrin
NMF	Natural moisturising factor
NO	Nitric oxide
OR	Odds ratio
QoL	Quality of life
PAMP	Pathogen-associated molecular pattern
PAR2	Plasminogen-activator type 2 receptor
PCA	Pyrrolidone-5-carboxylic acid
PD40Rrs5	Dose of methacholine causing an increase of 40% in Rrs5
POEM	Patient Oriented Eczema Measurement
PPB	Parts per billion
Rrs5	Resistance of the respiratory system
S.aureus	*Staphylococcus aureus*
SC	Stratum corneum
SG	Stratum granulosum
SCORAD	SCORing atopic dermatitis
SD	Standar deviation
SFTP	S100 fused-type protein
SNP	Single nucleotide polymorphism
SP	Serine protease
SPINK5	Serine Peptidase Inhibitor Kazal Type 5
SPRR	Small proline-rich protein
SPT	Skin prick test
SRI	Skin-related infection
STAT	Signal transducer and activator of transcription
S100	Calcium-binding protein
TAC	Tacrolimus
TCI	Topical calcineurin inhibitor
TCS	Topical corticosteroid
TEWL	Transepidermal water loss
Th	T helper
TJ	Tight junction
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cell
TSLP	Thymic stromal lymphopoietin
UCA	Urocanic acid
UV	Ultraviolet
ΔRrs5	Change in the resistance

ABSTRACT

Atopic dermatitis (AD) is one of the most common chronic skin diseases. The highest incidence of AD is in infancy and early childhood. While many experience remission, later relapses are possible. Some may continue on a more severe pattern. Early-onset, severe AD is associated with the highest risk of atopic sensitisation, particularly food sensitisation. Later allergic rhinitis and asthma are also common. It is unclear whether, or how, the possible atopic multimorbidity risk-pattern, the sequence of atopic conditions, can be predicted, and which of the conditions are, in fact, linked.

The aim of this thesis was to study whether secondary prevention, effective eczema treatment, could have an inhibitory effect on possible AD progression. Specific aims were 1) to collect to collect evidence and safety data for topical tacrolimus in small children and compare its usage with topical corticosteroid in moderate-to-severe AD, 2) to determine whether eczema treatment affects airway inflammation or bronchial hyperresponsiveness, and 3) to investigate the effect of coding variation in *FLG* and 13 other genes with epidermal barrier or immune function in children with moderate-to-severe AD.

A prospective, open-label comparative long-term study was conducted at outpatient clinic of Skin and Allergy Hospital in Helsinki, Finland during 2013-2019. In total, 152 children (varied from 75 to 152 between studies), aged from one—to three-year-old with moderate-to-severe AD, participated in the trial. Eczema treatment consisted of either topical mild-to-moderate corticosteroids (TCS) or 0.03 and 0.1% tacrolimus (TAC). Patients were followed up with study visits until 36 months. Data were collected on eczema efficacy parameters, infections, growth parameters, vaccination responses, and other relevant laboratory tests and outcomes. Atopic sensitisation was determined, and respiratory function tests performed to assess airway inflammation and bronchial hyperresponsiveness. Blood samples for genotyping were gathered.

Changes in eczema efficacy parameters were similar in TCS and TAC groups during the study. More potent ointment regimen was required by nearly 70% (52/75) of the patients. No signs of skin atrophy were documented, and none suffered from any serious adverse event like serious infections or malignancies. More than half of the patients were sensitised to food thorough the study. Aeroallergen sensitisation increased from one third to nearly half of the patients at study-end. Allergic rhinitis was documented in around every other patient. One third of the patients who completed the study (122) had exercise-induced bronchoconstriction (EIB) at the end, often combined with allergic rhinitis (67%) and parental atopy (88%). There were no differences between TSC and TAC treatments according to allergic sensitisation, allergic rhinitis, or airway inflammation and bronchial hyperresponsiveness.

In the genotype analysis, *FLG* loss-of-function (LoF) variant was found in 20 of 140 patients with significantly higher variant frequency than in controls (7.14 % vs. 2.34%) and the previous Finnish adult AD cohort (5.6%). Carrier status was associated with higher transepidermal water loss values.

In conclusion, this study found both eczema treatments, TCS and TAC, to be effective and well tolerable without differences in efficacy in treatment of childhood moderate-to-severe AD. The use of both 0.03 and 0.1% concentrations of TAC from one year's age onwards was demonstrated safe as was the use of both mild and midpotent TCS in long-term treatment of the same age group. There were no serious adverse events in either group. Atopic sensitisation was common in both groups and one third of the patients had exercise-induced bronchial hyperresponsiviness at the end of follow-up. In general, there were no differences in sensitisation patterns or airway outcomes between treatments. *FLG* LoF- variant was found in 14.3% of the patients which could contribute to epidermal barrier impairment due to lack of moisturizing effect of filaggrin protein.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Atooppinen ihottuma (AD) on yksi yleisimmistä kroonisista ihosairauksista. Ihottuma alkaa usein jo varhaislapsuudessa. Vaikka ihottuma rauhoittuisikin sen jälkeen, saattaa se ilmaantua myöhemmin uudelleen. Toisilla ihottuma ilmenee alusta alkaen hankalampana. Tällaisella ihottumalla on todettu suurin yhteys atooppiseen eli allergiseen herkistymiseen. Erityisesti riski herkistyä eri ruoka-aineille on kohonnut. Myös allergista nuhaa ja astmaa saattaa ilmetä. Täsmällistä tietoa näiden eri atooppisten sairauksien samanaikaisuudesta, ilmaantuvuusajankohdasta tai -järjestyksestä ei ole. Tarkkaa tietoa ei ole siitäkään voidaanko muiden atooppisten sairauksien ilmaantuvuuteen vaikuttaa.

Tämän työn tavoitteena oli tutkia, voisiko ihottuman tehokkaalla hoidolla olla estävää vaikutusta mahdolliseen muuhun atooppiseen sairastavuuteen. Tavoitteena oli erityisesti 1) kerätä tietoa takrolimuusivoiteen (TAC) käytön turvallisuudesta pienillä lapsilla ja verrata sen käyttöä kortikosteroidivoiteisiin (TCS) keskivaikeassa tai vaikeassa AD:ssa, 2) selvittää, vaikuttaako ihottuman hoito hengitysteiden tulehdukseen tai keuhkoputkien supistumisherkkyyteen ja 3) tutkia ihon läpäisyesteeseen tai tulehdusvasteeseen vaikuttavien geenien, kuten filaggriinin ilmaantumista lapsilla, joilla on keskivaikea tai vaikea AD.

Tutkimus suoritettiin vuosina 2013-2019 Iho- ja allergiasairaalassa Helsingissä. Rekrytoimme kaikkiaan 152 lasta (vaihdellen 75:stä 152:een tutkimusten välillä), jotka olivat iältään 1–3-vuotiaita, ja joilla oli keskivaikea tai vaikea AD. Ihottuman hoito koostui joko paikallisista miedoista tai keskivahvoista kortikosteroideista (TCS) tai 0,03- tai 0,1 %- vahvuisista takrolimuusivoiteista (TAC). Potilaita seurattiin vastaanottokäynnein 3 vuoden ajan. Tietoja kerättiin ihottumahoidon tehokkuudesta, infektioista, kasvusta, rokotusvasteista ja muista keskeisistä laboratoriotesteistä ja tuloksista. Atooppinen herkistyminen määritettiin. Seurannan lopuksi suoritettiin hengitysteiden toimintakokeet hengitysteiden tulehduksen sekä keuhkojen supistumisherkkyyden määrittämiseksi. Geenimäärityksiä varten otettiin verikoe.

Ihottumahoidon tehossa ei ollut eroa TCS- ja TAC-ryhmien välillä tutkimuksen aikana. Lähes 70 % (52/75) potilaista tarvitsi tehokkaampaa voidetta. Viitteitä ihon ohentumisesta ei esiintynyt hoitojen aikana. Vakavia haittatapahtumia kuten infektioita tai pahanlaatuisia kasvaimia ei esiintynyt. Yli puolet potilaista oli herkistynyt ruoka-aineelle. Herkistyminen ilmaväitteisille allergeeneille lisääntyi joka kolmannesta lähes puoleen potilaista tutkimuksen lopussa. Allergista nuhaa esiintyi noin joka toisella. Kolmannella tutkimuksessa loppuun asti mukana olleista potilaista (122) ilmeni rasituksen aiheuttama keuhkojen supistumistaipumus (EIB), johon usein liittyi allerginen nuha (67 %) ja vanhemmilla esiintyvä atopia (88 %).

TSC- ja TAC-hoitojen välillä ei ollut eroja allergisen herkistymisen, allergisen nuhan tai hengitysteiden tulehduksen tai keuhkoputkien yliherkkyyden suhteen. Genotyyppianalyysissä *FLG*:n ns. nollavariantti (LoF) esiintyi 20/140 potilaalla. Esiintyvyys oli merkitsevästi korkeampi kuin verrokkeilla (7,14 % vs. 2,34 %) ja verrattuna vastaaviin suomalaisiin aikuispotilaisiin (5,6 %). Kantajuus liittyi ihon lisääntyneeseen veden haihtumiseen.

Yhteenvetona voidaan todeta, että molemmat ihottumahoidot, TCS ja TAC, olivat yhtä tehokkaita ja hyvin siedettyjä lapsuuden keskivaikean ja vaikean AD:n hoidossa. Sekä 0,03- että 0,1 %-vahvuisen TAC:n käyttö oli turvallista yhden vuoden iästä alkaen. Myös miedon ja keskivahvan TCS:n käyttö oli turvallista saman ikäryhmän pitkäaikaishoidossa. Kummassakaan ryhmässä ei esiintynyt vakavia haittatapahtumia. Atooppinen herkistyminen oli yleistä molemmissa ryhmissä, ja kolmanneksella potilaista esiintyi seurannan lopuksi rasituksen aiheuttamaa keuhkoputkien suptstusherkkyyttä. Yleisesti ottaen hoitojen välillä ei ollut eroja allergisessa herkistymisessä tai hengitysteiden toimintaa koskevissa tuloksissa. *FLG* LoF-variantti löytyi 14,3 %:lla potilaista, millä saattaa olla vaikusta ihon läpäisyesteen toimintaan kosteuttavan filaggriiniproteiinin puuttuessa.

REVIEW OF THE LITERATURE

1 ATOPIC DERMATITIS

Atopic dermatitis (AD, also known as atopic eczema) is a chronic inflammatory skin disease, and the most common form of eczema. It was named in the 1930s after Greek word *atopos*, "without place".[1] AD is characterized by intense itching and eczematous lesions that can be acute, subacute, or chronic. The lifetime prevalence among children and adults varies around 10-20% with an increasing incidence in many developing countries.[2,3] %).

The highest incidence of AD is in infancy and early childhood. The onset during the first year of life (early-onset AD) occurs in approximately 60% of individuals and up to 70% experience remission of the eczema in adolescence although later relapses are possible. Early-onset AD is associated with the highest risk of atopic sensitisation, in particular food sensitisation, as well as later allergic rhinitis, and asthma.[4,5]

1.1 CLINICAL PICTURE OF CHILDHOOD ATOPIC DERMATITIS

Eczema lesions typically show an age-dependent distribution. Infantile eczema presents with erythema, oedema, excoriations, and exudate with wide distribution. AD usually affects face and scalp, then spreads to extensor surfaces of the limbs and trunk in a symmetrical distribution. From 2 years onwards, eczema tends to affect flexor surfaces and becomes more localised and chronic. Lichenification may occur as a result of prolonged rubbing. Nummular form of AD presents with long-lasting circular lesions most often located on the trunk and distal extensor limbs (Figure 1).[6]

Specific frequently involved sites in childhood AD are lips with cheilitis, the retroauricular region (often affected by fissures), and infraorbital folds (Dennie – Morgan lines), often combined with blepharitis. Keratosis pilaris may exist in arms, legs, and buttocks, sometimes even in cheeks. Palmar hyperlinearity might be evident. Pityriasis alba and follicular eczema are more common in children, the latter especially in skin of color. Atopic winter feet-symptom is usually seen in school-aged children.[7]

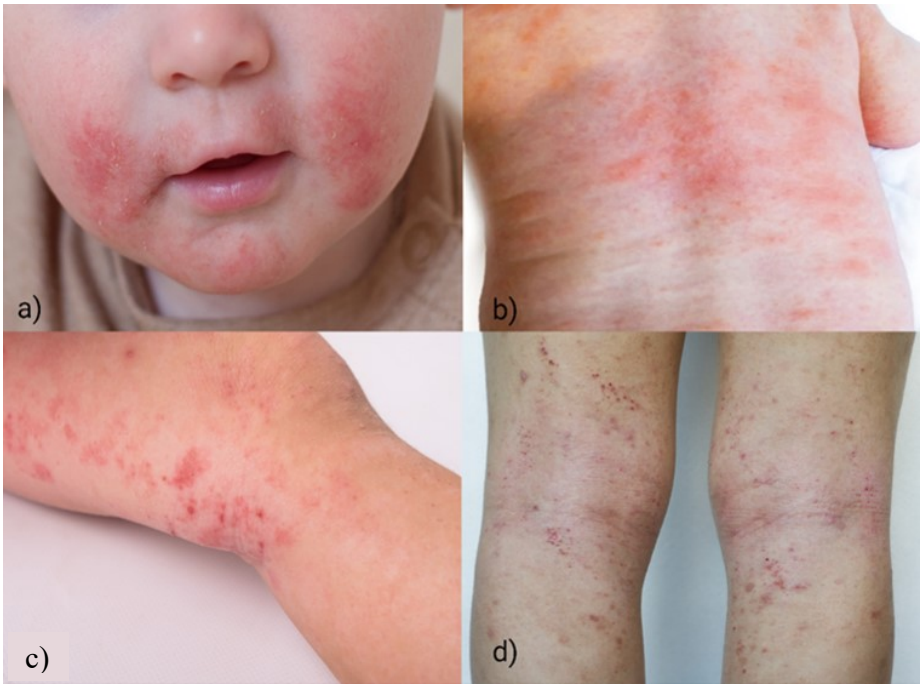

Figure 1 Clinical manifestations of childhood atopic dermatis in infants (a-b), and from 2 years onwards (c-d). Photo: iStock by Getty Images.

1.1.1 Laboratory tests in atopic dermatitis

Although the diagnosis of AD is based on clinical features and no specific laboratory or histological findings determine the diagnosis, high total or allergen-specific serum IgE concentrations and eosinophil counts are noted in many patients. %).

According to WAO, eczema is divided into two subtypes: atopic and non-atopic that differ in the level of total immunoglobulin (Ig) E and signs of sensitisation to common allergens. Contrary to atopic eczema, non-atopic eczema is characterized by a low level of total IgE, negative skin prick tests, and undetectable specific IgE antibodies. Previously, IgE-related AD was defined as extrinsic, and non-IgE related as intrinsic form of AD.[8] However, unlike specific IgEs, serum total IgE levels may normalize after suitable treatment.

1.2 DIAGNOSTICS AND SEVERITY MEASUREMENTS

The diagnosis of AD is based on morphology and distribution of the skin lesions accompanied by the presence of associated clinical signs, and a characteristic medical history. Many sets of diagnostic criteria exist.[9] In 1980

Hanifin and Rajka published diagnostic criteria for AD that are still in use in clinical studies. They are based on the presence of three of the following four major criteria: 1) pruritus, 2) typical morphology and distribution (flexural lichenification or linearity in adults/facial or extensor involvement in infants and children), 3) chronic/chronically relapsing dermatitis, and 4) personal or family history of atopy such as asthma, allergic rhinitis, or atopic dermatitis. In addition, at least three minor signs of 23 other signs are required for the diagnosis.[10] %).

In epidemiological studies, the more simplified criteria of the UK Working Party by Williams et al[11-13] are often applied. According to these criteria, AD diagnosis requires an itchy skin disease along with three or more of the following: 1) history of involvement of the flexural regions, 2) history of asthma or hay fever, 3) history of generally dry skin, 4) onset at less than 2 years of age, or 5) visible flexural dermatitis.

1.2.1 Differential diagnoses

Dry skin and eczema-like symptoms may be associated with other conditions as well. Common differential diagnoses of childhood AD include other eczema-like conditions like seborrheic eczema, nummular and infectious dermatitis, contact dermatitis, dyshidrosis, psoriasis, and even scabies and insect bites. %).

Dermatitis herpetiformis, ichthyosis vulgaris, acrodermatitis enteropathica (zinc deficiency), Hyper-IgE syndrome, Netherton syndrome, Wiskott-Aldrich syndrome, and Omenn syndrome are considered as rare differential diagnoses.[3] It is important to exclude immunodeficiency in case of severe eczematous rash, failure to thrive, and recurrent infections, or petechiae.

