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ATOPIC DERMATITIS AND DISTRESS

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Atopic Dermatitis and Distress

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By

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To the spirit of my father

Popular science summary

Atopic dermatitis (AD) is a chronic, inflammatory skin disease characterized by dry skin and severe itch. Up to 15% of Swedish children will develop AD, which also affects 2–3% of adults. Stress is known to trigger flare-ups of eczema. Virtually all patients with moderate-to-severe AD have been reported to have symptoms of anxiety and depression. AD is often worsened by stress and anxiety and there is a close relationship between emotional distress, pruritus and scratching.

For this reason, the importance of stress, anxiety and depression was investigated in patients with AD. Other aspects that were studied included information on the types of stress that patients identified as particularly linked to their eczema, what treatments patients recognized as useful, and the treatments they had been offered. This was first done through patient interviews in small groups and in the next step an online survey was used to further investigate the same questions. In a third study, functional magnetic resonance imaging (fMRI) was used to study which brain circuits were activated in AD patients when subjected to stress, and compare with healthy controls. Thereafter, the expression of one neuropeptide, calcitonin gene-related peptide, in the skin was investigated in a smaller patient cohort and connected to each patient's psychological profile regarding stress, anxiety, and depression, and their clinical status.

The purpose of this research project was to gain an increased understanding of stress and stress-related psychological conditions like anxiety and depression, as well as possible underlying mechanisms, both central and peripheral. Stress was found to be of great importance regarding worsening of patients' eczema. Differences in the central processing of stress were observed with fMRI. Also, increased levels of CGRP were found in inflamed skin of AD patients.

Abstract

Atopic dermatitis (AD) is a chronic, inflammatory skin condition characterized by itching, redness, and skin lesions, affecting approximately 10–20% of children and 1–3% of adults worldwide. Distress associated with AD can negatively impact quality of life, work, and daily activities.

The research aims were to explore the role of stress in AD patients and to further investigate brain activity during stress in AD patients compared with controls. Further, one possible mediator related to skin inflammation and stress, calcitonin gene-related peptide (CGRP), was studied in skin from AD patients and controls, and related to psycho-demographic measurements.

The initial parts of the project involved focus groups with patients and an online survey. Results from the focus group study and the survey study underlined the importance of stress as a trigger and worsening factor for patients with AD. Both studies indicated that stress, especially chronic stress, could be an important worsening factor. Decision-making and unforeseen events were often mentioned as stress triggers. In both the focus groups and the survey study, patients rated stress as of greater importance than climate factors. Itch was reported to be a result of stress and the type of stress possibly affected the nature of the pruritus experienced by patients. Furthermore, physical exercise was reported to have beneficial effects, something that was found in both the focus groups and the survey.

Differences were found in possible mechanisms for stress processing in AD patients compared with controls. Reduced deactivation in the default mode network in response to stress (an arithmetic test) indicated that there is likely a cognitive functional variability in AD patients compared with healthy control subjects, manifested as lowered inhibition ability under psychological stress. This was also supported by different correlations between brain activities and various psycho-demographic data. Findings from a functional magnetic resonance imaging study indicated that psychological stress affected brain activities in the motor cortex, the somatosensory association cortex, and perception and sensory integration processing among AD patients.

An immunohistochemical study showed an increase of CGRP in nerve-like fibers and inflammatory cells in inflamed skin of AD patients compared with non-lesional skin. The increase of CGRP-positive nerve-like fibers in skin correlated with depressive and anxiety scores in the patients.

The results showed that psychological stress was an important trigger factor for AD and both differences in central processing of stress in AD and peripheral changes in CGRP levels in skin were observed. This emphasizes the importance of a holistic approach to treatment of AD, with the possibility for healthcare to offer more individualized treatment depending on each patient's challenges and needs.

List of scientific papers

I. Lönndahl L, **Abdelhadi S**, Holst M, Lonne-Rahm SB, Nordlind K, Johansson B. Psychological stress and atopic dermatitis: A focus group study. Ann Dermatol. 2023 Oct;35(5):342-347.

