

OC01

Lower risk of atopic dermatitis among infants born extremely preterm compared with higher gestational age

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Extremely preterm infants (< 29 weeks of gestation) have a functionally immature skin barrier at birth, which can take more than four weeks to develop postnatally (1). In addition, the immune system of preterm infants is not fully developed, affecting the formation of tolerance and sensitization. Given the key role of skin barrier in atopic eczema (AE), extremely preterm infants might be at risk for developing AE. So far, AE risk has not been assessed in a large sample of extremely preterm infants and it is still not yet known whether the risk of developing (AE) is influenced by extremely preterm birth.

Here, we investigated the relationship between gestational age (GA) and AE using data from two independent french population-based cohorts. In EPIPAGE cohort, a total of 1,836 preterm infants were included in the analysis of whom 391 were born < 29 weeks. In LIFT cohort, a total of 493 preterm infants were included in the analysis of whom 88 were born < 29 weeks. We used a parental report of physician-diagnosed AE within the first five (LIFT cohort) or two years of age (EPIPAGE cohort) as the outcome variable.

There was a lower percentage of children with AE in the extremely preterm group compared to those born at a greater GA (EPIPAGE cohort, 2-year outcome: 13.3% for 24-28 weeks, 17.6% for 29-32 weeks, and 21.8% for 33-34 weeks [P = .002]; LIFT cohort, 5-year outcome: 11.4% for 24-28 weeks, 21.5% for 29-32 weeks, and 19.6% for 33-34 weeks [P = .11]). After adjusting for confounding variables including birth weight Z-score, cesarean section, age, use of systemic antibiotics during the neonatal period, family history of allergy, level of maternal education, and using the 33-34 week category as a reference, a lower GA (< 29 weeks) was significantly associated with decreased risk of AE in the EPIPAGE cohort

(aOR: 0.57 [95% confidence interval (CI): 0.37-0.87]; P = .009) and the LIFT cohort (aOR: 0.41 [95% CI: 0.18-0.90]; P = .03).

Among preterm infants, there is an association between very low GA (<29 weeks) and a decreased risk of AE compared with higher GA (29-34 weeks) and full term birth. Further studies are needed to confirm these results, and to understand how these findings are influenced by factors, such as the environment, nutrition, immune system development, and skin barrier function of very preterm infants.

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OC02

Detection of barrier-related gene mutations in atopic dermatitis with reverse blot hybridization assay

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Among the barrier-related genes, mutations of the filaggrin gene (*FLG*), serine protease inhibitor Kazal type5 (*SPINK5*), Kallikrein 7 (*KLK7*), and others, have been demonstrated in atopic dermatitis (AD). Because AD-related gene mutations vary significantly across ethnicities, we tried to find gene mutations in *FLG*, *SPINK5* and *KLK7* from Korean AD patients by utilizing data reported by Asians including Koreans. In addition, we aimed to develop a reverse blot hybridization assay (REBA) to apply to AD-related genes for the first time, and determine whether the REBA could be used to easily, cheaply and quickly detect complex barrier-related gene mutations like AD biomarkers.

We divided the subjects into moderate to severe AD and mild AD groups by eczema area severity index (EASI) score. We also divided the subjects into extrinsic and intrinsic AD groups. We collected blood samples, and then we extracted DNAs, amplified them through PCR, and checked on gene mutations using the REBA. The statistical difference was analysed between each variable.

The mutant type (MT) of *KLK7* was significantly more frequent in AD subjects than in control. Among AD subjects, MT frequency was higher in the moderate to severe group compared to the mild group. The MT frequency was not different between the intrinsic and extrinsic AD groups. MT was substantially more frequent in subjects with a higher EASI score. In *FLG* mutations such as c3321delA and pK4022X, the most frequent *FLG* mutations in Korean AD (>95%), there was no difference between AD and control subjects. In the *SPINK5* mutations, AD subjects more frequently had the mixed type of 603-49A>T, and the MT of 1188T>C and 2475G>T compared to control, but there was no difference between intrinsic and extrinsic AD. The MT of 2475G>T of *SPINK 5* was frequent in AD subjects with onset over 2-years-old. Among AD subjects, the moderate to severe AD group had more gene mutations compared to the mild group.

We found a correlation between the *KLK7* mutation (3'UTR AACC ins 5874) and AD, which has not been reported in Asians including Koreans, and a correlation between the mutations in 603-49A>T, 1188T>C and 2475G>T of *SPINK5* and AD, which also was not previously observed in Koreans. Furthermore, we verified that the REBA is fast, simple, and accurate, and can be applied to detect the barrier-related gene mutations in AD, which could be used as biomarkers to predict AD occurrence.

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OC03

Environmental factors have a profound effect on disease severity in skin barrier deficiency and eczema.

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Atopic dermatitis (AD) is one of most common skin disorders seen in childhood. It is recognised that epidermal barrier dysfunction is part of the complex aetiology. The double mutant (DM) flaky tail (maft) mouse is a model of skin barrier deficiency with, among other genetic mutations, an analogous mutation to the loss-of-function mutations in the human *FLG* gene, the most robust genetic risk for AD. The effects of different environmental conditions

on skin barrier deficiency are investigated in this study. Two different environmental conditions; individually ventilated (IVC) and conventional open (CON) cages, were used to house DM *maft* and control WT mice. Interventions involving altering conditions (CON-IVC environments), and antibiotic treatments (CON A/B), were assessed with respect to phenotype severity. Clinical dermatitis scoring, lung function tests, skin/lung histology, and antibody/cytokine ELISAs were recorded at 1, 4, 8, 20 and 32 weeks.

Clinical scoring was increased in DM *maft* mice from the CON group compared to those from the IVC ($p=0.02$) and CON-IVC ($p=0.02$) groups. Total IgE was higher in the DM *maft* CON group compared to the IVC ($p=0.01$) and CON-IVC (ns) groups. There were reduced cutaneous and lung inflammatory histological changes between DM *maft* mouse groups. Cytokine analysis, in particular that from the skin-draining lymph nodes, showed that DM *maft* mice had increased expression of IFN γ ($p = 0.02$) and IL-17 ($p = 0.02$) when bred and housed in CON conditions compared to their IVC littermates. There were no changes in airway hypersensitivity between DM *maft* and WT mice, increased lung compliance ($p < 0.05$) was noted in all groups of DM *maft* mice compared to WT.

This study suggests, that in the context of murine skin barrier deficiency, environmental conditions have an important influence on the development and severity of cutaneous inflammation. The research implies that such conditions will also affect systemic spontaneous atopy with long-term consequences for the lung phenotype in skin barrier deficiency. The thesis explores the interaction of the environment with the skin barrier and also highlights how altering the microbiome can influence and increase spontaneous atopy. The research discussed in this thesis illustrates how the DM *maft* mouse is a robust model of AD and is a useful tool for future research into the pathogenesis and treatment of this disease.

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OC04

The effect of controlled exposure to grass pollen in an environmental challenge chamber on dermal symptoms in patients with atopic dermatitis

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It has frequently been speculated that pruritus and skin lesions develop after exposure to aeroallergens in sensitized patients with atopic dermatitis (AD). The aim of the present study was to assess the cutaneous reactions to grass pollen in adult patients suffering from AD with accompanying IgE-sensitization to grass allergen in an environmental challenge chamber in a single center, double-blind, placebo-controlled study.

Subjects were challenged on two consecutive days by exposure to either 4000 pollen grains/m³ of dactylis glomerata pollen or clean air (placebo). The severity of AD was assessed at each study visit, prior and up to three days post challenge by clinical scores such as objective SCORAD (primary endpoint), IGA (Investigator Global Assessment) and “local SCORAD”. By objective SCORAD, which is a well-defined scoring system for AD, the extent and intensity items are considered while subjective symptoms are left out. Separate evaluation of air-exposed and textile covered skin areas was performed by IGA and “local SCORAD”. By “local SCORAD” the intensity of an air-exposed and a covered target lesion that had been defined prior to exposition was assessed. In addition, serum CCL17 (TARC) levels were determined by ELISA.

Exposure to grass pollen induced a significantly higher increase of objective SCORAD from pre-challenge to post-challenge day 3 in the verum group compared to the placebo group. Only in the verum group a significant worsening of air-exposed areas in comparison to textile covered skin could be observed. With exposure to grass pollen a trend of increased CCL17 could be observed.