1.2.2 Severity measurement tools

The clinical presentation of AD varies from mild to severe eczema. To define severity of AD, different tools have been developed for both doctors and patients. Most widely used tools for clinicians are EASI, SCORAD, The Rajka & Langeland criteria, IGA, as well as DLQI, and for infants IDQoL.[14-19] %).

The EASI assessment integrates body surface and the intensity of lesional skin into one composite score with a total range of 0–72.[14] SCORAD also assesses xerosis, pruritus and sleeplessness.[15] Rajka & Langeland criteria grade AD to either mild, moderate, or severe based on scores for eczema extent, course, and intensity (nocturnal sleeplessness due to itch).[16,17] IGA represents a measure of disease severity assessed by clinician at a single timepoint. The validated 4-point (from 0 “clear” to 4 “severe”) IGA for AD (vIGA-AD™) is further developed to assess AD in clinical trials.[18] (18). Body

surface area (BSA) is an assessment of the percentage of BSA involved with AD. Dermatology Life Quality Index (DLQI) and the infants' IDLQoL are questionnaires assessing health-related quality of life over the last week in patients and families with dermatological diseases.[19,20] Patient-Oriented Eczema Measure (POEM) is a patient-reported questionnaire assessing AD-specific symptoms over the last week.[21]

1.3 PATHOGENESIS

The etiology of AD is based on a complex interplay between genetic, immunological, and environmental factors resulting into impaired immunity and skin barrier.[22] The "outside-inside" hypothesis emphasizes a primary barrier defect as a generator of AD with accompanying antigen penetration and immunological cascades, whereas the "inside-outside" theory is based on a secondary barrier impairment due to inflammatory responses.[23] Also, a combined model has been suggested as the "outside-inside-outside" one, where above-mentioned theories exist simultaneously.[24] Nevertheless, skin barrier dysfunction and inappropriate immune mechanisms are both characteristic for AD and interact closely with each other. Other known contributors to pathogenesis are filaggrin deficiency, abnormal amounts of antimicrobial peptides, lipids and lipid synthesis proteases and their inhibitors, as well as defects in tight junctions (TJ).

1.3.1 Skin-barrier structure and function

Skin acts as an effective barrier between the internal and external environments providing both support and protection. It consists of three layers: epidermis, dermis, and subcutaneous fat layer. The deepest layer of the epidermis is called basal layer, where also melanin- producing melanocytes, and antigen-presenting Langerhans cells (LCs) are scattered. New keratinocytes slowly migrate from basal layer up towards the surface of the epidermis through stratum spinosum, stratum granulosum (SG), stratum lucidum, and finally to stratum corneum (SC) (Figure 2).

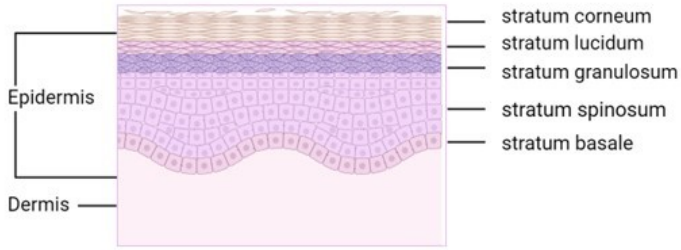

Figure 2 Epidermal layers of the skin. Created with BioRender.com

In healthy epidermis, there is a balance between the keratinocyte proliferation and desquamation processes. Both inherited and acquired modifications in lipid composition or epidermal differentiation, such as reduced expression of ceramides or Filaggrin gene (*FLG*), result in altered barrier function as seen in AD. An altered homeostasis of the SC is seen both in lesional and non-lesional AD skin leading to increased TEWL and penetration of allergens.[25]

1.3.1.1 *Main barrier*

The main barrier exists in the uppermost SC. Important functions of this epidermal barrier are to provide an outside-inside barrier to protect against mechanical, chemical, and microbial injury, as well as to control diffusion of molecules across the skin from inside to outside, measured as transepidermal water loss (TEWL). Unlike in clinical practice, measurements of TEWL are often used in eczema studies where increased values are indicative for skin damage such as eczema.[26] %).

While moving from the basement membrane towards the SC, keratinocytes differentiate into corneocytes with simultaneous accumulation of lipids. SC is thereby formed of keratin-filled corneocytes and intercellular lipid matrix. Keratins are structural proteins that are compound together into a weblike pattern. Corneocytes are surrounded by densely linked proteins forming a cornified cell envelope. Corneodesmosomes form bridges between adjacent cells. This kind of structure is often referred to as the 'brick and mortar'-structure of the skin, with corneocytes representing the bricks, and the lipids representing the mortar. Corneodesmosomes get eventually degraded by enzymatic activity leading to desquamation of the outermost denucleated corneocytes.[25]

1.3.1.2 *Second barrier*

While the first-line barrier is found in SC, a second-line barrier is thought to exist in the lower SG, formed by TJs. These include molecules such as occludin, claudin and zonal occluding proteins that are suggested to contribute to the paracellular diffusion barrier by mediating cell adhesion.[27] When TJs are impaired, LCs can reach out their dendrites through TJ barrier to sample surface antigens and allergens.[28] The observation that downregulation of filaggrin also influences TJ proteins indicates a mutual influence and interaction of SC and TJ barriers.[27] (See also chapter "Epidermal differentiation complex").

1.3.1.3 *Intercellular lipid matrix*

The intercellular lipid matrix is normally enriched with ceramides, cholesterol, and free fatty acids (FFAs). These lipids are synthesized in the keratinocytes, stored in organelles called lamellar bodies, and finally delivered into SC interstices together with antimicrobial peptides (AMPs) and enzymes like sphingomyelinase. Lamellar bodies and their contents influence thus both SC permeability and desquamation, as well as antimicrobial defense.[29] Lipids counteract the water and salt loss in addition to penetration of water-soluble substances. Important lipid abnormalities leading to SC impairment and enhanced molecular penetration are changes in free fatty acid and ceramide chain length, but also in sphingomyelin.[30,31] Both inherited and acquired factors may lead to impaired epidermal barrier structure and function. In either case, increase in pH, due to lack of NMF, activates serine proteases (SPs) and enhances barrier impairment while simultaneously decreasing ceramide-promoting enzymes sphingomyelinase and β-glucocerabrosidase.[32] Disturbed epidermal barrier leads to increased TEWL and manifests as dry skin enhancing penetration of irritants and allergens into the skin.

1.3.1.4 *Filaggrin*

Filaggrin is a structural protein that plays a central role in the development and maintenance of the skin barrier homeostasis. It is expressed in the upper layers of the SC and encoded within the epidermal differentiation complex (EDC).

Filaggrin arises from profilaggrin, which is encoded by *FLG* gene and contained in keratohyaline granules in the SG. Filaggrin aggregates keratin filaments into tight bundles and causes the before-mentioned collapse of the cells into flattened corneocytes, forming the SC barrier.[33] Profilaggrin is enzymatically cleaved into filaggrin monomers of either 10, 11, or 12 repeats as so-called copy number variation (CNV).[34]

Filaggrin contributes to SC hydration and water retention with its breakdown products, forming a major part of the natural moisturizing factor (NMF).[34] NMF has important function in regulation of the activity of enzymes crucial for maintaining the epidermal barrier homeostasis. Furthermore, components of the NMFs contribute to the elasticity of the skin through interaction with keratin and keratin filaments.[33] One of the filaggrin breakdown products is histidine and its further product transurocanic acid (*trans*-UCA). Upon UV exposure, it is converted into *cis*-UCA with immunosuppressive properties. Another amino acid derivate, glutamine, is converted into pyrrolidone-5-carboxylic acid (PCA), a potent humectant of the SC together with urea and lactic acid. They maintain an acidic pH and counteract invasion of microbes.[33-35]

1.3.1.5 *Epidermal differentiation complex*

Epidermal Differentiation Complex (EDC), located on chromosome 1q21, comprises along with *FLG* also many other genes encoding structural and regulatory proteins. These proteins are crucial for keratinocyte differentiation and SC properties, and include three protein clusters: 1) Cornified envelope (CE) precursor proteins involucrin, loricrin, the small proline-rich proteins (SPRRs), and the late cornified envelope proteins (LCEPs); 2) calcium-binding proteins (S100); and 3) intermediate filament-associated, the so-called S100 fused-type proteins (SFTPs) like filaggrin, filaggrin-2, trichohyalin, trichohyalin-like protein, hornerin, repetin, and cornulin.[36] Filaggrin-2 (encoded by *FLG2*) is also expressed by upper granular cells and deposited in the SG and SC in the epidermis similarly to filaggrin. It is involved in the cornification process and may have some synergistic roles with filaggrin in the formation of the epidermal barrier.[37,38] However, the timing of filaggrin-2 induction in the epidermis may differ from that of filaggrin.[37] In African American children, *FLG2* variants rs12568784 and rs16833974 were associated with more persistent AD.[39] Decreased levels of *FLG2, FLG*, and hornerin-encoding *HRNR* have been detected in adults with AD.[40]

1.3.2 Genes involved in atopic dermatitis pathogenesis

In general, genes of the known contribution to AD pathogenesis can be divided into different groups (Table 1)[41-45]

Table 1 Presentation of genes with a known relevant association with AD.

Group	Gene examples	Effect
Epidermal barrier genes	*FLG, SPINK5, DSC1, SERPINB7*	Variants leading to epidermal barrier dysfunction.
Genes of the innate and adaptive immune systems	*TLRs* *IL-4, IL-5, IL-13, IL2RA, IL-13RA, IL5RA, TSLPR,* *IL-4R, IL-18, IL-31, IL17A, TNFα, IL-22, STAT6*	Impairment of innate or adaptive immune responses
Genes encoding keratinocyte-derived alarmins	*IL-25, TSLP, IL-33*	Enhanced production of alarmins due to stress e.g., UV radiation or mechanical trauma

Polymorphisms of Th2-related genes *IL-4, IL-13* and *IL-31* and their receptors (*IL-4Rα, IL13Rα1*), for instance, have been implicated in the AD pathogenesis in diverse ethnicities.[44] A recent genome-wide meta-analysis of AD including Finnish patients found new missense variants in desmocollin 1 (*DSC1*) and serpin family B member 7 (*SERPINB7*) that likely disrupt the proper folding of epidermal proteins.[43]

1.3.2.1 *Filaggrin loss-of-function variants*

In 2006, *FLG* loss-of-function (LoF) or "null" variants were showed to associate with AD.[46] In general, LoF variants in *FLG* are present in up to 10% in the Northern European population and are also the cause of ichthyosis vulgaris. The most common variants in Europe are R501X, 2282del4, R2447X, and S3247X.[47]

However, reduced filaggrin levels are also observed in AD patients without these variants both in lesional and non-lesional skin, indicating other factors contributing to low filaggrin levels, such as skin dysbiosis, inflammation, and metabolic changes.[35,48] One possible additional factor to reduced filaggrin levels could be abnormal processing of precursor profilaggrin.[40] Regardless of cause, filaggrin deficiency promotes abnormalities in corneocyte and lamellar body function, as well as changes in lipid matrix and corneodesmosome structure.[33]

Interestingly, unlike with Asian and European AD patients, most African American patients have normal filaggrin levels.[49] At birth, filaggrin synthesis is low, increasing slowly thereafter - reflecting maturation of the epidermis. Despite ethnic background, normal filaggrin levels are often found in childhood AD.[50] However, a strong disposition to AD exists when mother is carrying a *FLG* LoF variant.[51] A prospective study in Caucasian AD children suggested that paediatric AD with *FLG* LoF variants represents a specific endotype with early- onset dermatitis and a more severe disease course with increased asthma risk.[52]

1.3.2.2 *Genetic variance of Epidermal differentiation complex*

There´s a dysregulation of many EDC genes both in lesional and non-lesional AD skin. Hornerin is part of the CE. Downregulation of hornerin contributes to lack of mechanical resistance in SC, and therefore to epidermal barrier defects associated with AD.[53] When *FLG2* was downregulated in cultured keratinocytes, also loricrin and involucrin were shown to be decreased both in lesional and non-lesional AD skin.[38]. In turn, deficient loricin led to reduced expression of occluding in the skin of flaky tail mice.[54] Occludin levels have shown to be decreased also in filaggrin–deficient human epidermis.[55]

Downregulation or loss of claudin-1 in knock-out mice has shown to result in alteration of both SC proteins and barrier function, as well as in increased uptake of allergens. The skin of these mice was hyperkeratotic and wrinkled.[56] Claudin-1 deficiency has also been found to exist even in non-lesional AD skin indicating an important role in TJ function and keratinocyte proliferation.[27] Variants in claudin-1, encoded by *CLDN1*, have been associated with early-onset AD (under 5 years of age) in an Ethiopian cohort while keratins K6/K16 have shown to be upregulated in AD.[57,58]

1.3.2.3 *Other known involved genes*

The fact that filaggrin expression is reduced in AD patients not only, but also, without *FLG* variants indicates that the expression of filaggrin and filaggrin-like proteins in AD skin is modulated by inflammatory responses.[35,40,48] IL-4 and IL-13 are known to increase SP kallikrein 7-expression and activity, a major contributor to corneodesmosome degradation.[59,60] Overexpression of kallikreins reduces the stratum corneum cohesion resulting in epidermal barrier deficiency.[60] Variations of single nucleotide polymorphism (SNP) in the SP inhibitor gene *SPINK5* (leading to inhibition of kallikrein 5 and 7), and the SP *KLK7* (encoding kallikrein 7) have been associated with AD.[22] Severe atopic symptoms are seen in Netherton syndrome where *SPINK5* variant is found in both alleles of the gene.[61]

Moreover, in human keratinocytes, IL-4, IL-13, IL-25, and thymic stromal lymphopoietin (TSLP), as well as IL-22, IL-17A, and IL-31 reduced the EDC molecules filaggrin, hornerin, involucrin, and loricrin.[40,48,62,63] IL-4 and IL-13 have been shown to downregulate the expression of EDC molecules via STAT6 and STAT3 activation.[62,63] In AD, the amounts of IL-4 and -13 are elevated. A SNP in IL-13 and a haplotype consisting of SNPs in IL-13 and IL-4 were strongly associated with the development of AD at 2 years of age.[64]

1.3.3 Immunology and inflammation

The immune defence of the skin includes immediate, nonspecific mechanisms (innate immunity), and delayed, stimulus-specific responses (adaptive immunity), both contributing to protect from invading microorganisms. Primary inflammation in AD results from both inherited and acquired barrier insults.

1.3.3.1 *Initial inflammation*

Raised skin pH, abnormal SP activity, and reduced skin microbiome diversity are hallmarks for AD skin. In AD-prone skin, AMPs like cathelicidin (LL-37), and human β-defensins (hBDs) are downregulated.[65] AMPs are important in

facilitating an innate chemical defence against microbes. When the antimicrobial barrier is compromised, patients are susceptible to skin infections due to e.g., *Staphylococcus aureus (S. aureus)*, *Herpes simplex virus (*HSV*)*, or *Malassezia* yeasts.[66]

It is thought that increased SP activity enhances primary cytokines, IL-1α and IL-1β (upon PAR2 signalling), from corneocytes to generate the first step of the inflammatory cascade in AD.[65] IL-1 cytokines are produced by cells belonging to the innate immune system, including macrophages and monocytes. A secondary step by Th2-associated proinflammatory cytokines then follows. Kallikreins bind to PAR2, which downregulates lamellar body secretion and initiates the primary inflammatory cascade.[29]

Due to exposure to either mechanical injury, microbes, or allergens (eg, house dust mite), TSLP is then activated from keratinocytes together with other AD-alarmins, IL25 and IL33, which, in turn, activate the switching towards Th2 inflammatory responses.[45,67] These alarmins activate dendritic cells (DCs) and LCs to induce the differentiation of naive CD4(+) T cells into Th2 cells with production of cytokines like IL-4, -5, and -13, IL-31, and IL-33, and downregulation of IL-10 and IFNγ.[22] Proinflammatory alarmins IL-25, -33, and TSLP also activate local group 2 innate lymphoid cells (ILC2s). ILC2s are upregulated in lesional AD skin and participate in type 2 immunity by increasing cytokines such as IL-5 and -13.[22,68]

Acute AD lesions are characterized by T-cell infiltrates with increased Th2 (IL-4, IL-5, IL-13, IL-31, CCL18), and Th22 (IL-22, S100A proteins) cytokines.[69] These proinflammatory responses downregulate terminal differentiation genes and TJ products, such as claudins, and contribute to barrier defect in AD.[69,70] The Janus kinase (JAK) pathways are activated resulting in inflammation and itch.