II. **Abdelhadi S**, Nordlind K, Holst M, Lönndahl L. Atopic dermatitis and distress – an inquiry study. In manuscript.

III. Jonsson T, Li T-Q, **Abdelhadi S**, Lönndahl L, Theodorsson E, Nordlind K. Atopic dermatitis and stress - A functional MRI study of female patients with atopic dermatitis using an arithmetic task. Submitted.

IV. **Abdelhadi S**, Nordlind K, Johansson B, Theodorsson E, Holst M, Lönndahl L. Expression of calcitonin gene-related peptide in atopic dermatitis and correlation with distress. Immunopharmacol Immunotoxicol. 2023 Sep 7:1-6.

Contents

List of abbreviations

AD	Atopic Dermatitis
BOLD	Blood-Oxygen-Level-Dependent
CGRP	Calcitonin Gene-Related Peptide
DLQI	Dermatological Life Quality Index
fMRI	Functional Magnetic Resonance Imaging
HADS	Hospital Anxiety and Depression Scale
HPA	Hypothalamic-Pituitary-aArenal
HR	Histamine Receptor
HC	Healthy Control
IgE	Immunoglobulin E
IL	Interleukin
KEDS	Karolinska Exhaustion Disorder Scale
LCs	Langerhans' Cells
PO-SCORAD	Patient Oriented SCORing Atopic Dermatitis
PSS	Perceived Stress Scale
PsTA	Psychic Trait Anxiety
RIA	Radioimmunoassay
ROIs	Regions Of Interest
SARS-CoV-2	Severe Acute Respiratory Syndrome CoronaVirus 2
SS	Stress Susceptibility
SCORAD	SCORing Atopic Dermatitis
STA	Somatic Trait Anxiety
Th	Helper T cell
TI	Transient Irritability
VAS	Visual Analogue Scale

1 Literature review

1.1 The skin

- The skin is the largest (up to 2 m^2) and heaviest (4–5 kg) organ of the body.
- The skin is the main interface between an individual and the environment; thus, it is a perfect organ for studying both innate and specific immunity.
- The skin is an important sensory organ, advising us when it is warm or cold, steering us away from painful experiences and providing sensory pleasure.
- Keratin helps the skin form a strong and flexible protective covering. It is also a key constituent of nails, hairs, beaks, claws, fur and feathers [1].

The epidermis, the outermost layer of the skin, is made up of multiple layers of self-renewing cells. Cells in the innermost or basal layer divide to produce the keratin-producing cells, keratinocytes, which undergo terminal differentiation as they migrate towards the surface of the skin. So-called epidermal stem cells are also found in the basal layer [1]. These cells divide, giving rise to keratinocytes capable of differentiation, as well as additional primitive stem cells. Epidermal stem cells can also develop from even more primitive cells. Good markers of epidermal stem cells are not available, but the cells are an attractive potential approach for skin replacement, for instance in patients with widespread burns. If they could be identified and enhanced, they should offer the best way of rapidly generating new, nearly normal epidermis.

The epidermal turnover time is about one month. Keratinocytes reach the interface towards the outermost layer, the stratum corneum, after about two weeks; the final compressed version of the cell is known as a corneocyte. Another two weeks are normally required for the corneocytes to reach the surface of the stratum corneum and be shed into the environment. The turnover time can be dramatically decreased, for example in psoriasis. Deviations in the process of keratinization are responsible for a wide variety of disorders, including ichthyoses and palmoplantar keratoderma. The keratinocytes are held together by complex structures known as desmosomes. When these structures are damaged, the keratinocytes become separated, which is seen in a range of autoimmune disorders.

1.2 Atopic dermatitis – characteristics and disease mechanisms

1.2.1 Atopic dermatitis

Atopic dermatitis (AD) is a chronic inflammatory skin disorder, distinguished by pruritus and eczematous lesions. It is a multifactorial disorder, impacted by immune dysregulation, environmental stimuli, genetic predisposition, and compromised skin barrier function [2]. AD is the most prevalent skin disease among children and has increased consistently over the past three decades. It is a relapsing condition and has a substantial impact on children who are afflicted, their families, and the broader community [3]. A multitude of inflammatory markers have been proposed as potential objective indicators of disease activity.