This pilot study demonstrates that controlled exposure to airborne allergens of patients with an “extrinsic” IgE-mediated form of AD (as demonstrated by the presence of IgE for dactylis glomerata) induced a worsening of dermal symptoms. This proof-of-concept implies the need for allergen avoidance as a preventive measure in these patients. Aeroallergen challenges with airborne allergens might be a useful model to investigate novel drugs in AD.

OC05

Exome sequencing among Ethiopian Atopic Dermatitis patients

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Filaggrin is a key protein involved in maintaining the skin barrier function and stratum corneum hydration. Mutations in the filaggrin gene (FLG) cause Ichthyosis Vulgaris (IV) and are a major predisposing factor for atopic dermatitis (AD) in individuals with European and Asian descent. We have previously shown that FLG mutations are rare among Ethiopian AD patients (1). The purpose of this study is to identify other mechanism as causative for the barrier impairment in Ethiopian individuals with AD.

We performed whole exome capture with Agilent SureSelect Human All Exon 50M (Agilent) in 22 Ethiopian AD patients with severe AD/ IV phenotype. Exome capture libraries were sequenced on Illumina HiSeq2000 (Illumina). We analysed the data with our in-house pipeline and filtered the variants to determine candidate genes. After identifying candidate genes we confirmed our result in an Ethiopian case (n=159) control (n=197) study using the TaqMan (Life Technologies) genotyping method.

We showed heterozygous variants in several candidate genes involved in skin barrier function such as SPINK 5, evoplakin, DSG1, keratin 7 and claudin.

SPINK 5 mutations is underlying Nethertons disease and have previously been associated with AD in Caucasian (2) and Asian populations (3) but has to our knowledge not been investigated in African AD patients.

If a genetic cause underlying AD is separate in different populations this can

1. give us important knowledge of the global pattern of disease causing genes
2. give us information about the pathogenesis of AD
3. have an impact on personalized treatment strategies.

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OC06

“Hard domestic water increases the risk of developing infantile eczema”

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Cross-sectional studies from the UK, Japan, and Spain have demonstrated a positive association between domestic water hardness (calcium carbonate concentration) and eczema risk in schoolchildren. Similar associations have also been described for chlorine. However, it remains unclear whether the relationship is causal and whether high domestic water calcium carbonate and chlorine concentrations are triggers of eczematous skin inflammation in early life, potentially through gene-environment interaction. 1303 infants were recruited from the general population throughout England and Wales at 3 months of age and examined for eczema and disease severity (SCORAD index) and also screened for the 6 commonest filaggrin mutations (R501X, 2282del4, R2447X, S3247X, 3673delC, and 3702delG). Transepidermal water loss (TEWL) as an expression of skin barrier function was measured on unaffected forearm skin in gram/(m²×h). Furthermore, we gathered data on domestic water calcium carbonate (CaCO₃ mg/L) and chlorine (Cl₂ mg/L) concentration provided by local water suppliers. We considered sex, parental socio-economic status, number of siblings, a family history of allergic disease, FLG mutation inheritance, TEWL, installation of a water softener, frequency of bathing, use of moisturisers and topical corticosteroids as potential confounders. 23.7% (309/1303) of all participating infants had eczema by 3 months of age, with a median SCORAD of 7.5 (range 3.5-75.0). FLG mutation carriage was positively associated with eczema risk (OR=3.22 (95% CI 1.74-5.96) and raised TEWL in unaffected skin (OR=2.18, 1.27-3.74). Domestic water hardness was non-normally distributed with a median of 257 mg/L (IQR 162.25-286.00). Chlorine concentration showed a median of 0.37 mg/L (IQR 0.26-0.48). Using the median CaCO₃ concentration as cut off between ‘hard’ and ‘soft’ water, there was a significant positive association with eczema by 3 months, even after adjustment for confounders (adjusted OR=1.47, 1.12-1.92). FLG mutation inheritance and

installation of a water softener did not significantly alter these risk estimates, and eczema severity and TEWL were not influenced by water hardness and chlorine concentration. Domestic water chlorine concentration showed a non-significant positive association with eczema risk (adjusted OR=1.25, 0.94-1.66). We found a significant positive association between domestic water hardness and eczema risk already by 3 months of age, suggesting that higher levels of cutaneous exposure to CaCO₃ may be involved in the initiation of eczematous skin inflammation. An intervention trial is now required to see whether installation of an ion-exchange water softener around the time of birth may be able to reduce the risk of developing infantile eczema.

OC07

A canine model of atopic pruritus

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We aim at establishing a canine model of atopic itch, as dogs are the only large animal species that naturally develop a skin disease that closely resembles atopic dermatitis (AD) in humans. As a result, a dog model of atopic itch has a high translational potential to study the human disease.

Four Maltese beagle atopic (MBA) dogs were sensitized against house dust mite (HDM) allergens. Hypersensitivity was confirmed by the development of elevated HDM-specific IgE serum levels and atopic skin lesions after topical HDM challenge (1, 2). After successful sensitization was achieved, the right side of the dog's abdomen was painted with 25 mg of HDM, and the animals were monitored by video for 24 hours. This challenge was repeated three times at daily intervals.

There was a significant increase in itch manifestations (scratching, biting, licking, chewing) at the site of HDM challenges (median of ~ 1 min/24 hrs before versus ~ 30 mins/24 hrs after the first challenge). Although the cumulative scratching behaviour 24 hrs after challenge did not increase with each of the additional two allergen applications, there was an increase in the onset of scratching behaviour within the first 20 min of each successive challenge, which suggested the possibility of a cutaneous sensitization phenomenon. There was also a significant increase in night-time activity after HDM challenge. In two dogs, pilot studies utilizing a high cut off intradermal microdialysis technique (MW ≤ 100 kDa) did not suggest

a pivotal role of typical pruritogenic peptides and proteins in the acute induction of itch behavior. Indeed, substance P, cathepsin S, interleukin 31, endothelin-1, nerve growth factor or thymic stromal lymphopoietin were all below the recovery and/or detection thresholds of mass spectroscopy within the first hour after HDM challenge.

These results suggest that a canine model is suitable to study atopy-associated pruritus and to evaluate new targets for the treatment of itch in humans as well as in dogs.

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OC08

Experimental model of peanut allergy in dogs after epicutaneous sensitization

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Food allergy can be a trigger for atopic dermatitis (AD). Among foods, peanut allergy is important as it is common and can lead to severe reactions. It is proposed that food allergy and eczema may represent manifestations of common disease and that epicutaneous exposure exists for both. Epicutaneous sensitization to foods is speculated in humans and demonstrated in mice. However, AD does not naturally occur in mice, no mouse model replicates AD upon challenge, and sensitization requires tape stripping.

Dogs naturally develop AD which is strikingly similar to human AD. A colony of atopic beagles that naturally develops AD, has skin impairment and is epicutaneously sensitized to environmental allergens has been identified. Developing a canine model of food-induced AD is useful to test treatments that are risky in humans.

This study addressed 3 questions:

1. Can epicutaneous peanut exposure sensitize atopic dogs without artificial skin damage?

2. Can epicutaneous sensitization occur also in normal, non-atopic dogs, without skin damage?

3. Can flare-up of dermatitis be triggered after oral challenge in individuals epicutaneously sensitized?

Peanut paste was applied epicutaneously on 5 atopic and 5 normal beagle dogs twice weekly for 8 weeks. Paste was applied under occlusion to avoid ingestion. Blood was collected on days 0, 28 and 56 for allergen-specific IgE. Clinical signs were scored on days 0, 28 and 56. Skin biopsies were taken on day 0 and 56 for H&E and immunofluorescence for IgE. On day 56 dogs were challenged orally with peanuts and clinical signs scored. Intradermal skin test (IDT) with peanut allergen was done on day 66 in all dogs plus 3 atopic dogs never exposed to peanuts.

With sensitization pruritic erythematous macules, papules developed in both atopic and non-atopics on the area of paste application. After oral challenge pruritic dermatitis worsened and generalized in both groups. On day 56 serology and IDT was positive in 9/10 dogs and none of the atopics naïve to peanuts. H&E showed marked superficial perivascular eosinophilic dermatitis in atopics and mild eosinophilic dermatitis in 1 of the non-atopics. IgE were detected on skin biopsies of both groups but more intensely in atopics.

Epicutaneous sensitization to peanuts can occur in atopic and non-atopic dogs and does not require skin impairment. Once sensitization has occurred oral challenge triggers generalized erythematous pruritic dermatitis.