1.3.3.2 *Sustained inflammation*

With time, recruitment of Th1, Th22, and Th17 cell subsets with increased production of cytokines like IL-2, IL-12, TNFα, and INF occurs, characteristic for chronic AD. IL-22 is a mediator of epidermal hyperplasia while IL-31 is associated with itch and disease severity.[71,72] IL-1 contributes to Th17 and Th2 cell development and to the chronification of AD lesions through an increase in Th1 shifting. An upregulation of keratins contributes to epidermal thickening and abnormal keratinocyte proliferation.[6,73]

Th17 cells are important contributors in innate immunity, particularly in neutrophil recruitment. Recently, Th17 cells have been implicated in allergic disorders.[74] IL-17, which is expressed mostly by the Th17 cells, regulates AMPs in keratinocytes. Downregulation of IL-17 may hence contribute to increased skin infections in AD patients.[75] Th1- and Th 17-mediated responses have been reported specifically in children and people of Asian descent.[73,74]

1.3.3.3 *Antigen exposure and Immunoglobulin E production*

Sustained antigen exposure trough impaired barrier in AD stimulates additional inflammatory responses, resembling a vicious circle. Interaction of the impaired barrier with activated immune responses also facilitates percutaneous allergic sensitization through promotion of IgE-class switching in B cells, and production of antigen-specific IgE, as well as eosinophilia.[6,67]

DCs express FcεRI, a high-affinity receptor for IgE. In AD, expression of FcεRI is increased resulting in enhanced binding of specific IgE and increased antigen presentation. This further leads to additional cytokine release and intensifies the inflammatory reaction.[76] However, not all patients have elevated IgE levels. For instance, in the absence of the FcεRI receptor, skin hyperplasia and cellular inflammation decreased along with lower expression of Th1, Th2, and Th17 cytokines.[72]

1.3.3.4 *Inflammatory markers and characteristics in atopic dermatitis*

Th2 cells express many markers for homing and skin recruitment. One of the skin-homing molecules is chemoattractant receptor-homologous molecule (CRTH2), a marker on Th2 cells.[77] CRTH2 is a receptor for prostaglandin D2 and T cells, and is also expressed on eosinophils, basophils, and keratinocytes.[78] Most circulating T cells are likely CD4+ CRTH2+. Their amount has shown to be increased in AD patients compared to healthy controls.[79] Another T-cell marker is cutaneous lymphocyte antigen (CLA), a molecule that helps to recruit T cells into the skin in case of foreign antigen penetration.[80] Antigen-primed T cells persist as effector memory cell reservoir in local skin pools and exert rapid recall responses when needed.[80]

LCs are epidermal DCs that can induce, not only immunological responses, but also responses enhancing tolerance.[81] Inflammatory DCs are also found in the epidermis contributing to inflammation in both acute and chronic AD.[76] In addition, regulatory T cells (Tregs) are able to regulate T cell activity by influencing effector T cells and antigen presenting cells. The circulating amount of Tregs is increased in AD patients.[82] Also, in a mouse model of AD, the Treg cell population with accompanying Th2-cytokine profile was increased, indicating that pathogenic Treg cells are aggravated to exacerbate AD symptoms.[83] T-cell responses are estimated to be directed towards self-antigens as IgE autoreactivity in up to a third of patients.[84]

DCs express also toll-like receptors (TLRs) that recognize foreign antigens. They function to promote innate immune response to pathogenic microorganisms. In particular TLR2 and TLR4 recognize microbial components or PAMPs (pathogen-associated molecular patterns). Recognition of PAMPs evokes a cascade that results in the release of proinflammatory cytokines, chemokines, antimicrobial peptides, and other molecules involved in innate and adaptive immune responses.[85] Increased

TLR2 activity has shown to strengthen TJs through activation of *CLDN1* and AMPs in keratinocytes.[86] On the contrary, an altered expression and function of TLR2 exists in AD skin.[22]

1.3.3.5 *Immunological characteristics of childhood atopic dermatitis*

Apart from clinical presentation, childhood and adulthood AD may differ from each other also in terms of genetics and immune dysregulation. Immune changes predisposing to AD development are present very early in life. As mentioned, young AD children have increased amounts of Th2 cells but not yet other T-cell subsets in peripheral blood, in contrast to adults.[87] Already before the actual onset of AD at 2 months of age, TSLP levels were shown to be increased in non-lesional infant skin.[88] Moreover, keratinocyte hyperactivation is evident even in infantile AD skin: hyperplasia and correlations between hyperplasia and Th2 cytokines might be due to increased IL-19 levels, thereby possibly crosslinking the Th2 and Th17 axes.[50] TJs and barrier-associated lipid genes may be downregulated in childhood AD.[89] Conversely, skin-homing central and effector memory T-cell counts have shown to be higher in children with AD compared to controls, decreasing thereafter with age.[75]

1.3.3.6 *Itch*

Itch is another hallmark for AD causing sleeplessness and impaired quality of life. TSLP, an important itch-mediator, stimulates IL-31 that further induces nerve fibers to release more pruritogens resulting in additional neurogenic inflammation.[90] Like IL-31, also IL-4, and -13, histamine, proteases, and neuropeptides are considered as pruritogens. The majority of these pruritogens bind to nonhistaminergic nerve fibers.[91] Pruritogens like TSLP are released not only by inflammation but also by scratching. This may lead to hypersensitisation of nerve fibers as itch–scratch cycle.[91] This neuro-immunological interaction is supported by the observation that itch relief is gained through inhibition of IL-4Rα by dupilumab or JAK inhibition.[92,93]

1.3.4 Environment

Rising global prevalence of AD indicates that along genetics, also environment plays a role in the disease pathogenesis. Environmental factors may trigger or protect from symptom development in hereditary predisposed individuals.

1.3.4.1 *Impaired barrier and pH*

The synthesis of lipids and the differentiation of keratinocytes are regulated by enzymes, pH and calcium gradient.[94] Acidic skin pH is important for functioning barrier. Normal skin surface pH ranges from 4 to 6 while in AD patients it is often increased. Soap, detergents, and exogenous proteases enhance protease activity to break down the skin barrier. In impaired barrier, the penetration of irritants and allergens increases, further triggering inflammation and protease activity in the skin.[23] Less acidic areas of skin, like cheeks in infants, flexural and intertriginous sites in adults, are more susceptible to AD.[95]

Alkaline detergents in skin care products may further unfavourably alter skin's pH. Increased pH reduces antimicrobial defence of the skin, causing patients with AD to be more susceptible to infections by *Staphylococcus* and other neutral-pH-favouring microbes. In most AD patients, the skin is colonized with *S. aureus*, as compared to about 5% of healthy subjects.[96] Patients with more severe disease have been shown to have higher levels of *S. aureus* in their home environments as well.[97] In over half of the patients, *S. aureus* strains produce superantigens such as staphylococcal enterotoxins A and B, and toxic shock syndrome toxin-1.[98] These toxins are immunomodulatory and able to penetrate and damage skin barrier. They induce superantigen-specific IgE production, release of inflammatory cytokines, and activation of T cells with simultaneous inhibition of Treg cells.[82,98-101] In addition, they are able to induce mast cell degranulation and contribute to pruritus.[102] Children colonized with toxigenic *S. aureus* strains have higher disease severity compared with the nontoxigenic and *S. aureus*-negative controls.[98]

Yeasts can be involved in chronic inflammation of AD. *Malassezia sympodialis* (*M. sympodialis*) favours to grow in higher pH and is often found in patients with head and neck distribution of AD.[103] IgE antibodies against *M. sympodialis* may exist, but even these patients are thought to have no additional benefit of topical antifungal therapy when combined with topical steroids.[104,105]

1.3.4.2 *Skin pruritogens and irritants*

Other contributing factors to AD pathogenesis and expression include skin irritants, climate, pollutants, tobacco smoke, water hardness, urban living, and diet. These factors are thought to contribute to AD by acting as pruritogens and irritants, upregulating inflammatory processes or worsening the skin barrier function. Jakasa et al[106] demonstrated that compared to healthy controls, there is higher permeability for sodium lauryl sulphate (SLS) even in uninvolved skin of AD patients. Exposure to this sulphate leads to skin irritation and removal of lipids from SC resulting in increased TEWL.[107] In

addition, products containing fragrances may act as pruritogens, even without any patch test positivity.[108]

1.3.4.3 *Epigenetic alterations*

Environmental maternal exposures during pregnancy may play a role in AD through epigenetic alterations, including microRNA and DNA methylation. Prenatal tobacco exposure is thought to increase risk for AD in the offspring of smoking mothers during pregnancy through methylation of *TSLP* gene.[109] Maternal stress during pregnancy might modulate the hypothalamic–pituitary axis and developing immune system in the fetus.[110] However, there can be many simultaneously affecting confounding factors such as lifestyle ones. Also, prenatal exposure to antibiotics might alter fetal skin and gut microbiome.

1.3.4.4 *Early-life environmental exposures*

The prevalence of AD seems to be higher in urban compared to rural areas.[111] Early-life exposure to dirt and pathogens may protect against developing AD and allergic disease, a theory known as the 'hygiene hypothesis'.[112,113] However, the protective effects may be transitory. Biodiversity, on the contrary, may adversely affect the human microbiota and its immunomodulatory capacity. Compared with healthy controls, atopic individuals had lower environmental biodiversity in their home surroundings. Moreover, they had lower diversity of gammaproteobacteria on their skin, which are able to express IL-10, known as a significant anti-inflammatory cytokine in immunologic tolerance.[114] In general, there´s a lower microbial diversity in AD skin, especially during flares.[115]

Urban living can be associated with increased stress and environmental pollutants.[111] There is a strong known association between paediatric asthma and environmental tobacco smoke.[116] Though air pollutants are known risk factors for asthma, their role in AD is not as well established. Patients with AD relating to airborne proteins usually have lesions on air-exposed skin areas. It is likely that multiple outdoor and indoor pollutants may trigger and exacerbate AD. Children with a genetic susceptibility to AD are more likely to have the disease when exposed to environmental risk factors.[117] Different combinations of genetic and exogenous factors can initiate a breakdown of epidermal function.

2 ATOPIC SENSITISATION AND COMORBIDITIES

2.1 ASSOCIATION OF CHILDHOOD ATOPIC DERMATITIS, FOOD ALLERGY, ALLERGIC RHINITIS AND ASTHMA

AD is associated with a higher prevalence of asthma, allergic rhinitis (AR), and food allergy (FA). Children with AD bare a greater risk for sensitisation and later respiratory symptoms, especially when polysensitised in early age.[118,119] Atopic sensitisation is defined by specific IgEs for aero- and/or food allergens in sera or by skin prick tests (SPTs).

2.1.1 Suggested pathomechanisms

The "allergic" or "atopic march" is a postulate progression of infantile AD with possible food allergies to later asthma and AR.[120] Based on this hypothesis it is thought that due to impaired epidermal skin barrier, epicutaneous sensitisation follows with subsequent induction of systemic Th2 immunity. This then predisposes to allergic nasal responses and promotes airway hyperreactivity.[121,122] Sequential exposures to allergens activate mast cells and basophils, leading to release of allergic mediators such as histamine, and manifestation of allergic symptoms.[121] (Figure 2)

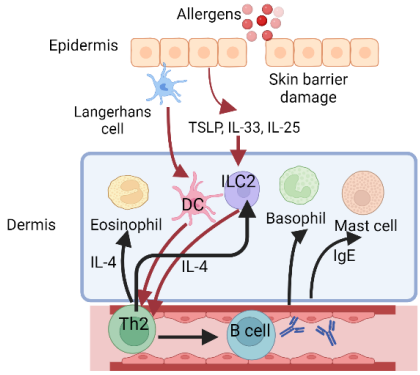

Figure 3 A model of atopic sensitisation from allergens entering the skin through barrier damage to activation and stimulation of immune cells with cytokine secretion. TSLP= thymic stromal lymphopoietin, IL=Interleukin, IgE= Immunoglobulin E, ILC2= Group 2 innate lymphoid cells, Th2= T cell type 2. Created with BioRender.com.

However, not all children with AD develop comorbidities, and some may even outgrow from eczema.[4] The thought of one straightforward atopic march-

pattern has nowadays developed to a more comprehensive theory, where atopic diseases more likely coexist.[123] The risk for the development of atopic comorbidities is complex and likely influenced by genetic and environmental factors. It is mostly determined by parental history of atopy, age of eczema onset, eczema severity, and the presence and burden of atopic sensitisation.[124] As inflammatory changes are present already in non-lesional infantile AD-prone skin, this early subclinical inflammation is thought to contribute to a comprehensive inflammatory burden documented as skin -derived circulating biomarkers.[125] Early existence of other atopic comorbidities increases the risk of future multimorbidity.

2.1.1.1 *Mathematic models*

It is suggested, that possessing a distinct phenotype increases the likelihood for other atopic comorbidities.[125-127] Machine learning models in two birth cohorts revealed eight latent profiles of symptom development between eczema, wheeze, and rhinitis from birth to school age.[126] Each profile had different temporal patterns of comanifestations and distinct genetic associations.[126-128] In four U.K. population–based birth cohorts, most children with eczema (75.4%) did not progress to any multimorbidity pattern. Mathematic modelling revealed five latent states where only one-fifth of those with eczema transitioned to multimorbidity. Carrying a *FLG* variant or rs7216389 increased the risk.[129] *FLG* LoF variants have previously been associated with early-onset, persistent, and more severe AD, as well as increased risk of asthma in patients with AD.[121]

2.2 FOOD ALLERGY

AD with FA is distinguished as a unique endotype with decreased filaggrin and ceramide levels as well as increased TEWL and type 2 immune activation.[130] These children may be in greater risk of developing other atopic comorbidities[131,132] In a prospective study carried out at a university hospital paediatric dermatology clinic, every third 3-year-old child with moderate-to-severe AD had symptomatic FA. The diagnosis was verified by food challenge or history of significant reaction after ingestion.[133] The most common food allergens in children with AD are cow's milk, egg, wheat, soy, and tree nut or peanut.[134,135] However, foods as such are not commonly considered as eczema triggers. On the contrary, impaired AD skin itself is believed to elevate the risk for FA. Exposure to peanut in surrounding household dust through a compromised infantile AD skin increased the risk for both peanut sensitisation (OR 1.97) and peanut allergy (OR 2.34).[136]

2.3 ALLERGIC RHINITIS

Allergic rhinitis (AR) is an inflammation on nasal mucosal membranes caused by IgE-mediated reactions to inhaled allergens. The overall prevalence of AR has increased being already 5% at 3 years of age, and 8,5% at 6-7 years age. [137] In sensitised individuals, allergens, such as pollen and animal dander, provoke an allergic response characterized by symptoms including sneezing, nasal itching, rhinorrhoea, and nasal congestion, or obstruction.[138] Pollen-sensitised individuals may also present with oral symptoms such as itchy mouth and throat after ingestion of the pollen-associated cross-reactive food.[139]

In a similar matter as with AD, upon mucosal impairment, nasal epithelium releases alarmins and initiates type 2- immune response. This leads to IgE-class switching and mucosal inflammation. Additional environmental factors and genetic predisposition are likely involved. Polysensitisation and multimorbidity are characteristic for the AR phenotype, which is associated with early-onset activation of *IL-5*, *IL-33,* and eosinophils.[140] Many patients with AR, especially when polysensitised, have coexisting asthma. Genome wide association studies (GWAS) have demonstrated some shared susceptibility genetic loci for allergic diseases: *IL33*, *IL1RL1*, and *TSLP* for instance may play a role in the development of atopic multimorbidity pattern.[140]

2.4 ASTHMA AND HYPERREACTIVITY

Asthma is a chronic inflammatory airway disease characterised by variable symptoms of shortness of breath (dyspnoea), wheezing, chest pain, and cough. In addition to airway inflammation, it is associated with reversible airflow obstruction (hyperreactivity), and structural remodeling.[141,142] Most children with asthma have the allergic type with aeroallergen sensitisation, eosinophilic airway inflammation, and tissue remodelling. Remodelling includes both increased thickness of the basement membrane as well as airway smooth muscle mass leading to structural changes of the airway wall.[143] These children commonly have positive personal and familial history for atopic disorders.