1.2.2 Prevalence

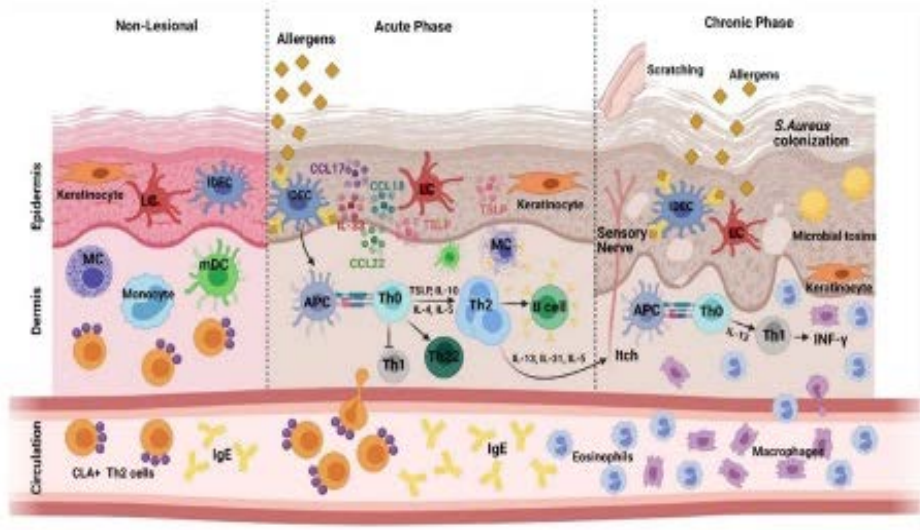
AD is a prevalent childhood disease, with an estimated prevalence of around 13% of children in the United States and a global occurrence of 15–38% in children under the age of 5 years [4].

The prevalence of the disease varies significantly between countries, affecting an estimated 20% of adolescents and 7%–10% of adults in the United States and Europe [5]. According to recent research, there is a significant degree of variability in the age at which AD begins to manifest. AD may manifest either during adolescence or maturity, with roughly 25% of persons with AD reporting the debut of their skin condition in adulthood [4].

1.2.3 Pathogenesis

Itching in AD is a result of various factors, including keratinocytes, the immune system, and non-histaminergic sensory nerves (Fig. 1). Emotional stress, sleep, and alcohol consumption can intensify pruritus in AD patients. Pruritogens, such as interleukin (IL)-31, IL-4, and IL-13, stimulate the brain and trigger motor activity, leading to an "itch-scratch cycle." Keratinocytes release pruritic factors, such as alarmin thymic stromal lymphopoietin, which indirectly contributes to itching. The role of histamine in itching in AD is unclear, but simultaneous blockade of the histamine H1 and H4 receptors is more effective in reducing pruritus and inflammation than blockade of either of them alone [6]. Non-sedative antihistamines used in clinical practice are not very effective in relieving pruritus in AD, suggesting a non-histaminergic mechanism. Inflammation also produces endogenous and exogenous pruritic factors, such as ILs, leukotrienes, and endothelins. AD patients have increased sensitivity to itching due to neuronal sensitization, which is based on increasing

sensitivity to stimuli. The main cause of sensitization is the alteration of neurons at the peripheral level, possibly due to local inflammation. Anti-inflammatory therapies, including topical and conventional inflammatory drugs, have been proven to improve the skin condition and reduce itching in AD patients [7].



Kader et al. Current Insights into Immunology and Novel Therapeutics of Atopic Dermatitis. *Cells*, **2021**

Figure 1. Different types of immune cells respond differently and play different roles at different stages of AD, as shown by the disease's stage-based pathophysiology. Epidermal dendritic cells with specific immunoglobulin E (IgE) molecules attach to high-affinity IgE receptors, and Langerhans cells and dermal dendritic cells take up allergens and antigens during an allergic response. Through stimulation of sensory nerves, pruritus is directly promoted by type 2 cytokines, B cells, and other Th cytokines. A variety of pruritogens, including cytokines produced by keratinocytes and immune cells, contribute to the development of chronic itching.