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OM01

A novel acoustic evaluation system for scratching behaviour in itching dermatitis: rapid and accurate analysis for scratching of atopic dermatitis patients

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Quantitative analysis of itching in patients with itching dermatitis including atopic dermatitis is indispensable for the evaluation of the disease activities and responses to various therapies. However, the objective evaluation system for itching is limited.

We have already reported an evaluation system for the AD model mouse scratching movements detecting their air-conducted sounds in a box. However, detection of the open air conducted sound of the human scratching is impossible. Then, we have developed a new objective and quantitative scratching sound detection system using a wristwatch type sound detector. The scratch sound detected on the wrist is acquired into personal computer through the filtering, squaring and smoothing process by specific hardware. Subsequently, the data was automatically processed and judged for the scratching movement using specific software based on the periodicity and the energy of signal. Twenty-four measurements for volunteers with healthy condition and atopic dermatitis by this system are evaluated comparing simultaneously recorded video analysis system.

Ratio of the scratching time in sleeping time evaluated with these two systems was almost identical. Normal healthy subjects scratch their skin around 2 minutes during 6 hours sleeping time. In contrast, the mean scratching time of AD subjects was 24 minutes in their sleeping time. In contrast time consuming video analysis system, this system takes only several minutes for evaluation of an overnight record.

This scratch sound detection system is expected for a new objective evaluation tool for the itching dermatitis, i.e., atopic dermatitis.

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OM02

Th17 cells and tissue remodeling in atopic and contact dermatitis

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Eczematous skin lesions of atopic dermatitis (AD) as well as allergic and irritant contact dermatitis (ACD, ICD) are characterized by the same typical clinical signs, although due to different causes. In both AD and ACD, the presence of T helper 17 cells which play an important role in host defense, has been reported. Furthermore, IL-17 is involved in tissue repair and remodeling.

This study aimed to investigate IL-17 expression in acute eczematous skin lesions and correlate it with markers of remodeling in AD, ACD and ICD.

Skin specimens were taken from positive patch test reactions to aeroallergens, contact allergens and irritants at days 2, 3 and 4. Inflammatory cells as well as the expression of cytokines and extracellular matrix proteins were evaluated by immunofluorescence staining and confocal microscopy.

ACD and ICD were characterized by IFN- γ expression, whereas in AD lesions, IL-13 expression and high numbers of eosinophils were the prominent phenotype. Expression of IL-17, but also IL-21 and IL-22, was observed in all eczema subtypes. The number of IL-22+ T cells correlated with the number of eosinophils. Markers of remodeling such as MMP-9, procollagen-3 and tenascin C were observed in all acute eczematous lesions, while a correlation of IL-17+ T cell numbers with tenascin C-expressing cells and MMP-9+ eosinophils was apparent.

The expression of IL-17 and related cytokines, such as IL-22, was demonstrated in acute eczematous lesions independent of their pathogenesis. Our results suggest a potential role for IL-17 in remodeling of the skin.

OM03

Evaluation of the atopy patch test as an *in vivo* model for the induction of atopic dermatitis

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The current understanding of the pathogenesis of atopic dermatitis (AD) is largely based on results from studies using the Atopy Patch Test (APT) as an *in vivo* model for AD. The model is assumed to induce similar immunological changes as observed in AD. However, comprehensive molecular profiling of the APT reaction has not been performed.

We sought to validate the APT as model for the induction of AD by comparing APT skin biopsies to chronic lesional skin samples from individual AD patients. We performed a broad gene expression (microarray) analysis comparing APT and lesional AD skin profiles.

We found high levels of similarity in gene expression profiles between APT (24 and 48 hours) and lesional AD skin samples. Genes were clustered in modules of signaling pathways. In 9 out of 11 modules the overall gene regulation in APT and lesional skin occurred in the same direction, including cell cycle, IL4/IL13, IL17/IL22, and the interferon responsive genes. The analysis also showed several differentially regulated genes when comparing the APT samples to the lesional skin. Furthermore, we observed similar T helper (Th) cell polarizations, with an upregulation and intensification of Th2 and Th22 responses in APT samples and lesional skin.

These results confirmed our hypothesis that the APT is an appropriate model for the induction of atopic dermatitis. In addition, we found differentially expressed genes in APT versus chronic lesional skin biopsies that may help us to explain the self-limiting nature of the APT. These latter genes may represent interesting new therapeutical targets.

OM04

Proteome analysis of stratum corneum from atopic dermatitis patients by hybrid quadrupole-orbitrap mass spectrometer

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The stratum corneum (SC) barrier is crucial for evaluation of AD. Although filaggrin, proteases, and other SC constituents are known to participate in the barrier, comprehensive evaluation of their abnormalities in individual AD patients remains to be addressed.

We sought to identify and quantify wide-ranging proteins and metabolites in SC by mass spectrometry to obtain a comprehensive and quantitative protein profiling. We collected SC by non-invasive tape stripping technique from the flexor surface of the forearm and upper arm of patients with extrinsic and intrinsic AD, ichthyosis vulgaris, and healthy subjects. All adhesives were dissolved and any attached SC was suspended in toluene. SC proteins were extracted and analyzed by hybrid quadrupole-orbitrap mass spectrometer. Substances were detected by Mascot search engine against SwissProt database.

We identified and quantified 440 proteins and/or metabolites. The substances were divided into five categories. Considerable amounts of inflammation-associated plasma substances (category 1) were present. The other main protein groups were SC barrier constituents (category 2), proteases participating in SC protein cleavage and profilaggrin processing (category 3), antimicrobial peptides (category 4), and others (category 5). To accurately evaluate each protein amount, we used the compensation value, which represents (amount of each protein) / (amount of keratin 1+keratin 10). Concerning category 1, albumin and various immunoglobulin fragments were elevated in extrinsic AD than intrinsic AD. In category 2, FLG was significantly reduced in extrinsic AD compared with intrinsic AD and controls. In category 3, kallikrein (KLK) 5 and KLK7 were increased at higher levels than controls. In category 4, dermcidin was reduced, while S100A7 and cathelicidin were elevated, suggesting that the impaired defense may be attributable to reduced dermcidin. In category 5, GCDFP-15 and suprabasin, produced by sweat glands, was reduced in AD compared with controls, indicating low sweating in AD.

Our proteome study allows us to quantify wide-ranging proteins in SC. Information obtained from this comprehensive study is useful not only for evaluation of the patient's SC condition, but also for detection of critical proteins or metabolites involved in the pathogenesis of AD.

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OM05

Cytokine effects induced by the human autoallergens thioredoxin and α -NAC

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Autoallergy is a phenomenon found in a subgroup of atopic dermatitis (AD) patients that mount specific IgE and T cell responses against autoantigens (autoallergens). One of these autoallergens is human thioredoxin (hTrx), a protein that shows high homology to the corresponding protein Mala s 13 from the skin-colonizing yeast *Malassezia sympodialis*. Sensitization to *Malassezia sympodialis* is specific to AD and sensitization to hTrx may be a consequence of the high cross-reactivity of Mala s 13 and hTrx, which has been shown at IgE and T cell level. For other autoallergens, such as α -NAC (Hom s 2), no such cross-reactivity has been described. Since there are first reports on different immunomodulatory properties of exogenous allergens and autoallergens, this study aimed to compare cytokine induction by hTrx, Mala s 13 and α -NAC.

We analyzed cytokine induction by Mala s 13, hTrx and α -NAC in PBMCs, monocytes and CD4⁺ T cells via ELISA and RT-qPCR, comparing different donor groups: IgE-sensitized AD patients, non-sensitized AD patients and controls. Western blots were performed to identify the activated signalling pathways. Blocking antibodies and siRNA were applied to identify important molecules and mediators.

All allergens induced the expression and secretion of pro-inflammatory cytokines in immune cells from AD patients as well as from control donors. Most cytokines were secreted in comparable amounts by PBMCs of both groups. However, this was not the case for IL-13 which was elevated in supernatants from *Malassezia*-sensitized donors and for IL-10 which

was reduced in supernatants from donors sensitized to the respective allergen. The main source of IL-10 were monocytes, which also produced pro-inflammatory cytokines such as IL-1 β and upregulated expression of the inflammasome-associated molecule NLRP3. Stimulation with Malassezia 13, hTrx and α -NAC resulted in differential phosphorylation of STAT3, NF- κ B and the MAP-kinase p44/42 in PBMCs. The application of blocking antibodies and siRNA revealed some of the involved receptors and signalling molecules. The induction of pro-inflammatory cytokines by the autoallergens thioredoxin and α -NAC might contribute to disease exacerbation in AD where both molecules could be released from damaged keratinocytes in inflamed skin lesions. The resulting Th1, Th2, Th17 and Th22 responses have been shown to play a role in the pathogenesis of AD.