Patients with asthma and AR share common physiology including increased bronchial hyperresponsiveness (BHR) and reactivity to a variety of stimuli like allergens, smoke, pollutants, or microorganisms. BHR is characterized by increased responsiveness of bronchial smooth muscle to non-specific constrictor stimuli.[144,145] Unlike in asthmatic adults, BHR is not necessarily present in wheezy infants; transient early-life wheeze is often a presentation of congenitally narrow airways, especially during viral infections. Growth of the airway calibre with time resolves wheeze in most cases. Therefore, wheeze alone is insufficient to predict progression to asthma. Recurrent wheezing is common in preschool children.[146]

However, along with genetics, atopy, persistent wheezing, and several respiratory infections have been identified as major risks factors to develop

asthma.[128,145,146] Reduced lung function by the age of 6 was noted among children with recurrent, severe, early-life wheeze.[147] Low lung-function association remained even up to early adulthood.[127,148]

2.4.1 Pathomechanisms

Interactions between genetic predisposition and the environment influence the course of the disease. Bronchial epithelial cells from atopic asthmatic children, for instance, have shown to have impaired interferon-β and -γ levels in response to rhinoviruses infection.[149] Smoking during pregnancy, and environmental tobacco smoke exposure during infancy have been documented to have negative effect on developing airways.[150] Conversely, compared to nonfarm rural mothers, mothers living on farms have shown to have significantly higher levels of transforming growth factor beta (TGF-β) and IL-10 in their colostrum and mature milk. TGF-β is known to reinforce innate immunity while IL-10 has widespread antiallergic activity, possibly strengthening infant's immune balance.[151]

Immunopathology in allergic asthma and AR is similar with the predominance of type 2 inflammation and tissue eosinophilia. The airways of a sensitised child with asthma release mediators, damaging the epithelium.[152] TSLP synthesised by bronchial epithelial cells is an essential trigger of allergic (Th 2-driven) airway inflammation.[153] Eosinophils in the lungs are increased through production of IL-5, while IL-9 is involved in mast cell activation. IL-4 drives B-cell isotype switching and IgE synthesis.[154] IL-13 is able to induce JAK-STAT6 signalling leading to smooth muscle contraction.[155] In knockout mice, STAT6 contributed to IgE synthesis, BHR, and airway remodelling upon allergen sensitisation.[156] In murine models IL-33 has been associated with airway remodeling.[157] S100A8 and S100A9, in turn, can induce mucin production in the airways through TLR4-linked pathway during asthma attacks.[158] Strong TLR signals are considered protective against allergic airway disease, while low airway amounts of TLR ligands may cause airway sensitisation and Th2-type immunity.[156]

2.4.2 Diagnostic considerations in childhood asthma

To diagnose asthma can be challenging in young children. The asthma predictive index (API) was developed to identify preschool children who may develop asthma at school age. It is based on large longitudinal data of respiratory illnesses.[159,160] The criteria of the index are frequent wheezing during the first 3 years of life and either one major risk factor (parental history of asthma or eczema), or two of three minor risk factors (eosinophilia, wheezing without colds, and AR). Eosinophilia was considered if eosinophils were $\geqslant$ 4% of the total white blood cells.[159] A modified API from 2004 requires

more than 4 episodes of wheezing and replaces AR with sensitisation to aeroallergens as a major criterion, and sensitivity to milk, eggs, or peanuts as a minor criterion.[161] However, API cannot be used to rule out asthma development. Some children will go on to develop asthma later in life with only mild wheezing history in early life.

Asthma diagnosis in preschool children is based on characteristic symptom patterns, presence of risk factors, variability in airflow limitation in the presence of airway inflammation, ruling out alternative diagnoses, and response to treatment.[162] There are several guidelines to help diagnose childhood asthma such as the one from The Global Initiative for Asthma (GINA).[163] In Finnish national guidelines from 2022 (Current Care Guidelines) filling at least one main criterion or two minor criteria is indicative for asthma in infant with recurrent wheeze (Table 2).[164]

Table 2 Finnish national guidelines (2022) for asthma diagnostics in preschool age with recurrent wheeze.

Main criteria	Minor criteria
Parental asthma, documented by physician	Food sensitisation
Atopic dermatitis, documented by physician	Wheezing apart from colds
Sensitisation to aeroallergens	Eosinophilia > 4% or > 0.3 x 10^9/L

2.4.2.1 *Lung function tests*

In addition to above-mentioned signs and symptoms indicative for asthma, lung function tests are used to confirm the diagnosis. Without objective measurements wheeze alone, although typical for paediatric asthma, can be misdiagnosed as asthma.

Impulse oscillometry with exercise and/or bronchodilatation tests is useful for young children over 3 years of age as an objective assessment for reversible airflow obstruction.[152] Change of resistance (Rrs5) in oscillometry of at least 40% is indicative for asthma while 35-39% is suggestive.[165,166] Another way to diagnose lung function abnormalities is spirometry with bronchodilatation test, which commonly requires more co-operation and is usually suitable from 5 years age onwards or at school age.[167] Variable expiratory airflow limitation, characteristics for asthma, is documented by spirometry as an increase in forced expiratory volume in 1 second (FEV_1) 15 minutes after administration of bronchodilator. In children, an increase from baseline of more than 12% of the predicted FEV_1 value is indicative for asthma.[164] Peak expiratory flow (PEF) tests are not considered reliable in children under 12 years of age.

Other tools have been developed as well. Fractional exhaled nitric oxide (FeNO) measurements reflect the eosinophilic inflammation in the airways in allergic asthma. As a result of airway inflammation, inducible NO synthase increases the production of NO, which can be measured in exhaled breath and as exhaled NO fraction (FeNO).[168] Although increased FeNO is suggestive for asthma, the diagnosis cannot be based exclusively on FeNO[169] Children often manifest with some degree of BHR in general. Increased BHR and FeNO levels have been documented also in children with AR, but not asthma, indicating sub-clinical bronchial inflammation and involvement of both the upper and lower airways.[170]

Methacholine Challenge is a bronchoprovocation test with high negative predictive power when ruling out asthma.[171] The diagnostic reference values are lacking for children under 12 years of age and the method is not recommended for common asthma diagnostics in children. Nevertheless, lack of BHR on methacholine test is suggestive for not having asthma.

In uncertain cases in infants, after a careful evaluation by a paediatrician along with objective tests, a medication can be initiated, commonly with inhaled corticosteroids (ICS) as follows: ICS treatment is recommended if asthma is likely, and the child has uncontrolled symptoms and/or ≥3 wheezing episodes per year.[163,164] A trial of ICS can also be recommended if the symptoms occur more than every 6–8 weeks.[163,164] Usually, a clinical improvement is observable during 2–3 months of treatment. Children should be reviewed within a few months after initiating treatment to confirm the diagnosis. If the treatment has no expected effect, alternative diagnoses and investigations should be considered. Tertiary health care units and specialists are responsible for preschool asthma diagnostics in Finland.

2.4.2.2 *Other diagnostic considerations*

Since wheezing is common in infants and often associated with atopy, SPTs or specific IgEs to common aero and food allergens are performed to evaluate atopy.[164] Total IgE and allergen specific IgEs have been associated with asthma in over 80% of children with actual diagnosed asthma.[172] In a birth cohort from New Zealand a significant correlation of IgE levels with BRH to methacholine challenge was documented[172.]

There exists a correlation between blood eosinophil count, asthma severity and airway hyperresponsiveness in children.[157] Eosinophilia is suggestive for increased asthma risk[173] Despite this fact, eosinophilia in serum does not always correlate with airway eosinophilia.[157] Blood eosinophils contribute to airway remodelling, subepithelial membrane thickening, and goblet cell metaplasia.[157,158] However, there is no standardised cut-off for eosinophilic inflammation, although a blood eosinophil count of 300 cells·μL^{-1} has often been used as a threshold.[174] Eosinophil counts are suggested to be measured

at the beginning, but also later in the diagnostics to determine the phenotype of asthma.[164]

2.5 OTHER COMORBIDITIES OF ATOPIC DERMATITIS

AD has been linked to several comorbidities. Skin diseases like ichthyosis vulgaris share similar genetic origin with AD.[175] Alopecia areata and vitiligo are known autoimmune conditions linked to AD[175]. Secondary bacterial (most often *Staphylococcus aureus*) infections can lead to impetigo, and even erysipelas and septicaemia.

Characteristic cytokine milieu and impaired plasmacytoid DC recruitment in the skin predisposes AD patients to viral skin infections.[176-178] Eczema herpeticum (EH) is a potentially life-threatening disseminated HSV infection that may occur in 10–20% of patients with AD.[176] Those with early-onset, severe AD in head and neck region are in greater risk. Also, previous EH infection elevates this risk.[178] *Molluscum contagiosum* infection due to poxvirus is also common in childhood AD.[6,7]

Mental health comorbidities, particularly attention-deficit hyperactivity disorder, anxiety, depression, and even eating disorders seem to be more common with AD.[179,180] AD seems to result in significant overall morbidity including obesity, cardiovascular diseases, and gastrointestinal immune-mediated disorders like celiac disease and dermatitis herpetiformis.[181,182] Severe long-term AD has been associated with adult lymphomas.[181]

3 TREATMENT OF CHILDHOOD ATOPIC DERMATITIS

Treatment of AD aims to control the active disease. However, it should not just concentrate on controlling acute flares, but rather build up on a long-term approach with comprehensive disease management. Effective therapy normalises the skin barrier function and should be efficient and correctly applicated. Since patients are children, it is important to educate parents and caregivers on the treatment.

Basic management strategies in AD include showers or baths with mild detergents, and using propriate emollients or moisturisers, as well as avoidance of irritants and temperature extremes.[183] Emollients are lipid-based products, whereas moisturisers add water and moisture to the skin.[184] Moisturisers contain active ingredients like humectants or ceramides. Humectants aid water retention to skin while ceramides aim to repair the reduced intracellular lipid amount in the SC.[185] This way moisturisers can act as either hydrophilic or lipophilic. Urea is a humectant that also induces NMF ingredient expression in the skin.[186] However, in infantile skin it may cause irritation and hence glycerol as a humectant is better tolerated in very young children.

Safety is especially important in the long-term management of a chronic disease. One aspect to consider when treating children is the fact that children under 2 years of age have thinner skin and a higher body surface area to weight ratio, making the skin in general more prone to significant percutaneous absorption and greater risk for even intoxication.[187] Maturation of the skin can last for several years after birth.[190] Moreover, children's immune system is still under development.

3.1 TOPICAL CORTICOSTEROIDS

Topical corticosteroids (TCS) have been widely used to treat AD since 1955. They are commonly used “as needed” when the skin is inflamed but can in certain cases be used also as maintenance therapy.

3.1.1 Mechanisms of action

TCS are anti-inflammatory with wide impact on both immune and skin barrier function. They act by reducing pro-inflammatory cytokines and immune effector cells. Moreover, TCS also influence antigen processing. Treatment normalises TEWL in lesional skin and upregulates the expression of *FLG* and *LOR*.[189]

3.1.2 Usage

TCS are considered as first-line therapy in AD and classified as mild, mid-potent and potent based on their potency to work. In Finland they are classified into four different groups from mild (group I) to very strong (group (IV). Group I-II agents (mild-moderate) are generally used for children. TCS are available in different formulations like creams, liniments, foams, and ointments. In addition to age, treated body site along with lesion severity are mostly the determining factors when choosing a suitable formulation. TCS differ from another also by the vehicle used which may affect their ability to penetrate the skin barrier.[190]

3.1.3 Concerns

Possible risks with the usage of potent long-time TCS are skin atrophy, telangiectasia, acne, perioral dermatitis, and striae.[191-193] Decreased cell epidermal cell size, suppression of cell proliferation and collagen synthesis including decreased fibroblast growth and reduced production of acid mucopolysaccharides have been documented.[192,193] Thin skin areas like eyelids are at greatest risk for side-effects such as glaucoma.[193,194]

Long-term application of potent agents can lead to systemic absorption and suppression of hypothalamic-pituitary-axis.[195] The cumulative effect of many simultaneous steroid therapies may increase the risk for adverse effects. For instance, growth delay has been reported with simultaneous use of topical and inhaled corticosteroids for AD and asthma.[196] While TCS show relatively fast eczema improvement, after long treatment cured areas show structural peculiarities like reduced NMF that may contribute to a new flare.[197]

3.2 TOPICAL CALSINEURIN INHIBITORS

Topical calcineurin inhibitors (TCIs) are bacteria-derived macrolide agents that have immunomodulating properties. There are two types of TCIs: topical tacrolimus was launched in 2000, and pimecrolimus in 2001.[198,199] Tacrolimus is available as a 0.03% (approved for children 2 years of age and older) or as 0.1% ointment (approved for children over 16 years of age), and pimecrolimus as a 1% cream (approved for children 2 years of age and older).

3.2.1 Mechanisms of action

TCIs bind to intracellular protein macrophilin-12 (also called FK-binding protein) in T cells and prevent translocation of a transcription factor called the nuclear factor of activated T cells (NFAT). This leads to decreased calcineurin activity and reduced expression of many T cell activation cytokines such as IL-

2, IL-3, IL-4, IL-5, IL12, IL-31, TNF-α, and IFN-γ, as well as inhibition of Th1 and Th2 cells.[200,201] TCIs have also an inhibitory effect on many other cells like eosinophils, mast cells, basophils and even LCs, in addition to effect on cationic channels (TRPV1) expressed by cutaneous sensory nerve endings.[202] In a meta-analysis of 4 randomized controlled trials topical tacrolimus showed to be more potent than pimecrolimus.[203]

3.2.2 Usage

TCIs are commonly prescribed as second-line after topical steroids in moderate-to-severe AD twice daily until eczema clearance. Since TCIs do not cause long-term side effects like skin atrophy that TCS may do, they can also be used as first-line treatment to treat thin and sensitive skin areas like the eyelids, face, and skin folds.[204] Off-label use of TCIs in children below 2 years of age is very common despite age restrictions.

TCIs are typically considered as long-term AD management. Proactive therapy as twice-weekly treatment on previously affected areas has shown to prolong the time to relapse along with daily use of emollients or moisturisers.[205]

3.2.3 Concerns

Common adverse effects of TCIs are a burning or stinging sensation, irritation, and pruritus on the treated areas, which typically is transient and occur during the first week of treatment.[198,206] The incidence of *Herpes simplex* and *Molluscum contagiosum* infections may be higher compared to other treatments during the first treatment months.[207] Sometimes folliculitis is seen with long-term treatment.

In 2006, the FDA issued a black box warning for both TCIs and a recommendation to avoid use in children younger than age 2 based on a theoretical risk of malignancy including lymphomas.[208] However, systemic absorption with topical use, even in children under 1 year of age, has demonstrated to be very low and below immunosuppressive levels.[209-211] Numerous studies have failed to demonstrate a link between TCI use and lymphoma risk.[212,213] Although there are still only few studies in infants, especially on 0,1% tacrolimus, TCIs have shown to be safe both in short-term and long-term studies with no increased risk of cancer.[209-213]

3.3 OTHERS

3.3.1 Ultraviolet treatment

Phototherapy for school-aged children may be considered individually in severe cases. Phototherapy is thought to have immunosuppressive properties. Narrowband-UVB is usually the first phototherapy option in paediatric populations.[214] However, recurrence after treatment is prevalent. Phototherapy can be used as monotherapy or in combination with emollients and topical steroids. Risks and benefits including possible long-term consequences of phototherapy should be considered individually. Short-term risks of UVB phototherapy include erythema, photosensitive eruptions, and recurrent HSV infections.[215]

3.3.2 Systemic treatments

Most traditionally used for short-term use have been cyclosporine, methotrexate, mycophenolate mofetil, and azathioprine with significant country-specific variations partly due to the lack of controlled trials in children and hence mostly off-label treatments in differing healthcare systems.[216-218]

In line with recent developments in AD field, targeted, systemic therapies have been developed. Dupilumab is a monoclonal antibody that binds to IL-4 receptor α (IL-4Rα), blocking IL-4/IL-13 signaling.[219] It is available as a subcutaneous injection and approved for children from 6 months on in refractory moderate-to-severe AD.[220] Other emerging systemic biologic agents for paediatric AD include IL-13 inhibitor tralokinumab, IL-13Rα inhibitor lebrikizumab, IL-31Rα inhibitor nemolizumab, and IL-5Rα inhibitor benralizumab.[221] In Finland lebrikizumab is available from 12 years of age.

Oral JAK inhibitors may be an additional therapeutic option. They interfere the JAK-STAT signalling pathway in lymphocytes and inhibit cytokine activity. Baricitinib is available in moderate-to-severe AD for children from 2 years of age and upadacitinib from 12 years age.[222]

3.3.3 Other topical agents

Of the topical therapies, topical Phosphodiesterase 4 (PDE4) inhibitor, crisaborole, is licensed in the United States for mild-to-moderate AD, but not marketed in the EU. PDE4 regulates the production of inflammatory cytokines through the degradation of cyclic adenosine monophosphate.[223]

Many other topical agents are under investigation for children like a topical JAK1/JAK2 inhibitor (ruxolitinib), an aryl hydrocarbon receptor (AhR) agonist (tapinarof), an antimicrobial peptide, commensal skin bacteria, and

TRPV1 (transient receptor potential vanilloid subfamily member 1) antagonists.[224,225]

3.4 PREVENTION

In many children AD disappears around 3 years age whereas some may continue a more severe course.[4] It would be of importance to identify these children at risk for more severe disease pattern to ensure optimal treatment of the defective epidermal barrier, and thereby minimize transcutaneous penetration of irritants and allergens, and the risk of possible sensitisation.[24] It is unclear whether, or how, the possible atopic multimorbidity risk-pattern, the sequence of atopic conditions, can be predicted, and which of the conditions are, in fact, linked. Many trials on primary and secondary prevention have been initiated. Trials on secondary prevention have covered methods including topical treatment, emollients, probiotics or prebiotics, and environmental modifications.[3,226,227]

When emollients and moisturises are important in the management of already established AD, their role in primary prevention is controversial. The results from eczema prevention trials are controversial. A large Cochrane review reported that skin care interventions such as emollients likely have no influence on the development or time to onset of eczema in healthy term infants by age one to three years.[228] On the contrary, it was shown that at one year's age interventions may actually increase, not only the eczema, but also the risk of food allergy.[229,230] It is of notice though that Skjerven et al[229] used bathing rather than emollient as intervention. In general, there was a lack of documentation of both adherence and compliance with interventions. Further, caregivers were not advised to wash hands before applying skin care products to babies, which may have been one significant cause of documented tendency for food allergies through transcutaneous sensitisation.