OM06

Autoreactive CD8⁺ T cells show a terminally differentiated phenotype in the circulation of patients suffering from atopic dermatitis

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During the last years, several studies addressed autoreactivity in patients suffering from atopic dermatitis (AD). Since autoreactive IgE can be detected in 23-91% of AD patients, this phenomenon deserves closer investigation. For many autoallergens T cell proliferation has been described and it is known from T cell cloning that specific T helper cells as well as specific cytotoxic T cells are present in the circulation and lesional skin [1]. This study aims to investigate frequency and phenotype of these autoreactive CD8⁺ T cells directly *ex-vivo*. We identified MHC class I T cell epitopes of the autoantigen α -NAC, a subunit of the chaperone *nascent polypeptide-associated complex* by measuring cytotoxic T cell proliferation and peptide binding to HLA-A*02. Generated MHC-tetramers bearing these epitopes bind specifically within patients' CD8⁺ compartment. Differentiation status of tetramer⁺ T cells was assessed by surface markers using the Chipcytometry technique, where each cell is screened by a comprehensive set of markers.

While also in control donors small numbers of cells were stained, the tetramer⁺ T cell frequency was significantly higher in AD patients. Direct *ex-vivo* surface marker analysis detected significantly elevated numbers of effector/memory T_{EM} (CD8⁺/CD45R0⁺/CD45RA⁻/CD27⁻) and terminally differentiated T_{EMRA} (CD8⁺/CD45R0⁺/CD45RA⁺/CD27⁻) cells in AD patients compared to controls. These CD8⁺ subsets have been described to be the primary source of IFN γ (T_{EM}) and granzymeB / perforin (T_{EMRA}), respectively.

The elevated frequencies of autoantigen-specific effector cytotoxic T cells in the circulation of AD patients argue for an impact in the subacute or chronic phase of AD, where IFN γ is known to be overexpressed in the skin of AD patients.

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OM07

RNA-Seq profiling increases the atopic dermatitis transcriptome and identifies novel pro-inflammatory genes with potential therapeutic implications

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Genomic profiling of lesional and non-lesional atopic dermatitis (AD) skin using microarrays has led to increased understanding of immune and barrier abnormalities that contribute to disease pathogenesis, leading to identification of therapeutic targets. However, microarrays have limitations that might result in below reliable detection levels of potentially important AD genes. RNA sequencing/RNA-seq does not harbor these limitations. What did we do: In this study, RNA-seq and microarrays were used to identify the AD transcriptome, defined by differentially expressed genes (DEGs) in lesional versus non-lesional skin from 18 adults with severe AD. The performance of RNA-seq and microarrays in detecting low abundance genes was compared to RT-PCR. RNA-seq enlarged the AD transcriptome, identifying novel immune and epidermal genes. What did we show: RNA-seq achieved the highest sensitivity and specificity, and correlated best with RT-PCR data (r=0.64 vs 0.51 for arrays). Using a

cutoff of $FCH > 2$, $FDR < 0.05$, RNA-seq detected an AD transcriptome of 1109 DEGs (519 up and 590 down-regulated) while only 886 (511 up and 375 down-regulated) were identified on arrays, of which 209 were also identified by RNA-seq. Among the uniquely up-regulated DEGs by RNA-Seq we found a dendritic-cell receptor, TREM-1/ Triggering Receptor Expressed On Myeloid Cells 1, and its related molecules, including CCL2, CCL3, TNF, and SIGIRR/Single Immunoglobulin And Toll-Interleukin 1 Receptor (TIR) Domain that regulates IL-1 and TLR-mediated inflammation. Using RNA-seq profiling for the first time in AD, we detected increased expression of TREM-1 pathway genes. TREM-1 signaling was shown to amplify inflammatory responses to microbial infections. Since AD is characterized by excessive inflammatory responses to staph infections, TREM-1 might be a potential therapeutic target in this disease.

OM08

Inhibition of Epidermal Tight Junction Function by Histamine is mediated by H1 and H4 Receptors

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A growing body of evidence suggests that Atopic Dermatitis (AD) develops as the consequence of an acquired or genetic defect in skin barrier. Recent studies have highlighted that histamine inhibits human keratinocyte terminal differentiation and promotes proliferation [1, 2]. Histamine has also been shown to disrupt Tight Junction (TJ) in endothelial cells, but little is known about its actions on epidermal TJs. In this study we investigated the effect of histamine and selected histamine receptor (H1R, H2R and H4R) antagonists on epidermal TJ function and composition. We also quantified the expression of HRs in the skin of AD and non-atopic controls.

Ca⁺²-differentiated primary human keratinocytes (PHK) and epidermal explants were treated with Histamine (1-100 μ M) to determine the effect this had on TJ function. TJ integrity was assessed by trans-epithelial electrical resistance (TEER) and paracellular fluorescein flux (permeability). Selective antagonists were used for each receptor: H1R (Cetirizine 10 μ M), H2R (Cimetidine, 100 μ M), or H4R (JNJ7777120, 10 μ M). Keratinocyte

differentiation was assessed by examining filaggrin, loricrin and keratin 10 expression by Western blot. Expression of HRs were evaluated by qPCR in skin biopsies taken from lesional and nonlesional sites in AD subjects (n=6-8) and non-atopics (n=10).

Histamine significantly reduced TJ barrier function. In cultured PHK, we observed a dose dependent reduction of TEER (10 and 100 μ M, $P<0.001$, n=10) and enhancement of permeability (100 μ M, $P<0.001$, n=16). Using the *ex-vivo* model, we confirmed Histamine (100 μ M) reduced TEER (0.7 fold, $P<0.05$, n=3) and enhanced fluorescein permeability flux (1.3 fold, $P<0.05$, n=3) in epidermal explants. In PHK, H1R and H4R, but not H2R, antagonists blocked histamine-mediated TEER reduction. We confirmed that histamine selectively reduced filaggrin. Only H1R antagonist was able to prevent the histamine-mediated reduction of filaggrin expression. Interestingly, H1R was reduced in AD skin lesional and non-lesional ($P<0.01$). No significant changes in H4R expression were found.

Our studies revealed that histamine might contribute to epidermal barrier impairment observed in AD skin, by reducing TJ integrity (H1R and H4R dependent) and filaggrin expression (H1R dependent).

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OP01

Feasibility Study of Barrier Enhancement for Eczema Prevention (BEEP)

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Recent studies showing the association between filaggrin gene mutations and atopic eczema have increased interest in the potential of enhancing the skin barrier to prevent the development of eczema¹. A definitive RCT is required to determine whether emollients can prevent eczema but to inform such a trial, essential pilot work was carried out.

The Barrier Enhancement for Eczema Prevention (BEEP) pilot study was a 2-arm parallel group, assessor-blind, randomised controlled trial. Term infants at high risk of developing

eczema (parent or full sibling with eczema, asthma or allergic rhinitis) were recruited in four centres in the UK and one in USA.

Parents in both groups were given advice on infant skin care (avoiding soap and bubble bath, and using mild, fragrance-free synthetic cleansers and shampoos). The intervention group were offered a choice of three emollients (an oil, a gel/cream and an ointment) to be applied daily to the infant's entire body surface, starting within 3 weeks of birth until 6 months old of age. The primary outcome was the proportion of eligible families willing to be randomised.

A total of 295 eligible families were considered for the study, of which 124 (42%) were willing to be randomised (78 in the UK and 46 in the USA). The rate of withdrawal was 13%. The effectiveness of different recruitment options were tested in this pilot to inform the main RCT. Willingness to participate was higher and the withdrawal rate lower where recruitment took place during pregnancy rather than immediately post-delivery.

At 6 months, a statistically significant protective effect was demonstrated with emollient use on the cumulative incidence of eczema, with a relative risk reduction of 50% (RR of 0.5, [95% CI 0.3, 0.9], $p=0.017$). No effect modification was seen when results were analysed by filaggrin mutation status. The most popular emollient was the cream/gel formulation, chosen by 43/64 (67%) families. Approximately 85% of the intervention group reported they applied the emollient daily, and contamination of the control group (defined as 'regular, generalised application of emollient for reasons other than the treatment of cradle cap, nappy rash or eczema') was 8/60 (13%).

This study shows that parents are willing to participate in an emollient prevention trial and provides the first signal from an RCT that daily full body emollient application from birth can prevent eczema. These results supported a successful bid to the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) programme to conduct a definitive RCT of emollients for the prevention of eczema in the UK.