4 AIMS OF THE STUDY

The aim was to study whether we could, through secondary prevention, optimize AD management with a possible inhibitory effect on its progression. The hypothesis of atopic march from eczema to asthma laid a ground for the thought, whether the march could be prevented early during infancy with effective eczema treatment.

We conducted a prospective, open-label comparative long-term study where we followed originally 152 one—to three-year-old children with moderate-to-severe AD treated topically with either mild-to-moderate corticosteroids or tacrolimus (0.03 and 0.1%).

The specific aims of this study were:

1) To collect evidence and safety data for topical tacrolimus in small children and compare its usage with topical corticosteroid in moderate-to-severe AD (I, II, and III).

2) To determine whether eczema treatment affects airway inflammation or bronchial hyperresponsiveness (III).

3) To investigate the effect of coding variation in *FLG* and 13 other genes with epidermal barrier or immune function in children with moderate-to-severe AD (IV).

5 SUBJECTS AND METHODS

5.1 STUDY DESIGNS AND PROTOCOLS

The study was carried out at outpatient clinic of the Helsinki Skin and Allergy Hospital in Helsinki, Finland during 2013–2019. Patients aged from one to 3 years, suffering from moderate-to-severe AD, were enrolled.

Study I consisted of hospital visits at baseline and after 1 week, 1, 3, 6 and 9 months, and 1 year. Study III included visits in study I and thereafter at every 6 months until the study-end at 36 months. Study II consisted of safety data and infections collected during the 3-year study period. In study IV blood sample for genetic analyses were gathered at 3-months follow-up visit (Figure 4).

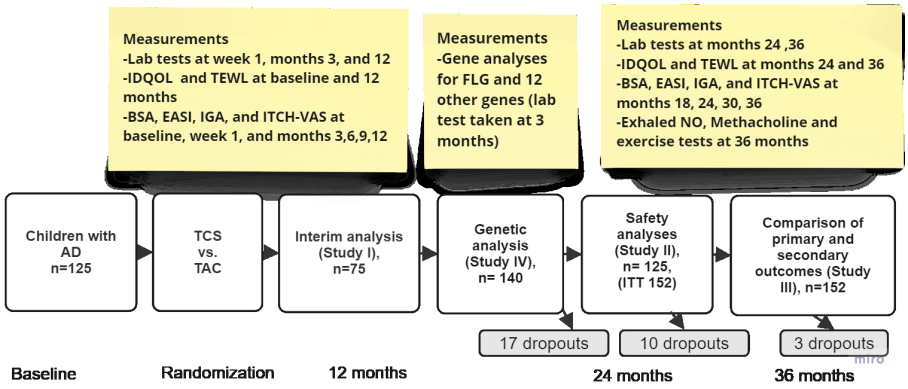

Figure 4 Flow chart of the study populations in studies I-IV.

5.1.1 Inclusion and exclusion criteria

In all studies patients were required to have an AD diagnosis according to the Hanifin and Rajka criteria.[10] They were required to have either moderate (score 4.5-7.5) or severe (score 8-9) eczema at baseline based on the Rajka and Langeland Eczema Severity Score[16], evaluated by a dermatologist.

Exclusion criteria were a skin disease, such as ichthyosis vulgaris. Also, patients with a constant need for inhaled corticosteroids or immunosuppressive medication were excluded.

5.1.2 Patients and ethics

Study I comprised those first 75 patients who had reached the 1-year follow-up. In study III altogether 152 patients were recruited. Study II consisted of safety data of 125 of these patients. In study IV a sample for genetic analyses was taken of 140 patients who were recruited for studies I-III.

Participation was voluntary. Parents of the participated children were asked for a written consent prior to participation. Research data was collected during patient visits at the Skin and Allergy Hospital.

The ethics committees of the Helsinki University Central Hospital and the Finnish Medicines Agency approved the study protocol for studies I-IV (222/13/03/03/2012, EudraCT2012-002412-95). All studies were conducted in accordance with the ethical principles of the Declaration of Helsinki and were conducted as single-centre investigator-driven studies.

5.1.3 Medication

Patients were informed to have a 2-week wash-out period before baseline. After stratified allocation, patients were randomised 1:1 to treatment with 0.03%, and if needed, 0.1% tacrolimus ointment (TAC group), or 1% hydrocortisone acetate ointment, and if needed, hydrocortisone butyrate 0.1% ointment (TCS group). AD was treated according to the treatment protocol of Skin and Allergy Hospital. In TAC group, treatment was twice daily until clearance was achieved, after which it continued as twice-weekly maintenance therapy. In TCS group, eczema lesions were treated twice daily for 3-7 days, followed by a treatment pause lasting a minimum of 3-7 days. In both groups daily treatment was restarted in case of a flare-up based on parents' or caregiver's decision.

To ensure adherence, study nurse weighted the treatment tubes at each study visit. Moisturising emollients were permitted. Treatments such as other corticosteroid regimens, systemic corticosteroids, ultraviolet therapies and systemic immunosuppressive agents or other systemic therapies were prohibited. Antihistamines and topical/systemic antimicrobials were permitted, if needed, after investigator´s consultation.

5.1.4 Eczema severity assessments

Treatment response to topical therapy was assessed by evaluating affected percentage of body surface area (BSA), Eczema Area and Severity Index (EASI), and Investigator´s Global Assessment (IGA) at baseline and at each follow-up visit.

Visual analogue scale (VAS) was used (0= no symptoms to 10= severe symptoms) to assess pruritus/itch. IGA was estimated on a 5-point severity

scale (0= clear, 1= almost clear, 2= mild disease, 3= moderate disease, 4= severe disease, 5= very severe disease).

5.1.5 Transepidermal water loss

Transepidermal water loss (TEWL) was measured by the same study nurse in all studies from the most severe eczema lesion at baseline, and after that at months 12, 24, and 36. At the final visit, eczema TEWL was measured from the same site as baseline. In Addition, control measurements were performed from eczema-free control site (left forearm). Measurements were made with yearly calibrated VapoMeter (Delfin Technologies, Kuopio, Finland) according to published guidelines.[231]

5.1.6 Quality of life

To study the impact of AD on patients' quality of life (QoL), we used The Infant's Dermatitis Quality of Life Index questionnaire[20] with the permission of, M.S. Lewis-Jones and A.Y. Finlay. It includes 10 questions, filled by parents or guardians, each graded from 0 to3 points. The IDQoL is calculated by summing the score of each question resulting in a maximum of 30 and a minimum of 0. The higher the score the more quality of life is impaired. The QoL was evaluated first at baseline and thereafter at months 12, 24, and 36.

5.1.7 Safety

Medical history and adverse events were recorded. Laboratory and other parameters were followed according to Table 3.

Table 3 Laboratory and other parameters during studies I-IV.

	Baseline	1 week	3 months	12 months	24 months	36 months
CRP, Blood count, kidney value, S-IgE, TEWL, QoL	x			x	x	x
S-tacrolimus (in TAC group)		x		x	x	x
S-cortisol			x	x	x	x
Blood sample for genotype analyses			x			
IgEs for food and aeroallergens, SPTs	x					x
Vaccination responses						x
IL-4, -10, -14, -31, IFN-γ	x					x

In the tacrolimus group, blood concentration of tacrolimus was measured by fluid chromatography-tandem mass spectrometry. Serum cortisone levels were measured in all patients by immunochemiluminometric assay.

In study II safety information was gathered on growth parameters (weight and height), infection rates (skin-related and non-skin related infections), and responses to vaccinations included in the general national vaccination program (IgG antibodies against Diphtheria, Tetanus, Rubella, Morbilli, and pneumococcal serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F).

Decreased vaccination response was determined, based on laboratory guidelines (HUSLAB® and the Finnish Institute for Health and Welfare, THL), as follows: for tetanus and diphtheria IgG antibodies <0.1 IU/ml, and for rubella <10 IU/ml.[232,233] For morbilli the absence IgG antibodies was considered pathological. Pneumococcal vaccine response was interpreted as normal if the concentration of antibodies in more than half of the 10 serotypes examined was greater than 0.35 μg/mL.[234]

Serum cytokine levels were measured with ELISA. All laboratory tests were performed in the laboratory of Helsinki University Hospital (HUSLAB®) and were based on accredited methods. Topical treatment of the blood test site was not allowed in the morning before laboratory testing.

5.1.8 Serum total and specific IgEs, skin prick tests

Total IgE was assessed by CAP system-specific IgE (fluorescence enzyme immunoassay, Phadia AB, Uppsala, Sweden). Age-related references for the upper normal limits for young children are for age 5 months to 1 year, 70kU/L; 2 to 3 years 110 kU/L; and 4 to 7 years 130 kU/L.[235]

We screened IgEs for aero- and food allergens by Phadia ImmunoCAP (Phadia AB, Uppsala, Sweden). If the result was positive (>0,35 kU/l), specific IgEs were performed, depending on result, either against food (peanuts, milk, egg white, soy, cod, or wheat) or aeroallergens (timothy, birch, mugwort, horse, cat, dog, *Cladosporium herbarum*, or house dust mite).

Skin prick tests (SPTs) were performed by qualified nurse on healthy skin on the forearms. They included histamine dihydrochloride 10 mg/ml as the positive control, saline as the negative control, and following allergens: birch, timothy, mugwort, cat, dog, horse, house dust mite, *Cladosporium herbarum*, egg white, milk, soy, cod, wheat, peanuts, or shrimp. A test reaction of at least 3 mm was considered positive if the histamine reaction was at least 5 mm and the saline control negative. Antihistamines were withheld for 5 days prior to testing.

5.1.9 Airway inflammation and lung function

Airway and respiratory symptoms were evaluated throughout the study. We screened for symptoms like night cough, dyspnoea, wheezing, and exercise-induced symptoms as well as symptoms equivalent with AR.

At study-end, all participating children underwent tests for lung function and airway hyperresponsiveness. Possible inhalation corticosteroids were discontinued for 4 weeks prior the lung function measurements. Also, the caregivers were instructed not to give short-acting bronchodilators for at least 1 day before the tests. In those having had respiratory infection less than two weeks prior lung function testing, the tests were postponed accordingly.

5.1.9.1 *Oscillometry and Exercise test*

To assess lung function, impulse oscillometry was performed (IOS, JAEGER GmbH, Germany), and the resistance of the respiratory system (Rrs5) and the reactance (Xrs5) determined with Z scores calculated based on height-adjusted reference values.[165] Airway responsiveness to exercise was defined after 6–8-minute running test by measuring the change in the resistance (ΔRrs5) after 1, 5 and 10 minutes. Measurement was repeated 15 minutes after inhalation with salbutamol (bronchodilation test). A positive test result was defined as an increase of 35% or more indicating exercise-induced bronchoconstriction (EIB).

5.1.9.2 *Methacholine challenge test*

The modified dosimetric methacholine test is a bronchial provocation test suitable for toddlers. [236] With this challenge method, the dose of nebulized methacholine causing an increase of 40% in Rrs5 (PD40Rrs5) was determined. Low cumulated methacholine dose indicates increased bronchial hyperresponsiveness.

5.1.9.3 *Exhaled and fractional nitric oxide*

Exhaled NO was measured by tidal breathing method in which the child breathes through a mask (excluding nasal breathing) connected to a chemiluminescence analyzer.[237] The device measures simultaneously on-line concentration of NO and exhalation flow with the help of ultrasound sensors. As a result, an average NO concentration (FENOmb) and production (V'NO = V'E * FENO) is documented after minimum 10 exhalations. Since this method was quite demanding, only few children managed to complete the test in the beginning of the study compared to study-end.

The exhaled NO level was also measured by standard single-breath analysis, where after 2-3 acceptable exhalations a mean concentration (Fractional NO concentration, FeNO) was recorded, and Z scores calculated by using height-adjusted reference values.[238] A higher-than-normal level of NO is associated with swelling (inflammation) of airways.[168]

5.1.10 Genetic analyses

At 3-months follow-up, a blood sample was taken from 140 participants for genetic analyses to examine for possible variants in *Filaggrin (FLG)* and other genes affecting skin barrier. Sequence variation in *FLG*, *FLG2*, and 12 other AD related genes were investigated. Sequencing and genotyping were performed at the Sequencing Core of the Technology Centre of the Institute for Molecular Medicine Finland (FIMM). Genotyping was performed using Sequenom MassARRAY system and iPLEX Gold assays (Agena Bioscience, San Diego, CA, USA). Control samples (N=1664) were obtained from the Health 2000 (H2000) GenMets Study.[239] Health 2000 Survey is a Finnish population-based study carried out in 2000-2001. GenMets subcohort about metabolic syndrome contains genome-wide SNP data of participating individuals.

Quality control and analyses for genotype data were performed with PLINK (open-source genome analysis tool, v1.90b5.3).[240] Analyses included samples with a maximum of 2 missing SNP calls and variants with missing call rates <0.1. Selected single nucleotide polymorphisms (SNPs) in the *FLG* and *FLG2* were genotyped, and their association tested with AD. The linkage between eczema severity and *FLG* LoF variants was examined.

As an exploratory candidate-gene study (for possible previously unreported monogenic-like causes of moderate-to-severe AD), we studied protein-coding regions of the following genes by amplicon sequencing: *FLG*, *FLG2*, *CLDN1*, *DOCK8*, *IL13*, *IL17A*, *IL22*, *IL31*, *IL33*, *IL4*, *IL4R*, *SPINK5*, *STAT6*, and *TLR2*. Amplicon sequencing was performed using Illumina Truseq Custom Amplicon Kit and the MiSeq system (Illumina, Inc., San Diego, CA, USA). Genes were selected based on matching one or more of the following criteria: 1) cause for monogenic immunodeficiency with AD-like features (eczema, asthma, or allergy); 2) previously reported association with AD, allergy, or asthma; or 3) known involvement in AD pathogenesis. Information for variants with previous associations to atopic dermatitis, eczema, allergy, IgE levels, or eosinophil counts was sought from the following publicly available online databases: FinnGen F6 (FinnGen, 2022)[241], UK Biobank (UK Biobank, 2017)[242], and GWAS catalog (GWAS Catalog, 2018)[243].

Reads were aligned to the GRCh37 human reference genome assembly utilizing Bowtie2.[244] Variant calling was done using an in-house pipeline.[245] VCFs were trimmed and combined using BCFtools.[246] To analyse germline variants, only variants with alternative/reference read frequency ratio of >0.2

were included in analyses. In addition, recurrent PCR/alignment-errors were removed manually after visual inspection of data on IGV.[247] Variants were defined as heterozygous when the alternative/reference read frequency ratio was between 0.2 and 0.8, and homozygous when the ratio was >0.8. Variant annotations were performed with ANNOVAR.[248] Rare variants were defined as having a frequency<0.01 in gnomAD exomes and genomes of Finnish origin.[249] Gene-wise rare variant frequencies in the cohort were also compared to rare variant frequencies in gnomAD to estimate enrichment of rare variation in the selected candidate genes. Variant frequencies in the study cohort were calculated by dividing the number of identified alternative alleles by the number of samples with a minimum 10x coverage at the site. Variant pathogenicity was estimated with CADD[250] and REVEL[251], and evolutionary conservation was evaluated with GERP++[252].

5.2 STATISTICAL METHODS

All analyses were performed with the statistical software package SPSS versions 21.0, 22.0, 24.0, 25.0, 26.0, or 27.0 (IBM Corp, NY, USA). Significance was set at $P < 0.05$. Sample size calculation for Study III was done with 80% power and 0.05 alpha error. Normality of distributions (parametrical vs non-parametrical) were assessed by histograms.

In Study I the variables had mainly non-parametric distributions due to relatively small patient numbers at the one-year follow-up point. The Mann-Whitney *U*-test served to test differences between treatment groups when continuous parameters were compared, and the Wilcoxon signed rank test for changes within a group during the first treatment year. In Studies II-IV continuous parameters were compared with Mann–Whitney *U*-test or independent samples *t*-test along the distributions. In all studies nominal/categorical variables between treatment groups were compared with χ^2 test or Fisher´s exact test.

In Study I, correlations between eczema severity parameters were analysed with Spearman´s correlation test. In Study III, correlations between baseline food or aeroallergen sensitisation and the degree of exercise induced bronchoconstriction (EIB) were based on Pearson´s correlation test.