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OP02

Primary prevention of atopic dermatitis by skin care with emollient: a randomized controlled study

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Childhood atopic dermatitis is a highly prevalent disease in many countries but primary prevention strategy has not yet established so far. Recently skin barrier function gains paid attention from preventive point of view. The aim of this study is to explore whether skin care treatment with emollient starting from early infancy is effective for prevention of eczema at the age of 32 weeks.

Infants born in our centre were enrolled within a week from their delivery to this study. They were randomly allotted into two groups. One is a proactive treatment group (P group) in which mothers of infants were advised to apply emollient after bathing every day. The other is a reactive treatment group (R group) in which mothers of infants were asked to apply emollient only after their skin showed dryness. P group consisted of 51 infants and R group did 48 ones. Their skin condition was examined at the age of 4 weeks, 12 weeks, 24 weeks and 32 weeks by paediatric dermatologists. Serum concentrations of antigen specific IgE at 12 weeks and 32 weeks or at the moment of eczema onset were evaluated by using a newly developed Diamond Like Chip method. Primary outcome was cumulative incidence of atopic eczema at the age of 32 weeks.

Cumulative incidence of eczema in P group at 32 weeks was 41.3% (19 out of 46) which was significantly lower than that of R group 62.2% (28 out of 45). Mean concentration of eggwhite specific IgE in P group was 8.6 ± 18.2 (mean \pm SD) Ue/ml and that of R group was $7.9 + 12.9$ Ue/ml. Cumulative incidence of Eggwhite sensitized infants at 32 weeks of age in P group was 67.3% and that of R group was 58.1%. Mean concentration of milk specific IgE in P group at 32 weeks was 0.23 ± 0.66 (mean \pm SD) Ue/ml and that of R group was 1.2 ± 3.31 Ue/ml ($p < 0.01$). Cumulative incidence of milk sensitized infants at 32 weeks was 22.4% and that of R group was 30.2%.

Proactive skin care treatment from early infancy showed protective effect of primary prevention from childhood atopic eczema.

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OP03

Childhood atopic dermatitis is not associated with short stature

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Atopic dermatitis (AD) is a chronic inflammatory disorder that is associated with other chronic diseases such as asthma and food allergy, major quality of life impairment, and use of potent topical and sometimes systemic corticosteroids, all of which might affect growth in childhood and adolescence. However, previous smaller scale studies found conflicting results whether childhood AD is associated with short stature.

We performed a meta-analysis of 9 large-scale US population-based studies (7 pediatric and 2 adult), including the 2003 and 2007 National Survey of Children's Health and 2008-2012 National Health Interview Survey. The final cohort included a nationally representative sample of 251,368 children and adolescents (0-17 years old) and 57,080 adults (18-85 years old). Height-for-age percentiles and height (inches) were modelled using generalized linear mixed models with Box-Cox data transformation and a random effect for each study. Models were also constructed with binary link function to model <5th height-for-age percentile. Multivariate models controlled for age, sex, race/ethnicity, household income, highest level of household education, birthplace and number of children in the home.

In univariate analyses of the pooled pediatric cohort, AD was associated with significantly lower height-for-age percentiles in children and adolescents ($\beta = -0.02$, $P < 0.0001$) or height in adults ($\beta = -0.53$, $P = 0.02$). In multivariate models, however, there was a significant statistical interaction between AD and current age as predictors of lower height-for-age percentiles. Adolescents age 13-17 years had significantly lower height-for-age percentile ($\beta = -0.01$, $P = 0.002$) and were more likely to have a <5th percentile height (adjusted odds ratio (aOR) [95% confidence interval (CI)]: 1.42 [1.12 – 1.79], $P = 0.003$), whereas children ages 2-5 and 6-12 years did not ($P > 0.05$). An association between AD and decreased height percentile

overall and/or specifically at age 13-17 years was observed in 5 of the 7 pediatric studies. In contrast, the 2 adult studies revealed that AD only had a marginal association with height-for-age percentiles in adults ($\beta = -0.008$, $P=0.07$), but was not associated with height ($\beta = 0.00009$, $P=0.34$) or odds of having $<5^{\text{th}}$ percentile height (aOR [95% CI]: 0.98 [0.86–1.12], $P=0.77$). Further, there were no significant interactions between AD, age and other socio-demographic factors .

These data suggest that AD is associated with a transient period of short stature during adolescence, which resolves by adulthood.

OP04

Psychological Impact of childhood Eczema: birth cohort study

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Childhood eczema is common yet there is limited research on its impact on childrens' long-term mental health. We used data from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study to explore associations between childhood eczema and adolescent mental health.

In questionnaires sent at 11 time points between birth and 12 years of age, parents were asked whether their child had an eczematous rash. Adolescent mental health was measured using the Strengths and Difficulties Questionnaire (SDQ) completed by the parent when the study child was 16 years old. Longitudinal latent class analysis was used to derive phenotypes of childhood eczema experience. This classification of childhood eczema was used as an independent variable in linear regression models of adolescent SDQ subscales at 16 years.

3243 children (22.7% of initial cohort) had complete eczema and SDQ data. Four phenotypes of childhood eczema were generated using latent class analysis – no eczema (n=1947, 60.0%), early onset/clearing (n=555, 17.1%), late onset (n=316, 9.7%) and persistent (n=425, 13.1%). Early onset/clearing and late onset eczema were weakly positively associated with the hyperactivity subscale of the SDQ (b=0.23, $P=0.02$ and b=0.24, $P=0.06$ respectively) and early onset/clearing eczema was weakly positively associated with the conduct disorder subscale (b=0.31, $P=0.02$) of the SDQ compared with those who had no childhood eczema.

There is weak evidence of an association between childhood eczema and mental health difficulties in adolescence. The association with early onset/clearing and late onset eczema yet not persistent eczema is counter-intuitive and contrasts with the finding of Schmitt *et al* who found that emotional problems increased with increasing eczema persistence. Additional analyses using “trajectory of SDQ scores” are underway that may shed further light on our finding and will be presented at the conference.

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OP05

Systematic review of what symptoms are measured in eczema treatment trials

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It was agreed by consensus as part of the Harmonising Outcome Measures for Eczema (HOME) initiative (www.homeforeczema.org) that symptoms should be one of the four domains included in the core outcome set for measuring eczema in clinical trials ¹. The first stage in establishing what symptom outcome measurement instrument(s) should be recommended for inclusion in the core outcome set was to identify how symptoms have been measured in published eczema treatment trials.

We searched the Global Resource for Eczema Trials (GREAT database) from 2000 onwards to identify randomised controlled trials (RCTs) that measured at least one eczema symptom. We included composite scales that included both symptoms and signs. The GREAT database (www.greatdatabase.org.uk) contains records of all RCTs of treatments for established eczema.

The primary outcome was the proportion of trials that reported eczema symptoms. Secondary outcomes included what symptoms were reported and what instruments were used to measure these symptoms.

We identified 319 RCTs on atopic eczema treatments published between Jan 2000 and May 2012. Of these, 243 (76%) reported symptoms, 65 (21%) did not and it was unclear exactly what was measured in 11 (3%) of trials. Of the trials that reported symptoms, virtually all reported itch (238/243, 98%) most commonly measured by a visual analogue scale (VAS).

Sleep loss was the second most commonly reported symptom (139/243, 57%) with several other symptoms such as stinging and pain being reported in a few trials.

Approximately half of trials that reported symptoms did so by using SCORing Atopic Dermatitis (SCORAD) (109 / 243, 45%). SCORAD is a composite score of signs and symptoms that includes sleep loss and itch as the two symptoms. However, most reported only the total (composite) score and not individual scores for signs and symptoms (100/109, 92%).

Symptoms are commonly reported in eczema treatment trials but often as part of a composite outcome. It has been agreed that symptoms should be a core outcome domain for eczema trials¹ but it is not possible to extract symptoms only data from most published studies. Future trials should present patient reported symptoms scores separately and in more detail.

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OP06

Eczema Signs and Symptoms: what is important to patients?

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We aimed to gain knowledge from patients' perspectives on the importance of individual signs and symptoms of AD in determining treatment response using an international survey.

Relevant signs and symptoms of atopic dermatitis (AD) were identified from previous studies on outcome measures (1, 2). A questionnaire evaluating the importance of these signs and symptoms in determining “response to treatment” from a patient’s perspective was developed. The questionnaire asked about 18 different signs and symptoms, plus basic demographic information; it was translated from English into Swedish, German and Portuguese and then back translated according to a standardized protocol. Patients and parents of children with AD were informed via Harmonising Outcome Measures for Eczema (HOME, www.homeforeczema.org) collaborators in Europe, Japan, China, Taiwan, Tanzania, Australia and the US and were self-selected to take part in the on-line survey (using SurveyMonkey®). Most patients answered using the English version of the survey, but translations were used when applicable.

Patients were asked “How important are these features in deciding whether or not a treatment is working?”, and rated the importance of the signs and symptoms on a 5 point Likert scale, ranging from “very important” to “not relevant to me”. To rate an item as adequate it was required that 80% of the responders must have rated the item as “important” or “very important”.

831 patients from 28 countries completed the survey, 450 (54.1%) adults and 351 (54.1%) parents to children with eczema; 520 (65.5%) were females and 274 (34.5%) were males. Skin colour ranged from light 537 (67.3%), over slightly coloured 190 (23.8 %), dark 62 (7.8 %), to very dark 9 (1.1%). Most patients (559; 70.4 %) developed eczema between age 0-2 years.

Itch and pain were most important items to patients, additionally scratch marks to parents of children with AD. Also assessed by 80% or more of the patients as important or very important were “skin feels hot or inflamed, bleeding, involvement of "visible" or "sensitive” body sites, cracks, sleep difficulties, amount of body affected and weeping”.

Symptoms are important for patients; signs can be useful for clinical assessment. The results of this study can be useful in determining the face validity of scales used to assess the core outcome domains of signs, symptoms and long-term control from a cross-cultural patients’ perspective.

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OP07

Investigator Global Assessment in randomized controlled trials in atopic dermatitis: a systematic review

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An investigator global assessment (IGA) ranks in the top three most commonly used outcome measures in atopic dermatitis clinical trials.¹ The Food and Drug Administration (FDA) requires an IGA for new drug approvals for atopic dermatitis in the U.S. Despite its prominence, few validation studies exist supporting its use. Our aim was to better understand the frequency of use of the IGA in clinical trials of atopic dermatitis and assess the degree of standardization of the instrument in regards to implementation and analysis.

We performed a systematic review of randomized, controlled trials using The Global Resource of Eczema Trials (GREAT) database - a database hosted by the Centre of Evidence-based Dermatology at the University of Nottingham. Global assessments were defined as ordinal scales assessing global disease severity as assigned by an investigator.

We identified 283 trials published between 2000 and 2012 after excluding conference abstracts. Approximately one third (n=97, 34%) of trials used some form of an IGA. North American-based studies used the IGA more frequently compared to European –based studies (77% vs. 30%, p<0.001). The IGA served as the primary outcome measure in 30% of trials. Of the 97 trials using an IGA, 62% used a static scale and 39% used a dynamic scale that referenced baseline severity, with one trial using both. Static IGA scales varied in size (between 4-6 point scales) with 80% using a 6 point scale. Instructions for performing the IGA appeared in 27% of the studies. The IGA score was analyzed as a continuous variable in 22% of studies.

The IGA plays a prominent role in clinical trials in atopic dermatitis, especially in North America. However, our data reveal the instrument lacks basic standardization with various scales and instructions being used across studies. Standardization of the instrument scale

and instructions is needed urgently. Standardization will allow proper study of the instrument's measurement properties, such as content validity and interobserver reliability.

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OP08

A Comparison Study of Outcome Measures for Atopic Dermatitis

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Atopic dermatitis remains a therapeutic challenge even for the seasoned dermatologist. There are a number of severity scoring systems available to assess patients' severity and response to treatment, playing a quintessential role in the continuous research to the treatment of atopic dermatitis.

Despite the variety of scoring systems available, there have been limited studies on their reliability, and none prospectively compare their inter-rater and intra-rater reliability, as well as examining their correlation with patient's quality of life.

Four most commonly used objective scoring systems - the SCOring Atopic Dermatitis (SCORAD) and Eczema Area and Severity Index (EASI), Three Item Severity score (TIS), Six Area Six Sites Atopic Dermatitis score (SASSAD) - were examined and their correlation with three quality of life instruments was assessed (Patient-Orientated Eczema Measure (POEM), (Child) Dermatology Life Quality Index (CDLQI/DLQI), and SkinDex-29.

12 atopic dermatitis patients having different degrees of severity were assessed on the same day by 5 independent trained dermatology assessors. Reliability was measured using the intra-class correlation coefficient calculated through one-way random effect ANOVA and Bland-Altman plots. Correlation between subjective and objective outcome measures was computed using the two-tailed Spearman's rho correlation with scatterplots.

There are significant differences between inter-rater and intra-rater reliability among the four scoring systems, making one more favourable among the others. EASI demonstrated a high

intra-rater and moderate inter-rater reliability, ICC=0.886 (95% CI: 0.744-0.952) and ICC=0.73 (95% CI=0.5 – 0.9), respectively. SASSAD showed moderate intra-rater and inter-rater reliabilities, ICC=0.720 (95% CI = 0.424-0.878) and ICC=0.68 (95% CI=0.44-0.88). TIS showed high intra-rater reliability ICC=0.886 (95% CI: 0.744-0.952) but low inter-rater reliability ICC=0.497 (95% CI=0.233–0.785). Objective SCORAD showed low intra-rater and inter-rater reliability, with ICC of 0.446 (95%CI=0.037-0.730) and 0.498 (95% CI=0.234–0.785), respectively. Only SASSAD demonstrated moderate correlation with SkinDex-29 $\rho=0.611$ ($p=0.035$).

EASI emerged as the more reliable scoring system, while SASSAD shows some correlation with patients' quality of life. However, there are major improvements to be made to produce a scoring system that reflects patients' disease activity and damage to provide physicians adequate information to assess their management efficacy.

OT01

Are the concepts of induction of remission and treatment of subclinical inflammation in atopic dermatitis clinically useful?

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Atopic dermatitis treatment is often initiated by symptoms or visible erythema. The role of induction of remission or treatment of inflammation that is not visible is unclear.

We investigated whether (i) the notion of “subclinical inflammation” is scientifically sound, (ii) treatment corrects subclinical inflammation and (iii) different strategies for initial clearance of atopic dermatitis impact on long-term disease control

Methods: We conducted a systematic review based on searching MEDLINE, Embase, CENTRAL, and the Global Resource of Eczema Trials (GREAT) from inception to the end of October 2012.

20 out of 26 included studies presented evidence of subclinical inflammation with a continuum of changes in skin barrier dysfunction, proinflammatory cytokine milieu, and lymphocytic infiltration from normal appearance, post-treatment lesional to active skin lesions in individuals with atopic dermatitis. Such subclinical inflammation is improved with proactive treatment aimed at maintaining remission.

Failure to get control of atopic dermatitis with initial therapy was associated with a higher risk of relapsing in 14 RCTs; RR = 1.31, 95% confidence interval (CI) = 1.02-1.68, for fluticasone; RR= 1.36, 95% CI = 1.12- 1.66, for tacrolimus. Three trials on systemic/phototherapy suggested that induction of remission resulted in long-term remission without maintenance therapy in around 15% of patients.

Induction of remission followed by maintenance therapy, may prove to be an integral part of disease-modifying strategy for treating atopic diseases.

OT02

Old drug, new hope for atopic dermatitis patients: Identification of the aryl hydrocarbon receptor as a pharmacological target of coal tar therapy

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Topical application of coal tar is one of the oldest therapies for atopic dermatitis (AD), a T helper 2 (Th2) lymphocyte-mediated skin disease associated with loss-of-function mutations in the skin barrier gene, filaggrin (FLG). Coal tar is a cheap, safe and effective therapy but its use in dermatological practice is declining due to safety concerns, cosmetic reasons, and the - until recently - unknown mechanism of action. With regard to the safety issues, we have conducted a large cohort study and we did not find an increased cancer risk associated with medicinal use of coal tar cream 1. This led to the present study in which we clarified the molecular mechanism of coal tar therapy in AD 2. Our findings provide a rationale to continue and promote this ancient therapy as a safe and effective therapy.

We identified the aryl hydrocarbon receptor (AHR) as the molecular target for coal tar treatment, using 3D-reconstructed skin models with genetically defined, patient-derived cells, and an in vitro siRNA-mediated knockdown approach.