In study III, an intention-to-treat (ITT) analysis with the last-observation-carried-forward method was performed where appropriate. ANOVA or independent samples *t*-test were used to determine the 95% confidence intervals (CIs) for the differences in means in Study III. In Studies III and IV repeated measures ANOVA (General linear models) was used to analyse mean changes in parameters during the study. In study III Bonferroni correction, and in Study IV either Bonferroni or FDR correction, was applied to adjust for multiple testing.

In Study IV association of *FLG* and *FLG2* variants with AD was tested using Fisher's exact test with Lancaster's mid-*P* adjustment and 95% CI in

PLINK (216). In all studies continuous variables are presented as medians with 25th to 75th percentiles (Q1-Q3) or as means with either standard deviations (±SD) or range. Categorical variables are presented as counts and percentages.

6 RESULTS

There were no baseline differences between study groups (Table 4).

Table 4 Baseline characteristics of the study populations

Variable	TCS, n =75	TAC, n=77
Sex, n (%)		
Male	44 (58.7)	35 (45.5)
Age (years), mean (SD)	1.8 (0.7)	1.8 (0.7)
Severity of AD, n (%)		
Moderate	42 (56.0)	41 (53.2)
Severe	33 (44.0)	36 (46.8)
Family history of atopy, n (%)	63 (84)	69 (89.6)
Elevated IgEs, n (%)		
Food	39 (52.0)	38 (49.4)
Aero	29 (38.7)	24 (31.2)
Positive SPTs, n (%)	45 (60)	46 (59.7)
Height (cm), mean (SD)	83.4 (7.4)	83.1 (7.0)
Weight (kg), mean (SD)	11.6 (2.4)	11.7 (2.1)
Parent(s) smoking, n (%)	24 (32.0)	21 (27.3)

TCS = topical corticosteroids, TAC = tacrolimus, IgE= Immunoglobulin E, SPTs = Skin prick tests

6.1 EFFICACY

6.1.1 Clinical parameters

No statistically significant differences occurred in eczema efficacy parameters EASI and BSA between TAC and TCS groups at baseline or during the follow-up. Mean EASI change from baseline until 36 months was 9.2 (±8.0) in TCS, and 9.4 (±8.7) in TAC. Mean BSA% change for TCS was 21.2 (19.1), and for TAC 23.6 (±22.0). (Figure 5).

6.1.2 Transepidermal water loss

TEWL at eczema site decreased in both treatment groups during follow-up, whereas at control site it had an increasing tendency in both groups towards the end. However, it increased more in the TCS vs TAC group (mean change difference, P=0.04). Control site TEWL was at lowest in both treatment groups after one year treatment. (Figures 5 and 6).

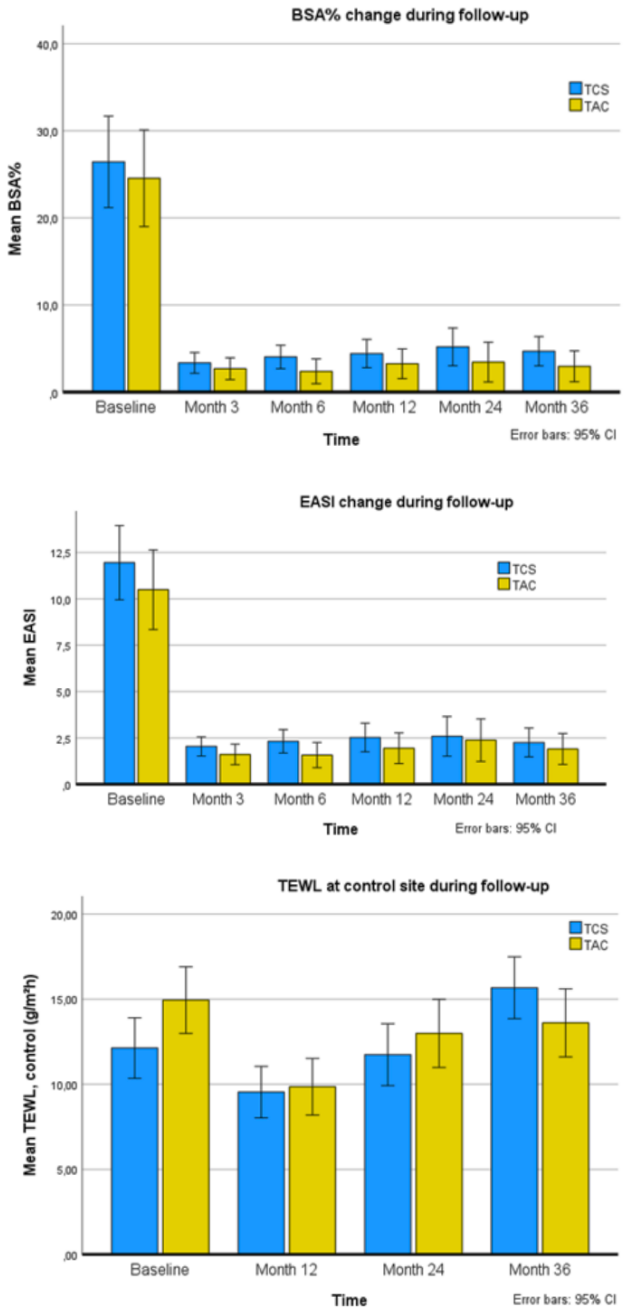

Figure 5 Mean changes in BSA, EASI, and control-site TEWL during the study. BSA, Body Surface Area; EASI, Eczema Area and Severity Index, TEWL, Transepidermal water loss. TCS= Topical corticosteroids, TAC= Tacrolimus. General linear models.

6.1.3 Other parameters

IGA and IDQOL changed similarly in both groups from baseline. Mean IGA decreased from baseline by 1.9 (±1.1) in TCS, and by 2.2 (±1.0) in TAC. Of the 122 study patients (after final 30 dropouts) at 36 months, 49.2% (n=29) in TCS had IGA 0 or 1 (clear or almost clear) compared to 66.7% (n=40) in TAC (P=0.053, χ^2). Of these patients, 9 in TCS, and 15 in TAC had had severe AD at baseline. Mean IDQOL change was 4.6 (±4.3) in TCS, and 5.3 (±5.1) in TAC. Change in mean Itch-VAS was the same (3.5 points) in both groups.

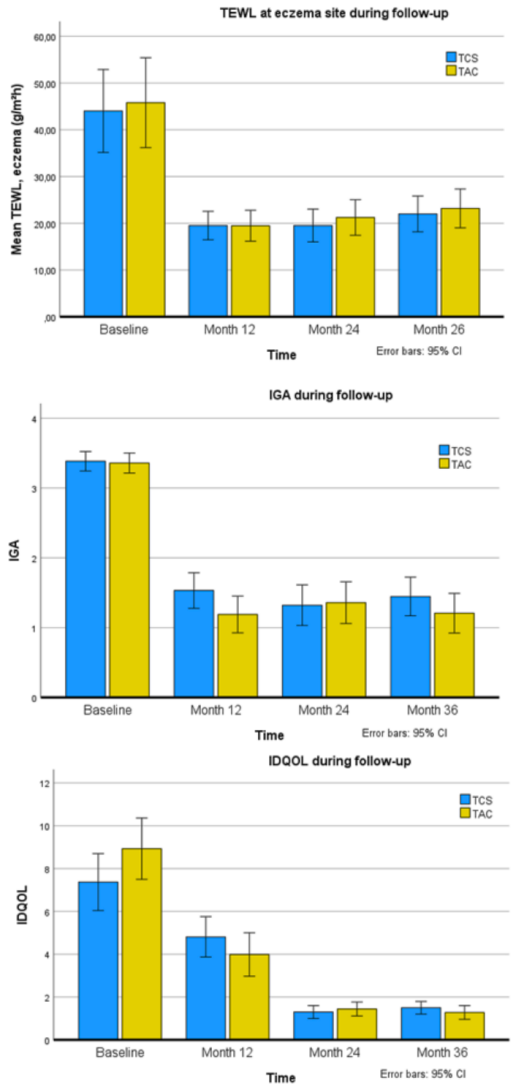

Figure 6 Mean changes in eczema-site TEWL, IGA, and IDQOL during the study. BSA, Body Surface Area; EASI, Eczema Area and Severity Index; IDQOL, Infants Daily Quality of Life Index; TEWL, Transepidermal water loss. TAC=tacrolimus, TCS=topical corticosteroids. General linear models.

6.1.4 Early versus other eczema treatment responders

We defined 'early' vs. 'other' eczema responders as differences in treatment response at 3 months in BSA (0–1.0% vs. ≥ 1.1%), EASI (0–1.0 vs. ≥ 1.1), and IGA (0–1.0 vs. ≥ 1.1). The only difference was seen in EASI: 32.9% of the patients (n=23) in TCS, and 52.2% (n=35) in TAC had EASI score <1 at 3 months (P=0.025, χ^2). Early eczema responders and other responders did not differ at baseline in terms of sex, disease severity, atopic sensitisations, eosinophil counts, or serum IgE levels.

6.1.5 Ointment usage

During the first 12 months in study I, 69.3% (52) of the patients had a need for stronger treatment option. In TCS 66.7% (24) of patients used class II steroid (0.1% hydrocortisone butyrate), and 71.8% (28) used 0.1% tacrolimus in TAC. After first year, ointment consumption was less accurate to define since many families forgot to bring the tubes to follow-up visits.

6.2 SAFETY

6.2.1 Adverse events

No signs of skin atrophy were observed with corticosteroid regimen. The most common adverse event with tacrolimus was an uncomfortable feeling after ointment application, reported by caregivers. This usually disappeared during the first days or week of the treatment. No patients showed clinically significant changes in serum chemistry or haematology, and none suffered from any serious adverse event like malignancies. Main safety outcomes are shown in Table 5.

6.2.2 Infections

In total 223 skin-related infections (SRIs) were observed of which 105 bacterial (impetigo, folliculitis, boils, and erysipelas), 88 viral (warts, molluscum, herpes simplex), and 31 others (tinea or *Candida,* based on clinical evaluation). No differences in infections occurred between groups.

Similarly, no differences emerged with 1357 non-SRIs where most of these were respiratory (n=980, upper respiratory infections, pharyngitis, tonsillitis, and laryngitis). Mean number of respiratory infections per patient was 6.6 with a range from 0 to 26 for 3 years. In addition, 74 viral rashes, 163 otitis media cases, and 139 other infections (mainly gastrointestinal and urogenital) were observed. No severe infections occurred in either treatment group.

Table 5 Safety outcomes of the study population.

VARIABLE	TCS	TAC	*P*-value[a]
SRIs, n			
Total	100	123	0.20
Bacterial	51	54	0.89
Viral	38	50	0.16
Other	11	20	0.34
Non-SRIs, n			
Total	671	686	0.50
Respiratory	498	482	0.27
Viral rashes	33	41	0.66
Otitis media	78	85	0.85
Other	61	78	0.50
Malignancies, n	0	0	
Decreased vaccination responses, n (%)			
Patients, total	15 (20.0)	13 (16.8)	0.62
- For 1 agent	11	6	
-For 2 "	3	5	
-For ≥3 "	1	2	
S-cortisol, median (IQR)			
Baseline	168.0 (138.0-218.0)	152.0 (123.5-187.7)	0.36
Month 12	173.0 (139.3-216.0)	182.0 (128.0-242.0)	0.84
Month 24	174.0 (134.0-218.0)	178.0 (129.0-254.3)	0.38
Month 36	206.0 (153.5-280.0)	170.5 (137.5-263.0)	0.23
Pathological at some point*	1 patient	0	
Pathological S-tacrolimus			
1 week	-	9 patients**	
Month 12	-	1 patient**	
Month 24	-	0	
Month 36	-	0	

[a]Mann-Whitney U-test. *Reference values for serum cortisol levels: 2 mo-1 y, 66-632 nmol/L; 2-13 y, 69-632 nmol/L. **All patients with detectable tacrolimus concentrations (≥1.5 μg/L) had used topical ointment on the same day blood samples were taken contrary to instructions. All had normal (undetectable) values <1.5 μg/L in control samples. SRI= skin-related infection, TCS=corticosteroids, TAC= Tacrolimus

6.2.3 Blood cortisone and tacrolimus levels

Serum cortisone levels were measured from all patients (both treatment groups) while tacrolimus concentrations were only examined in TAC group. No differences in cortisone levels were found between groups at any time

point. Only one patient in TCS group had pathological serum level below normal (56 nmol/L) during first year of treatment. For this individual, an ACTH test was performed with a normal result. Subsequent cortisone levels of the patient were all within a normal range.

In TAC group nine patients had detectable (≥1.5 μg/L) tacrolimus blood concentrations after 1-week, and one patient after 1-year topical treatment, all within a range of 1.5 to 5.6 μg/L. All patients had used topical TAC on the same day contrary to instructions. They all had non-detectable levels in control samples taken few weeks after hand. However, two patients were noticed to have ichthyosis vulgaris at the beginning of the study after elevated tacrolimus levels and following clinical check-up. They become hence dropouts. No detectable tacrolimus levels existed at any patients at months 24 and 36.

6.2.4 Growth parameters

Growth parameters were similar in both treatment groups both before and after treatments. Mean change in height from baseline until 36 months was 26.0 (±3.8) in TCS, and 25.8 (±3.8) in TAC. Mean hight parameters were below the SD for children in Finland (13) both at baseline (TCS -0.4, TAC -0.6) and after 36 months (TCS -0.2, TAC -0.4).

Weight measurements followed similar pattern. Mean weight change for TCS was 5.8 (±10.7), and for TAC 7.1 (±1.5) (p=0.8).

6.2.5 Vaccination responses

No differences in vaccination responses occurred between treatment groups as seen in Table 3. Altogether 28 patients manifested with decreased responses with no differences regarding AD severity, infections, or cytokines (IL4, -10, -13, -31, or IFN-ɣ) between treatment groups.

However, more infections were observed in patients with decreased or absent vaccination responses in both groups compared to patients with normal ones. In the TCS group there were mean 2.33 SRIs and 10.2 non-SRIS per patient with pathological vaccination responses compared to 1.35 SRIs and 9.07 non-SRIs per patient with normal responses. In TAC group on average 1.69 SRIs and 12.31 non-SRIs/patient with decreased responses were observed compared to 1.64 and 9.15 with normal vaccination responses.

6.3 ATOPIC SENSITISATION

6.3.1 Immunoglobulin E and eosinophils

Serum total IgE increased during the study with both treatments. Mean change in IgE for TCS was 171.3 (±474.1) and for TAC 623.0 (±2747.4), p=0.2. A couple of patients in TAC group had increased values as high as 20000 kU/L during the study. Blood eosinophil counts decreased in TCS group with mean change by 0.1 (±0.4) while the change for TAC was 0 (±0.5). However, the difference wasn´t statistically significant (p=0.4). Same patients in TAC group who had significantly increased total IgE during the study, also had simultaneously increased eosinophils towards the study-end. (Figures 7 and 8).

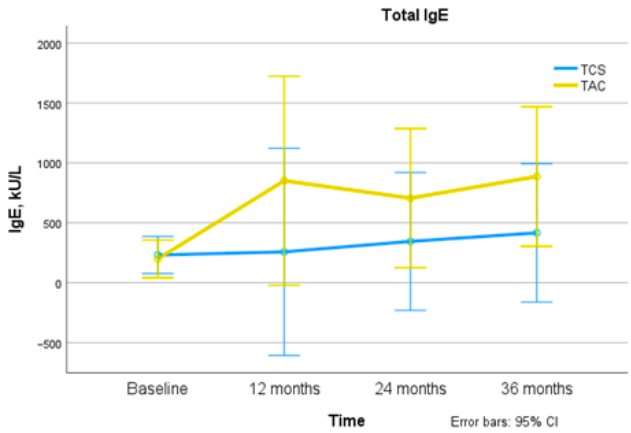

Figure 7 Mean changes in total IgE during the follow-up. TCS=Topical corticosteroids, TAC= Tacrolimus

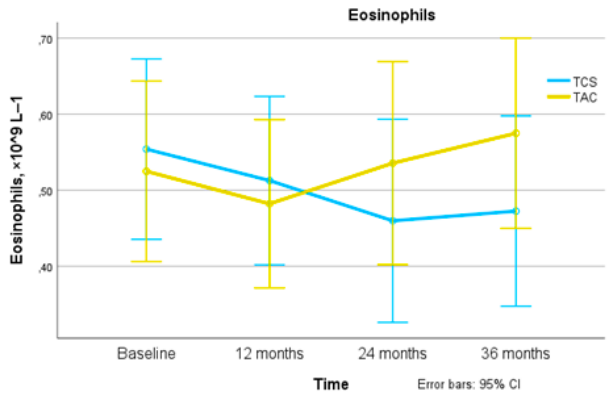

Figure 8 Mean changes in blood eosinophil counts during the follow-up. TCS=Topical corticosteroids, TAC= Tacrolimus

Nearly half of the patients (47%) in both groups had increased total IgE already at baseline. At 3 months, those with higher baseline IgE had also more eczema (BSA% > 1% vs. ≤ 1%), p=0.027. Around 60% had blood eosinophils ≥ 0.4 at baseline in both groups.