We found that coal tar activated the AHR, resulting in induction of epidermal differentiation. AHR knockdown by siRNA completely abrogated this effect. Coal tar restored filaggrin expression in FLG-haploinsufficient keratinocytes to wild-type levels, and counteracted Th2 cytokine-mediated downregulation of skin barrier proteins. In AD patients, coal tar completely restored expression of major skin barrier proteins, including filaggrin. Using

organotypic skin models stimulated with Th2 cytokines, we found coal tar to diminish spongiosis, apoptosis, and CCL26 expression, all AD hallmarks. Coal tar interfered with Th2 cytokine signaling by reducing STAT6 phosphorylation.

Now that we have identified the working mechanism of coal tar, we have isolated coal tar fractions with favorable properties (colorless, less smell) that retain the desired in vitro biological effects. Preliminary data on the biochemical interaction between coal tar and the AHR, demonstrates a pivotal role for the AHR in epidermal homeostasis and skin barrier development.

Our findings clearly have both a scientific and medical impact. Although pharmaceutical industry has ignored the AHR for many decades, recent data on the physiological role of AHR combined with our findings set the stage to seek alternatives for the classical coal tar therapy and will aid in drug discovery for eczema based on the rehabilitation of the AhR as a bona fide drug target.

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OT03

Systematic review of treatments for eczema

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Eczema (syn. 'atopic eczema' or atopic dermatitis') is a chronic inflammatory skin condition that affects around 20% of UK children. We sought to update the Health Technology Assessment systematic review of eczema treatments published in 2000¹ with the main aims of providing useful clinical information for healthcare professionals, commissioners, and people with atopic eczema and their families; and to identify research gaps for further primary, secondary or methodological research.

Six electronic databases have been searched from the end of 1999 to 31st August 2013, including EMBASE, MEDLINE and The Cochrane Library. Randomised controlled clinical trials (RCTs) comparing two or more treatments for established eczema involving people of

all ages with eczema were included. Outcomes of changes in patient-rated symptoms of atopic eczema such as itching (pruritus) or sleep loss were used where possible. Global severity as rated by patients or their physician was also sought. If these were not available, then global changes in composite rating scales were summarised. Adverse events were also included if reported. Studies published only as abstracts were excluded. Studies published in non-English languages were screened by international colleagues and included with full data abstraction, if eligible.

A total of 287 new trials, encompassing 92 different treatments for established eczema were included.

There was reasonable evidence of benefit for treatments such as topical corticosteroids; topical tacrolimus and pimecrolimus; topical corticosteroids, educational approaches; oral ciclosporin and azathioprine.

There was reasonable evidence to suggest no clinically useful benefit for treatments such as twice daily versus once daily topical corticosteroids; probiotics; and supplements rich in linoleic acid (evening primrose oil and borage oil).

Additional research evidence is needed for a number of commonly used approaches including emollients, bath additives, and complementary and alternative therapies.

There was no RCT evidence whatsoever for dilution of topical corticosteroids, impregnated bandages, soap avoidance, frequency of bathing, or routine patch testing.

The review has identified key areas for further research such as the optimum use of emollients, frequency of bathing and use of wash products, the role of allergy testing, and antiseptic treatments. There is a special need to conduct more studies in a primary care setting where most people with eczema are seen in the UK, and to conduct studies that use the same core set of outcomes that adequately reflect the chronic nature of the disease.

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OT04

Treatment of adult patients with moderate-to-severe atopic dermatitis with dupilumab in early phase clinical studies shows significant improvement in skin disease activity and pruritus: a review of study data

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Moderate-to-severe atopic dermatitis (AD) is not adequately controlled with topical agents alone. Dupilumab, a fully human monoclonal antibody targeting IL-4R α , blocks both IL-4 and IL-13 signaling implicated in AD pathogenesis. Studies were initiated to test the preliminary safety and efficacy of dupilumab.

Dupilumab was evaluated in 2 randomized, double-blind, placebo-controlled phase 1b trials involving adults with moderate-to-severe AD (combined N=67). A phase 2a randomized, double-blind study evaluated the safety and efficacy of dupilumab administered concomitantly with topical corticosteroids (TCS) vs. TCS alone in adults with moderate-to-severe AD (N=31). Treatment was administered for 4 weeks in all studies. Research sponsored by Regeneron Pharmaceuticals, Inc. and Sanofi.

Dupilumab demonstrated an adverse event (AE) rate similar to placebo (PBO); no identified dose-limiting toxicities or imbalance in serious AEs were observed. The most common AEs more frequently reported in dupilumab-treated patients vs. PBO were nasopharyngitis and headache. Overall, patients enrolled in the phase 1b studies had severe AD, with mean affected BSA of 48.8%, EASI score of 28.3, SCORAD of 63.3 and NRS pruritus score (0-10) of 6.0. Dupilumab reduced pruritus and improved skin disease activity in a dose-dependent fashion vs. PBO within 7 days after initial injection. The proportion of patients who achieved an EASI50 response at day 29 was 71.4% in the 300mg arm vs 18.8% for PBO (p=0.0025), and NRS pruritus score decreased by 45.4% vs 18.6% for PBO (p=0.0016). Reductions in serum thymus and activation regulated chemokine (TARC/CCL17) and IgE confirm the mechanism of action. In the phase 2a study, 100% of patients on dupilumab+TCS achieved EASI50 responses at Day 29 compared to 50% on PBO+TCS (p=0.0015). Furthermore, patients in the dupilumab arm of the combination study used approximately 50% less TCS during the treatment period (mean [SD] 48.7g [40.3] vs 99.4g [152.5]).

In early phase clinical studies dupilumab showed an acceptable safety profile and clinically meaningful improvements in both signs and symptoms in adult patients with severe AD. These changes were mirrored by reductions in TH2 biomarkers. In addition, relative to TCS alone, dupilumab+TCS showed enhanced efficacy, despite patients reduced use of TCS. Together, these findings support further evaluation of dupilumab for the treatment of AD.

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OT05

Are caregivers corticophobics ?

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Topical corticosteroids (TCS) form the mainstay of treatment for atopic dermatitis (AD). If used appropriately, TCS are safe and effective, however advice to patients given by doctors and pharmacists to apply TCS 'sparingly' or 'thinly' contributes to 'steroid phobia', increasing the risk of poor clinical response or treatment failure. After assessing the facets, origins and frequency of fears related to TCS use among patients with atopic dermatitis (1) we designed and validated (2) a scale named TOPICOP (TOPICAL CORTICOPHOBIA) consisting of twelve questions for assessing AD patients and their parents worries and beliefs about TCS. The objective of the present study was to evaluate the position of health care-givers in three groups (Dermatologists, Paediatricians and Pharmacists) in relation to the TOPICOP scale. We performed an analysis of variances (ANOVA) to assess discrepancies between the 3 groups. Fisher or χ^2 tests were used to analyse each item responses. 72 dermatologists, 29 paediatricians, and 29 pharmacists attending Patient Education Day meetings in France completed the TOPICOP questionnaire.

Concerning the beliefs (TCS pass into the bloodstream, TCS can lead to infection, TCS can make you fat, TCS damage the skin, TCS can affect future health and TCS can lead to asthma) the mean scores were respectively 41.61, 31.68, 23.42 for pharmacists,

dermatologists and paediatricians. The score was significantly higher for pharmacists ($p < 0.001$).

Concerning the worries (applying too much cream, putting cream on certain zones like eyelids, waiting before treating, stopping the treatment as soon as possible, being afraid of TCS without knowing side effect, prescribing as little TCS as possible) the mean scores were respectively 47.68, 31.55 and 18.87 for pharmacists, paediatricians and dermatologists. The score was significantly higher for pharmacists ($p < 0.001$).

The analysis of each item found that pharmacists have more fears than the other caregiver groups in relation to the quantity of cream to apply ($p < 0.001$), the advice to stop the treatment ($p < 0.001$) and about consequences on future health ($p < 0.001$).

This study confirms that dermatologists, paediatricians and especially pharmacists have worries and negative beliefs about TCS. Patient corticophobia could thus be induced by the exaggerated warning given by healthcare professional. Updated evidence based information defining a clearer risk/benefit ratio should be helpful in educating healthcare professionals.

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OPT06

Maximal Use Systemic Exposure (MUSE) Study Evaluating AN2728, A Novel Boron-Based Small Molecule, for the Treatment of Subjects 2 to 17 Years Old with Mild-to-Moderate Atopic Dermatitis

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AN2728 is a novel boron-based compound that inhibits phosphodiesterase-4 activity and reduces the production of pro-inflammatory cytokines that may be associated with atopic dermatitis (AD).^{1,2} The objective of this open-label, maximal use study was to evaluate the systemic exposure, pharmacokinetics, and safety of AN2728 Ointment, 2% applied twice daily for 28 days for the treatment of AD in children and adolescents.

The study enrolled 34 subjects with mild-to-moderate AD, defined as a score of 2 (mild) or 3 (moderate) on the 5-point Investigator's Static Global Assessment (ISGA) scale in three patient cohorts based on age and minimum percent of treatable body surface area (%BSA) affected: 2-5 years old ($\geq 35\%$), 6-11 years old ($\geq 35\%$) and 12-17 years old ($\geq 25\%$). During the first 8 days when pharmacokinetic assessments were performed, subjects were dosed in the clinic; dosing was performed at home thereafter. Disease severity was measured using ISGA (0, clear to 4, severe), signs/symptoms score (0, none to 3, severe) and %BSA affected.

At Day 29, 65% of subjects achieved ISGA scores of clear or almost clear and 47% of subjects achieved scores of clear or almost clear with a >2-grade improvement from baseline. Marked reductions from baseline were observed across all the individual signs and symptoms of AD (pruritus, erythema, lichenification, excoriation and exudation) throughout the treatment period. Notably, mean pruritus scores improved by approximately 60% from baseline as early as 5 days into treatment. The mean %BSA affected decreased by an average of 78% across all subjects after 4 weeks of treatment. The most common treatment-related adverse events were application site reactions (occurring in 12 subjects) which generally were mild or moderate in severity and resolved spontaneously. One patient withdrew from the study due to application site pain. Pharmacokinetic results demonstrated low blood levels of AN2728 similar to those previously observed in adults after adjusting for %BSA treated.

These results generated under maximal use conditions suggest that AN2728 Ointment, 2% may be safe and effective in subjects 2 years of age and older with mild-to-moderate AD.

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OT07

Safety and Efficacy of AN2728 Topical Ointment, 2% and 0.5%, in a Phase 2 Dose-Ranging Study of Adolescents with Mild-to-Moderate Atopic Dermatitis

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AN2728 is a novel oxaborole compound and phosphodiesterase-4 inhibitor with anti-inflammatory activity.¹ A phase 2 randomized, double-blind, bilateral, multi-center, dose-ranging, study was conducted to determine the safety and efficacy of AN2728 Topical Ointment, 2% and 0.5%, administered once daily (QD) or twice daily (BID) for the treatment of mild-to-moderate atopic dermatitis (AD) in adolescents.

The study enrolled 86 patients (40% male) aged 12-17 years with AD involving up to 35% of body surface area. Each patient had two target lesions of similar severity based on AD severity index (ADSI) score of 6-12, a maximum 1-point difference in ADSI score between the two lesions, and an erythema subscore of at least 2 (moderate). The index comprises the sum of scores ranging from 0 (none) to 3 (severe) for erythema, pruritus, exudation, excoriation, and lichenification. Patients were randomly assigned to a QD or BID treatment frequency. In addition, patients treated one target lesion with AN2728 Topical Ointment, 2% and the other with AN2728 Topical Ointment, 0.5%. Patients were evaluated on days 1, 8, 15, 22 and 29. Disease severity was determined based on the ADSI score on days 8, 15, 22 and 29. The primary endpoint was the change from baseline in ADSI score.

AN2728 Topical Ointment, 2% and 0.5%, were found to be generally safe and well-tolerated. No serious adverse events (AEs) were reported, and no treatment discontinuation occurred due to AEs. Application site symptoms were uncommon. Based on improved ADSI scores relative to baseline, a clear dose-response was seen across the four dosing regimens. The greatest improvement in ADSI score was noted with treatment with AN2728 Topical Ointment, 2% BID, which yielded a 71% improvement in ADSI score from baseline after 28

days, with 62% of lesions in this treatment group achieving total or partial clearance. This treatment group also demonstrated the greatest improvement across all five signs and symptoms of AD after 28 days, including a notable 79% reduction in pruritus severity.

Of the four dosing regimens examined in this phase 2 study of adolescents with AD, AN2728 Topical Ointment, 2% BID produced the greatest improvements in disease severity and was generally safe and well tolerated.

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OT08

New biomarkers for disease severity in atopic dermatitis

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Promising new treatments for atopic dermatitis (AD) are currently investigated. The question is whether they are more effective than established treatments. However, comparing the results of different trials in AD is difficult because of the large number of different clinical outcome measures that have been used. Therefore, there is an urgent need for valid, reliable and objective severity measures of AD that allow comparison of clinical trials and epidemiological studies.

A great variety of serum biomarkers for disease severity in AD has been reported. The most frequently reported biomarkers include serum ECP, serum IgE, serum IL-2R, and serum TARC/CCL17 levels. Correlations between these biomarkers and disease severity showed large differences between publications.

This was a pilot study in which we used a multiplex (Luminex®) approach we investigated 31 potential biomarkers for AD in sera from 17 adult patients (6 male; 11 female) diagnosed with severe AD. Blood was taken at admission to the hospital and after two weeks of treatment. Patients were treated with potent topical corticosteroids; patients on oral immunosuppressive drugs were not included in this study. Severity of AD at the time of blood sampling was assessed by using the Six Area Six Sign Atopic Dermatitis (SASSAD).

All patients showed significant clinical improvement. SASSAD scores decreased from 36.9 at baseline to 8.0 after two weeks of treatment. Biomarker levels at admission and after treatment were compared to determine if they are associated to disease severity. Of the 31 markers studied, seven showed a statistically significantly decrease after treatment and are therefore associated with SASSAD. This included TARC (CCL17), MDC, interleukin 22 (IL-22), PARC (CCL18), soluble interleukin-2 receptor (sIL-2R), E-selectin and interleukin 16 (IL-16).

Using stepwise regression analysis with cross validation, we found a combination of biomarkers (n=4) that shows a strong correlation to disease severity ($r^2=0.83$).

In this pilot study we show that a combination of biomarkers shows a stronger correlation to disease severity, compared to a single biomarker. We suggest to use this set of biomarkers for monitoring disease severity in future clinical studies to improve study comparability.

OT09

Establishing Biomarkers of Disease Activity and Therapeutic Response

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Atopic dermatitis (AD) is the most common inflammatory disease. Evolving disease models link changes in epidermal growth and differentiation to Th2/Th22 cytokine activation. Available therapeutics for moderate-to-severe AD patients are currently limited. We are currently experiencing a translational revolution in AD, with active testing of targeted therapeutics. Unlike psoriasis trials that show only minimal disease improvement in the placebo arm, there is up to 20% improvement in the placebo arm in AD trials. Since it is difficult to quantify improvement in erythema and lichenification in short term studies, reliable tissue biomarkers are needed for the testing of novel pathway-targeted therapeutics. We have compiled gene expression, cellular infiltrates, serum IgEs, eosinophils, and clinical response data (by SCORAD or EASI indices) from our past studies with two therapeutic agents for moderate-to-severe AD (NB-UVB, and CsA; 49 patients total, 39 with complete observations in most important markers across all studies). Spearman analysis was used for determining correlations with baseline disease severity (Scoring of AD/SCORAD index) and with improvement in SCORAD. To determine the best predictors of therapeutic response

several regression algorithms were employed. Sensitivity analysis was conducted with the best performing model, using the set of all complete observations with varying input numbers.

Several markers showed significant positive (i.e serum IgE, lesional skin mRNA expression of S100A12, S100A8, and IL-13) or negative (i.e periplakin/PPL) correlations with disease activity at baseline. Among the markers with highest correlations with disease improvement with treatment we found key inflammatory and Th2/Th22-related markers, including MMP12, IL-13, CCL26, CCL22, S100A7. The decrease in epidermal hyperplasia/thickness was best correlated with reductions in IL-13, S100A12, and MMP12. The best predictors of therapeutic response across all treatments were CCL26 (the single best predictor), IL-22, CXCL1, CCL22, and CCL13. As for predictors of disease activity at baseline, the best predictors were IgE levels, elafin/PI3, MX1, CCL17, K16, and CCL22. Although reduction in IL-13 mRNA was correlated with the therapeutic improvement, it was not chosen to be part of the minimum set of predictors of therapeutic response.

Using a large cohort of moderate-to-severe AD patients that participated in CsA and NB-UVB studies, we were able to validate biomarkers of disease activity and therapeutic response across, and define a set of predictors of therapeutic response across all treatments. This set of disease and therapeutic response biomarkers could be very useful in current trials with targeted therapeutics.