6.3.2 Food and aeroallergen specific Immunoglobulin Es

Food sensitisation (food specific IgEs) occurred at baseline in 51% and at 36 months in 53% of patients (in 41% of the ITT population) without differences between treatment groups. Most common ones at the beginning were egg, milk, wheat, and peanut. At the end there were equal amounts of sensitisation for egg and milk, followed thereafter by wheat and peanut. Polysensitisation (sensitisation to ≥ 3 food allergens) occurred both at baseline and at 36 months in 25% of patients (19% in the ITT population at study-end).

Apart from less animal sensitisation in the TAC vs TSC group (12 vs 25 patients) at baseline, there were no differences between the groups concerning aeroallergen sensitisation (aeroallergen specific IgEs). Sensitisations to birch or grass were the most common ones and were seen at baseline baseline in 21 (29%) patients in TCS, vs 18 (23%) in TAC group. At study-end, of the patients who completed the study, 34 (58%) were sensitised to birch or grass in TCS, and 29 (48%) in TAC group, p=0.31.

6.3.3 Skin prick tests

At baseline, 60% of patients had positive SPTs (at least one) whereas 67% of the patients who completed the study had SPT positivity at 36 months. When only the intention-to-treat (ITT) population was considered, 53% showed SPT positivity at final follow-up. No differences were observed between treatment groups in sensitisation patterns.

6.3.4 Early sensitisation

Early sensitisation was defined with one or more of the following: a positive SPT, specific IgE(s) to food or aeroallergens, elevated serum total IgE, or an elevated eosinophil count at baseline. Among those followed until 36 months, 60% children were early sensitised.

6.4 LUNG FUNCTION AND AIRWAY HYPERRESPONSIVINESS

6.4.1 Test results

At study-end, 63 patients were able to perform fractional exhaled nitric oxide (FeNO) test successfully, with no differences between treatment groups.

Significant exercise-induced bronchoconstriction (EIB) was observed in 22% of the 152 ITT patients (n = 18 in the TCS, and n = 15 in the TAC group, P=0.54), and in 28% of the 122 patients who completed the whole study, with no significant difference of change in resistance (ΔRrs5) between groups (Table 6). Both baseline food, and aeroallergen sensitisation correlated with the degree (change of Rrs5%) of EIB after running test (Pearson 0.36, < 0.001; and 0.31, P=0.001, respectively).

At the study-end, 102/122 patients performed the methacholine bronchial challenge test successfully. No significant differences were found between treatment groups.

Table 6 Comparison of airway outcomes during the study

Variable	TCS	TAC	95% CI[a] p-value	p-value
Mean (SD)				
Exhaled nitric oxide, ppb	9.8 (±10.0)	13.2 (±13.7)	3.4 (-9.5 to 2.6)	0.3
Exercise test,change of Rrs5Hz,%	28.8 (±28.9)	23.6 (±16.2)	5.2 (-3.4 to 13.9)	0.2
Methacholine test,PD40, µg	470.9 (±593.9)	556.0 (±678.7)	85.1 (-336.0 to 165.9)	0.5

[a]Independent samples T-test. TCS=corticosteroids, TAC=Tacrolimus.

6.4.2 Symptoms

The most common respiratory symptom was night cough (TCS group 53% and TAC group 48% of patients), followed by dyspnoea (TCS 29% and TAC 26%), wheezing (TCS 28% and TAC 14%, p=0.05) and exercise-induced symptoms (TCS 19% and TAC 9%). There were more wheezing episodes in TCS (n= 39) than in TAC (n=17) group (P=0.048).

AR symptoms were documented in 33 (44%) TCS vs 27 (35%) TAC patients in ITT population (P=0.35). In patients who were followed up until the study-end AR existed in 44% of TCS vs 43% TAC patients. Of the patients with severe AD at baseline 18 (53%) had AR in TCS group vs 7 (19%) in TAC, p=0.004. In general, AR coexisted more often with EIB (22/33; 67%) than without it (32/86; 37%, P=0.004).

6.4.3 Early sensitised patients

We observed few differences in eczema and airway inflammation parameters between TAC and TCS groups in a small exploratory analysis at 36 months in patients with either elevated serum total IgE (n = 71) or with aeroallergen sensitization (n = 53) already at baseline (Table 7).

Table 7 Comparison of eczema and airway outcomes at 36 months in patients with elevated total and aeroallergen specific IgEs at baseline.

Variable	Elevated total IgE[b]				Elevated aeroallergens			
Mean (SD)	TCS	TAC	95% CI[a]	p	TCS	TAC	95% CI[a]	p
Number of patients	35	36			29	24		
BSA%	7.7 (±8.8)	2.9 (±6.6)	4.8 (0.5-9.1)	**0.03**	8.8 (±9.6)	3.5 (±7.4)	5.3 (-0.2 to 10.8)	0.06
Exercise test,change of Rrs5Hz,%	40.4 (±37.8)	23.6 (±16.4)	16.8 (-0.3 to 33.8)	0.054	50.3 (±37.3)	27.0 (±18.1)	23.3 (3.3 - 43.3)	**0.02**
Methacholine test,PD40, µg	289.0 (±467.7)	642.2 (±800.6)	353.2 (-757.4 to 50.8)	0.09	247.3 (±448.4)	814.9 (±876.3)	567.6 (-1093.4 to -41.8)	**0.04**

[a]Independent samples T-test. TCS=corticosteroids, TAC=Tacrolimus

.[b]Reference values for total serum IgE: 6 mo-1 y, 0-70 kU/L; 2-3 y, 0-110 kU/L; 4-7 y, 0-130 kU/L.

6.4.4 Eczema response versus airway outcomes

There were no differences in airway outcomes between early eczema treatment responders vs. other responders. The only exception was the association between EASI response and exhaled NO (ppb). Mean ppb for early EASI responders (n=29) was 8.6 (SD ±8.2) compared with 14.3 (±14.7), p=0.02, for later responders (n=31). Early and other responders did not differ at baseline in terms of age, sex, or disease severity.

6.5 FILAGGRIN AND OTHER ATOPY-RELATED GENES

The effect of coding variation in *FLG* and a few other genes with epidermal barrier or immune function was investigated by genotyping and targeted amplicon sequencing (study IV).

6.5.1 Genotype variants

Variants detected by genotyping in the patient cohort included the four most prevalent European *FLG* LoF variants Arg501Ter, Ser761fs, Arg2447Ter and Ser3247Ter, in addition to rs12730241 (G>A) and three *FLG2* variants (Ser2377Ter, Cys298Ser, and Gly137Glu). Other genotyped loci included Gln1754Ter, Ser1020Ter, and Val603Met for *FLG* and Thr1314fs, Cys298Arg, and Leu168Phe for *FLG2*, but they were monomorphic in patients.

A *FLG* LoF variant (Arg501Ter, Ser761fs, Arg2447Ter, or Ser3247Ter) was identified in 20 of 140 (14.3%) patients. The combined *FLG* LoF-variant (all above mentioned variants together) frequency (7.14%) was significantly higher than in the general Finnish population (2.34%), p=2.72E-05, OR=3.2. When variants were tested separately, only Arg2447Ter (n=8) showed a significant association with AD (p<0.01, OR=5.8). In addition, a modest association with moderate-to-severe childhood AD was seen for rs12730241 (p=0.028, OR = 1.5) and rs6587667 (p=0.039, OR=3.6). The rs6587667 variant co-occurred with the rs12730241 variant in 19 of 20 controls and in six of six patients, indicating that the two variants are in linkage disequilibrium and not independent.

6.5.2 Effects of genotypes for transepidermal water loss

FLG LoF-variant carriers presented with higher TEWL values than noncarriers both at baseline eczema lesion (Ser761fs), (median 54.5 vs 38.2, p=0.021), and at previous eczema site at 36 months (combined *FLG* LoF), (23.9 vs 17.4, p=0.029), consistent with epidermal barrier impairment. Nevertheless, LoF-variant carrier status was not linked to disease severity (Figure 9). The rs12730241-A allele correlated with significantly lower TEWL (16.8) at study-end, p=0.036. There were no differences in clinical parameters between rs12730241-A allele homozygotes (n = 10) and heterozygotes (n = 49).

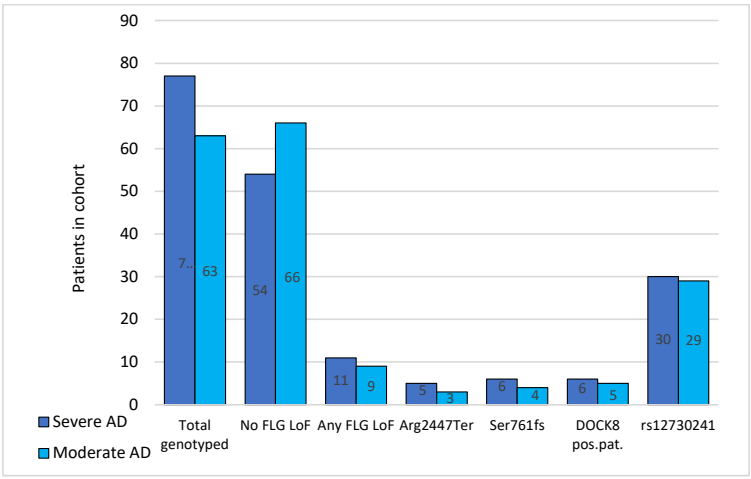

Figure 9 Severity of AD at baseline in relation to *FLG* and *DOCK8* variant status. The numbers in bars represent the number of patients in respective severity. Pos.pat= possibly pathogenic.

6.5.3 Candidate-gene study

An exploratory candidate-gene study by amplicon sequencing did not reveal LoF variants, previously reported pathogenic variants, or statistically significant enrichment in the 13 candidate genes studied.

However, most of the rare variation was found in *DOCK8* (21 missense variants at 13 loci in 19 patients). After applying a Combined Annotation Dependent Depletion (CADD)[250] score cutoff of 15 (the median value for all possible canonical nonsynonymous and splice variants in CADD), nine *DOCK8* missense variants in 11 patients were considered potentially harmful. Two patients had two such *DOCK8* variants. They presented with parental AD, moderate disease, normal serum total IgE levels, slightly elevated eosinophil counts (0.40–0.53 E9/l), and positive aeroallergen sensitisations at baseline. They had no severe or frequent infections.

In general, carriers of the potentially harmful *DOCK8* variants (n = 10) had significantly increased total IgE and eosinophil counts in comparison with noncarriers (n = 110) both at baseline (IgE: 374 vs. 70 kU/l, p=0.003; eosinophils: 0.71 vs. 0.44 E9/l, p=0.025) and at 36 months (IgE: 671 vs. 147 kU/l, p=0.002; eosinophils: 0.59 vs. 0.38 E9/l, p=0.032). *FLG* LoF carrier was excluded from this analysis.

6.6 DROPOUTS

There were two patients diagnosed with ichthyosis vulgaris at the beginning of the study after which they become dropouts. Before 24 months follow-up there was altogether 17 dropouts. Thereafter 10 more patients become dropouts. Before final 36 months follow-up additional 3 patients dropped out.

Reasons for quitting were as follows: Doctors' decision/lack of efficacy (11), unknown/lost to follow-up (8), prohibited therapy (5), moving away (3), and parents ´or caregiver´s withdrawal of consent (3). In sum, there we 30 dropouts of which 13 in TCS and 17 in TAC.

7 DISCUSSION

The natural history of AD remains partly unknown. It is characterized by highly variable disease course with alternating flares and remissions. Mathematic models and analyses have recently been used to identify disease subgroups based on similar AD activity patterns, or trajectories, over time. Here, early-onset AD seems to possess a specific, more severe disease pattern.[253] Emollient-based intervention studies as primary prevention have, at least not yet had convincing results.[228] Also, the impact of early microbial exposures as well as the role of probiotics and breastfeeding have been studied with relatively limited evidence.[254-256]

It has previously been estimated that over half of all children with early-onset atopic dermatitis are sensitised to one or more allergens.[257] The idea with secondary prevention in AD is to aim to optimize disease management with possible inhibitory effect on progression.[258] With this thought we followed originally children with moderate-to-severe AD, treated topically with either mild-to-moderate corticosteroids or tacrolimus (0.03 and 0.1%).

Both eczema treatments, TCS and TAC, were effective and well tolerated without differences in efficacy. There were no serious adverse events in either group. Atopic sensitisation was common in both groups and one third of the patients had exercise-induced bronchial hyperresponsiveness at the end of follow-up. In general, there were no differences in sensitisation patterns or airway outcomes between treatments. FLG LoF- variant was found in 14.3% of the patients.

7.1 EFFICACY

There was a rapid and sustained decrease in eczema efficacy parameters BSA and EASI with both treatments thorough the study. Traditionally, topical corticosteroids have been used since many decades to treat skin conditions like AD, although unlike with topical calcineurin inhibitors, there´s a lack of good-quality studies on their efficacy versus placebo in AD, especially on children.[259-262] However, especially when treating children and moderate-to-severe disease, active comparator trials are needed for clinicians to be able to compare real treatment outcomes and options.[263]

Comparative studies have shown TAC to be at least as effective as mild-to-moderate TCS.[206,264,265] This is in line with our findings. A recent randomized clinical trial showed children treated with 0.03% TAC, compared to ones with 1% hydrocortisone cream, to have lower inflammatory markers IL-10, IL-17, and IL-23 after 3 weeks. There was, however, no difference on the severity scale.[266]

Measurement of TEWL is often used in clinical studies as indicator of skin barrier function.[267] Higher values have been documented in AD patients both in lesional and clinically unaffected skin.[268-270] In our study after 12 months, control-site TEWL was at lowest in both treatment groups. This could reflect the more frequent follow-up visits and perhaps even compliance during the first year of the study.

In addition, we found that *FLG* LoF-variant carriers presented with higher TEWL values than noncarriers both at baseline and at previous eczema sites at 36 months, consistent with epidermal barrier impairment. This is in line with previous observations. Carrying a *FLG* LoF variant has shown to contribute to reduced amounts of natural moisturizing factor, and elevated TEWL in the skin.[271,272] *FLG* features also intragenic CNV with allelic variation of either 10, 11, or 12 FLG repeats, which may affect the expressed filaggrin amount, as well as the urocanic acid concentration in the epidermis.[273] The frequencies of CNV have been identified in Irish children as a risk factor for AD with a dose-dependent effect.[273]

Towards the study-end TEWL increased more in TCS than in TAC group. This could be due to different treatment regimens: TCS were advised to be applied as needed in case of flares, whereas TAC treatment was based on twice-weekly application after the initial remission. A small German study compared 0.1% TAC with mometasone furoate cream for 10 days and found significant TEWL and skin hydration improvement only in the TAC group.[274]

Also, different formulations may play a role: AD-induced mice treated with topical corticosteroid gels containing liposomes or nanoparticles maintained better TEWL values after TCS treatment than the ones treated with conventional formulation.[275] Both TCS and TCI have been documented to normalize the expression of filaggrin and loricrin, but TCS decreased also the epidermal structural protein involucrin compared to TCI.[276] In addition, unlike TAC, prolonged use of TCS reduced filaggrin in the skin.[277,278]

7.2 OINTMENT USAGE

In study I during the first 12 months (of the first 75 patients) as much as 69% of the children needed more potent treatment (0.1% hydrocortisone butyrate in TCS and 0.1% ointment in TAC group). Severe vs moderate disease groups did not differ in their response to treatment nor did the different treatment groups. The requirement of more potent treatment is interesting since before referring children to our tertiary clinic they had already been followed by several doctors and nurses at the primary care. The Current Care Guidelines for AD from that time recommended mild, but also moderately potent TCS in adequate amounts for treatment of children with AD.[279]

This finding leads to the thought that are children with more severe AD undertreated due to fear of more potent treatment options by parents or even health care professionals.[280,281] Parental education and a good doctor-patient

relationship is a cornerstone of treatment management in a childhood chronic disease like AD. It is valuable not only regarding symptom relief, but also concerning the quality of life.[282-285] A long-term strategy including established doctor–patient trust and possibly even a written plan, based on shared decision-making between health care personal and patient or caregiver, is of value.[286,287]

We followed the patients frequently during the first year with study nurse weighing the treatment tubes on every visit. This indicates that the need for more potent options with both TCS and TAC was due to a real need for more potent therapy and not due to non-adherence. Hence, the result can be considered as reliable. In addition, we noticed a great need for discussion and questions at every follow-up, especially in the beginning. Families were allowed to contact study nurse, and if needed, the dermatologist by phone during the 36-month follow-up.

7.3 SAFETY

Safety assessments showed only minor adverse events. No signs of skin atrophy were observed with TCS regimen. In literature adverse events such as skin thinning have occurred with low frequency and been associated with long-term treatment with potent TCSs.[288,289]

The most common adverse event with TAC was a burning sensation in the treated areas which usually disappeared during the first days or week of the treatment. This is in line with previous studies. In adult patients treated with 0.1% TAC, 47% experienced a burning sensation at treatment initiation.[203] In a paediatric study, 21% had transient application-site burning with 0.03% TAC at the beginning, and in a meta-analysis comparing pimecrolimus with 0.03% TAC, more pimecrolimus-treated paediatric patients experienced this adverse event.[210,290]

None in our cohort showed clinically significant changes in serum chemistry or haematology, and none suffered from any serious adverse event like malignancies.

In general, systemic absorption of topically administered tacrolimus has shown to be very low. Even frequent topical administration, as with continuous therapy, has not been associated with local or systemic adverse effects.[209,291-293] Nine patients in TAC group had detectable TAC blood concentrations (1.5-5.6 μg/L which all normalized in the control samples within few weeks. These patients had used TAC ointment on the sampling day, contrary to instructions. In addition, two patients with temporary detectable values were diagnosed with ichthyosis vulgaris. Ichthyosis vulgaris causes abnormal skin barrier function, keratinization, and desquamation.[294]

No clinically or statistically relevant differences emerged in growth parameters during the follow-up. Similarly, no differences concerning SRIs and non-SRIs between treatments were recorded. Impetigo, warts and

molluscum were common SRIs. The results are in line with the findings of others.[295-297] Skin infections in AD are commonly caused by *S. aureus,* and occasionally by *Streptococcus pyogenes* due to alterations in skin barrier, immune dysfunction, and skin microbiome.[298] A large cohort study found patients with AD to be 55% more likely to develop impetigo compared with controls.[299] Similarly, individuals with AD are thought to be susceptible to more severe and widespread molluscum infection, caused by poxvirus.[300]

Most frequent non-SRIs were respiratory infections. Mean number of respiratory infections per patient was 6.6 with a range from 0 to 26 during the 3-year follow-up. On average, Finnish children suffer 6–8 upper respiratory tract infections per year.[301] A Swedish prospective questionnaire study found an association between AD and respiratory infections in children up to 2 years of age.[302] In a Norwegian cohort, atopic disease was a strong determinant of all upper respiratory tract infections in 4-5 –year-old children.[303]

Decreased vaccination responses were documented in 18% of patients with no differences between treatment groups. Interestingly, more infections were observed in patients with decreased or absent vaccination responses than others. The amount was same in both treatment groups. Previous reports have not found signs of impaired vaccination responses with topical AD treatments.[304-306]

However, a couple of studies have reported impaired responses to vaccines in AD patients in general. A study of young infants aged 6-18 months with moderate-to-severe AD investigated mononuclear cell cytokine-responses and found impaired production of IL-10 regulatory cytokine to tetanus and diphtheria vaccines compared to unaffected children.[307] In another study investigating the immunogenicity of trivalent inactivated influenza vaccine, differences in antigen-presenting cell function between AD and non-AD individuals were found.[308]

Withdrawals from the study were mostly due to noncompliance, doctors' decision to change the treatment, use of prohibited therapy, or moving away.

7.4 ATOPIC SENSITISATION

Serum total IgE increased during the study. Around 50% of the patients were sensitised to food at baseline and remained so. Our results are consistent with previous findings whit 50-75% of children with early-onset AD being sensitised to one or more allergens.[257] There were no remarkable differences in sensitisation profiles between treatments.

Persistent total serum IgE levels in infancy have been associated with food sensitisation and the development of eczema in infants, and rhinitis and asthma later in early childhood.[309] In our study a significant increase in total IgE was associated with significantly increased blood eosinophils as well. Early manifestation of food allergy also increases the risk for later aeroallergen sensitisation.[310] While egg and cow´s milk sensitisations are common and

often transitory in most infants, sensitisations to fish and nuts are considered more permanent.[311]

In general, IgE levels seem to be only weakly or non-consistently correlated with eczema disease severity.[312] Many patients with AD are sensitised to foods without this playing a role in eczema activity.[136] However, impaired skin barrier can lead to increased allergen penetration and allergic sensitisation.[310] Early introduction of allergenic foods such as peanuts can enhance tolerance and decrease the risk for food allergies while exposures through compromised AD skin seems to increase the risk. [136,313]

Aeroallergen sensitization increased from one third at baseline to nearly half of the patients at study-end. In a recent European cross-sectional study of AD patients, sensitisation was most prevalent to *Dermatophagoides (D.) pteronyssinus* whereas sensitisation to birch pollen was detected in 20.5 %, and to cat epithelium in 18.2%.[314] In Finland and in our study most common sensitisations were birch, grass, and furry animals.[315] Unlike in some other countries, *D pteronyssinus* was not common in our study population, possibly due to different weather and indoor conditions compared to e.g. UK.

AR was documented in over 40% of the patients in both treatment groups with no differences between treatments. In a similar Nordic prospective study where AD children were followed up to 8 years of age, AR was found in 45% of the patients.[311] Having furry pets in the household and living in a diverse microbial environment has been associated with beneficial changes in the infant´s gut microbiota, possibly with a preventative effect on allergies.[316,317] However, to prevent AD, avoidance of neonatal cat, but not dog, exposure has been suggested, at least in individuals with ichthyosis vulgaris[294]

7.5 LUNG FUNCTION AND HYPERREACTIVITY

One third of the patients who completed the study had EIB. Baseline food or aeroallergen sensitisation correlated with the degree of EIB. In the previous adult study, BHR and eosinophilic airway inflammation were found to be more common in patients with AD than in controls. The prevalence of BHR was also higher in patients with AD, positive SPTs, and high serum total IgE levels.[318] Further, in a 10-year follow-up, eczema, respiratory symptoms, and BRH decreased with previous long-term TAC treatment.[319]

We found, however, no differences between TSC and TAC treated patients either with methacholine bronchial challenge test or oscillometry. It was previously thought, based on the atopic march-theory in transsectional studies, that up to one third of the children with AD at 4-year of age will develop asthma by the age of six.[320] In recent mathematic models and longitudinal studies, the amount for true atopic march has estimated to be less, highlighting instead the existence of different heterogeneous patterns or trajectories of atopic morbidities. Here, asthma and AR could exist even

without previous eczema.[126-129]. Also, with time, eczema can resolve spontaneously.[4]

In our patients with EIB, most (88%) had parental history of atopy. AR occurred in up to 67% of patients with EIB. These results are consistent with previous findings where AR or allergic sensitisation, and parental atopy are found to be risk factors for preschool asthma with simultaneous wheezing tendency.[321-324] Furthermore, increased asthma risk has been linked to the trajectory of early-onset, persistent AD with *FLG* LoF variants, and maternal asthma.[4,5,325-329] In our cohort we found no differences between TSC and TAC treatments according to allergic sensitisation, AR, BHR, or even asthma.

The development of asthma and airway hyperresponsiveness and inflammation is more complex than previously thought, influenced by interactions between genetic predisposition and the environment, such as viral infections. An atopic predisposition appears to predispose to development of allergic asthma, which is likely secondary to pathogenic inflammatory responses involving cytokines, chemokines and their receptors.[330]

We found in our long-term study, however, no evidence that we could interfere the development of airway hyperresponsiveness or inflammation with either one eczema treatment. In literature the evaluation of clinical predictors at already 4-5 years of age has found to be predictive for school-age asthma.[331] Asthma risk associated with early sensitisation may be related to early intense Th2-polarised long-term memory responses.[332] The association between asthma and sensitisation to aeroallergens continues through adolescence and even into the early adulthood. Therefore, it is critical that young children are identified who are at risk of developing asthma.

7.6 GENETIC ASPECTS

In recent years, research interest has been focused on identifying specific susceptibility genes of AD and other atopic diseases. New risk loci for AD have been found.[43,333]. Some of the newly found susceptibility genes that have previously been associated with allergic sensitisation, self-reported allergy, and asthma were found to have an association or genetic overlap with other inflammatory and autoimmune diseases, reflecting possibly the spectrum of comorbidities associated with AD.[333]

In amplicon sequencing of 13 AD-related genes, we did not identify any LoF variants, previously reported pathogenic variants, or statistically significant nonsynonymous coding region variants. However, we found it interesting that carriers of potentially harmful *DOCK8* variants had significantly increased total IgE and eosinophil counts in comparison with noncarriers both at baseline and at 36 months. All but one *DOCK8* carrier had allergic sensitisations at the study end. While DOCK8 deficiency due to recessive

damaging variants is a well-known cause of hyper IgE syndrome and a tendency to viral infections, the relation of DOCK8 with AD is less clear and sparsely reported thus far.[334-339].

In genotype study of 140 children having moderate-to-severe AD, *FLG* LoF variants were found in 14.3% of the patients. The combined FLG LoF variant frequency was significantly higher in study patients than in controls (7.14 % vs. 2.34%) and slightly higher than the 5.6% previously found in a Finnish adult AD cohort.[340] Homozygous or compound heterozygous *FLG* variants cause ichthyosis vulgaris and are known genetic factors predisposing to AD as well.[341] An increased penetration of allergens and chemicals has been documented in filaggrin-deficient skin.[342]

AD patients with *FLG* LoF variants represent a specific endotype[35] and were associated in our study, as mentioned, with higher TEWL values than noncarriers both at baseline and at previous eczema sites at 36 months.

The frequency of specific *FLG* LoF variants in people of African or Asian ancestry differs from those of European ancestry. Unlike in other ethnicities, Arg501Ter and Ser3249Ter are the two most common variants in northern Europe.[34,49,273,343-345] In general, the association between *FLG* variants and AD is less clear in people of African ancestry.[49]

7.7 STRENGHTS

Our study is one of the largest to follow long-term use of TAC in this age group. There have been studies comparing TAC with placebo, but not so much with active comparative treatments, especially in young children.

The prospective, investigator-initiated nature of the study can be seen as a strength. The study personnel remained the same during the 36-month follow-up and thus measurements and scoring of eczema and airway parameters can be considered more reliable. We could create a good relationship with families and provide information and advice when needed.

7.8 LIMITATIONS AND CHALLENGES

The restricted cohort size can be seen as a limitation regarding safety data. The cohort size was due to the study being a single-centre investigator-initiated clinical study with young infants and relatively frequent follow-up visits. The study cohort represented also selected patients of a tertiary-care centre. We could not analyse long-term data on child development, growth parameters, and AD disease course because the follow-up time was limited to 36 months. Possible delayed effects after the follow-up period, such as developmental problems or malignancy incidence could not be investigated.

Further, TEWL was measured from the most severe eczema lesion at baseline, and after that at 12 months from the currently most severe lesion. We estimated this to be the most representative. However, we took later notice

that it might not have been optimal due to possible site variation. This is why we performed following TEWL eczema measurements (including at 36 months) to be taken at the same site as at baseline. However, the control site TEWL (which showed most differences between the groups at 12 months and at study-end) was always taken at the same site (left forearm). Furthermore, it is a point of debate whether TEWL, reflecting the inside-out barrier, is a good-enough predictor of the ingress of skin irritants and allergens (the outside-inside barrier). An additional measurement tool of skin hydration such as Corneometer could have brought supplemental information.

Another limitation was that the infection data were retrieved mostly retrospectively at each follow-up visit, and that these data were based mainly on information from parents/caregivers. Thus, the type, severity and treatments of infections could not always be confirmed completely from health record data or other documented sources.

The lack of placebo group can be seen as a limitation. Due to the long-term nature of the trial, we considered a placebo group unethical since severe eczema causes a high disease burden. In addition, there had also been vehicle-controlled studies with TAC but less with active comparative treatment resembling real-life circumstances.

In genetic studies the small cohort size was a limiting factor. Also, due to technical challenges in primer designing for highly homologous regions, we failed to cover all *FLG* gene coding regions. Therefore, analysis of the *FLG* gene was limited to variants in nonhomologous regions at the ends of the gene, and to the variants covered by genotyping. In addition, since amplicon sequencing failed to cover most of the *FLG* gene, other rare or, to our knowledge, previously unreported *FLG* LoF variants could not be detected, and hence the reported total *FLG* LoF frequency found by genotyping in our cohort may be an underestimation. Also, it should be noted that when analysing FLG, analyses like copy number variation were not performed.

8 CONCLUSIONS

To our knowledge, this was the first long-term comparative follow-up study of TSC versus TAC in toddlers from the age of 1 year onwards. The current study demonstrated the safety of both eczema treatments, TCS and TAC, without differences in efficacy.

There were no signs of skin atrophy or growth suppression associated with long-time use of TSC during 3-year follow-up. Neither serious adverse events, like severe infections or malignancies, occurred during the 3-year follow-up with either treatments. More potent options, 0.1% TAC or mid-potent TSC, were well tolerated and needed in over two thirds of the patients suggesting that children with moderate-to-severe AD may often be undertreated and in need of stronger but nevertheless safe ointment options.

While AD disappears in many toddlers, some with severe early-onset AD may have a more difficult disease pattern, often associated with early sensitisations. Atopic sensitisation was common and detectable in over half of the patients both at baseline and at 36 months. AR occurred in 40% in both groups, but up to 67% of those with exercise-induced bronchoconstriction, reflecting the close relationship between upper and lower airways. One third of the patients had exercise-induced airway symptoms at the end of follow-up. In general, there were no differences in sensitisation patterns or airway outcomes between treatments.

FLG LoF- variant was found in 14.3% (20 of 140) of the paediatric AD patients. The combined FLG LoF variant frequency was significantly higher in study patients than in controls (7.14 % vs. 2.34%), and higher than the previously reported 5.6% frequency in a Finnish adult AD cohort. That *FLG* LoF-variant carriers presented with higher TEWL values than non-carriers, is consistent with epidermal barrier impairment due to lack of moisturizing effect of filaggrin protein. In general, TEWL was at lowest at 12 months in both treatment groups reflecting possibly the more frequent study visits and better compliance during the first year. That control site TEWL increased more with TCS than TAC towards the end could be due to different treatment regimens, or due to different effects on skin barrier in the long-term treatment.

Even though we didn´t find enrichment of rare pathogenic AD variants with amplicon sequencing, the carriers of potentially harmful *DOCK8* variants had significantly increased total IgE and eosinophil counts in comparison with non-carriers. This result suggests that role of *DOCK8* variation in AD should be further investigated in larger cohorts.

9 FUTURE PERSPECTIVES

There´s a new active era going on with AD research with new investigations, findings, therapeutic options, and promising scenarios, even for severe childhood AD.[346-349]

Although true atopic march seems to happen in less degree than previously thought in transsectional studies, it still represents a one possible trajectory of early-onset severe AD. Finding infants and children with this trajectory pattern is important. We have planned and initiated a following questionnaire-based study for the study cohort presented here to follow up children with possible risk of developing asthma in school-age.

The immunological activity already in nonlesional infant skin with AD could suggest the need for early (systemic) intervention at disease initiation.[49] In the future, maybe with the help of intelligent individual biomarkers and more precise endotyping prevention of atopic multimorbidity could be possible.[350]

Modulating Th2 signalling could represent an option for influencing the atopic multimorbidity pattern. A recent meta-analysis concerning dupilumab and the incidence of allergic events showed that the risk of new or worsening allergies was reduced by 34% with dupilumab treatment.[351] The effect was seen as reduced serum total IgE levels and EASI scores. Treatment benefit was greater for younger patients with severe early-onset (onset before 2 years age) AD.[351]

Immunotherapy could also be implemented even more in treatment of AD with aeroallergen sensitisation and/or AR, since aeroallergens may worsen flares in AD and even precede asthma.[352] Sublingual immunotherapy (SLIT) offers a practical and safe choice.[353] A large meta-analysis showed SLIT, particularly to house dust mite, to improve AD severity and quality of life.[354]

However, topical therapy is still the first-line, cost-effective treatment especially in young children with AD. Attention should be paid to efficacy, safety, tolerability, and hypoallergenic properties of components in topical treatments, vehicles, and skincare products. Caregivers should be advised to e.g., wash their hands before applying moisturisers and other products to children to avoid transcutaneous sensitization.[355,356] The use of mid- potency TCS for short courses during flares especially on lichenoid areas on limbs in children could support rapid symptom relief. For the optimal long-term treatment and to control and prevent flares, combining TSC with following TCI could be more effective than either alone.[357]

Furthermore, the findings of new AD associated loci with genetic overlap of other inflammatory and autoimmune diseases could contribute to new immune targeted therapeutic options along traditional therapies focusing on skin-barrier.[333] Although AD is a complex multifactorial disease, the increase in IgE and eosinophil counts documented in study in carriers of potentially

pathogenic DOCK8 missense variants suggests, that the role of DOCK8 variation in AD could be further investigated in larger cohorts.

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- Franklin Roosevelt

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12 ORIGINAL PUBLICATIONS I-IV