1.2.4 Clinical picture and scoring

The clinical presentation of AD is defined by sudden episodes of itchy, eczematous lesions on dry skin, particularly in flexural folds. Despite the uniformity of this presentation, the circumstances that trigger the illness are varied, and the underlying pathophysiological mechanisms involved are intricate [8].

AD is distinguished by its pruritic nature and chronic relapsing nature. It manifests within the first 5 years of life in 90% of affected individuals (although not in the initial weeks of life, as observed in the autosomal dominant hyper-immunoglobulin E (IgE) syndrome). The distribution of AD is age-dependent, with facial, scalp, and extensor involvement being

predominant in infants and young children, while flexural involvement dominates in older children and adults [9]. Anthropoietic dermatitis is characterized by xerosis and universal pruritus in children and youths. Acute lesions are distinguished by the presence of pruritic papules accompanied by erythema, excoriations, and serous exudate. In contrast, chronic AD is characterized by fibrotic nodules and lichenification, which are frequently accompanied by acute lesions.

Diagnostic criteria have been established in the absence of pathognomonic lesions that can be used to definitively diagnose AD. The Hanifin and Rajka criteria from 1980 and subsequent modifications, such as the UK Working Party's Diagnostic Criteria for AD, are the most frequently referenced. On the basis of these criteria, five main clinical characteristics are identified: (1) pruritus; (2) a chronic, relapsing course; (3) typical distribution; (4) a personal or familial history of atopy; and (5) onset prior to the age of 2 years. Minor criteria that are frequently observed in patients with AD are diagnostically useful [9].

1.3 Treatment

Treatment for AD depends on the patient's age, the number of affected body areas, other medical conditions, medications, the intensity of pruritus, how much the quality of life has been negatively impacted, and the patient’s own personal treatment goals. Emollients (with or without antipruritic drugs) and avoiding infections and trigger factors are recommended during all stages of the disease, including eczema-free intervals [10].

It is suggested that topical immunosuppressive medications be used first when eczema presents itself. Although the phosphodiesterase-4 inhibitor crisaborole has recently been licensed for use in the United States in the treatment of AD, its use remains restricted in several nations. Ultraviolet phototherapy is effective for mild eczema; however, it is not used in children or young adults due to the risk of skin cancer with prolonged exposure. Glucocorticoids, cyclosporine, and methotrexate are just a few examples of the typical systemic immunosuppressants that have been used to treat severe AD. These medicines may have serious side effects, such as liver and renal damage, and do not target particular areas of immunological dysregulation in AD [11, 12].

1.4 AD, anxiety and depression

Anxiety and depression symptoms have been documented in nearly all patients with moderate-to-severe AD [13]. Anxiety and psychological tension frequently exacerbate AD [14] and emotional distress, pruritus, and scratching are all closely related. It has been

reported that stress can occur up to 24 hours prior to the onset of AD deterioration [15]. Individuals diagnosed with AD exhibit increased vulnerability to anxiety and tension when confronted with circumstances such as overworking, social conflicts, or feelings of inadequacy. This implies the existence of a personality type associated with atopy [16]. Elevated anxiety levels may hasten the immune response of type 2 helper T cells (Th2), which is skewed in AD [17].

1.5 Stress and AD

As previously mentioned, AD is frequently exacerbated by psychological distress and anxiety. However, it is important to note that AD can also induce tension and negatively impact various aspects of life, including sleep, physical activity, mental health, and emotional well-being. The impact has been estimated to be on par with that observed in other chronic diseases, including diabetes and epilepsy [18]. AD may be triggered and exacerbated by stress. Although the term stress encompasses both physiological and psychological stress, the primary emphasis of this thesis is on the role of psychological stress in AD. Multiple studies have examined the correlation between AD and psychological stress, revealing that stress worsens AD and that patients with AD experience greater levels of psychological stress compared with healthy adults [19] [20]. The temporal relationship between psychological stress and eczema has also been examined [21], in addition to various facets of the nature of pruritus in relation to psychological stress.

Previous research has demonstrated that physical activity accompanied by sweating exacerbates symptoms of AD [22]. However, physical activity has been shown to reduce tension levels among Korean AD adolescents, according to a recent study [23].

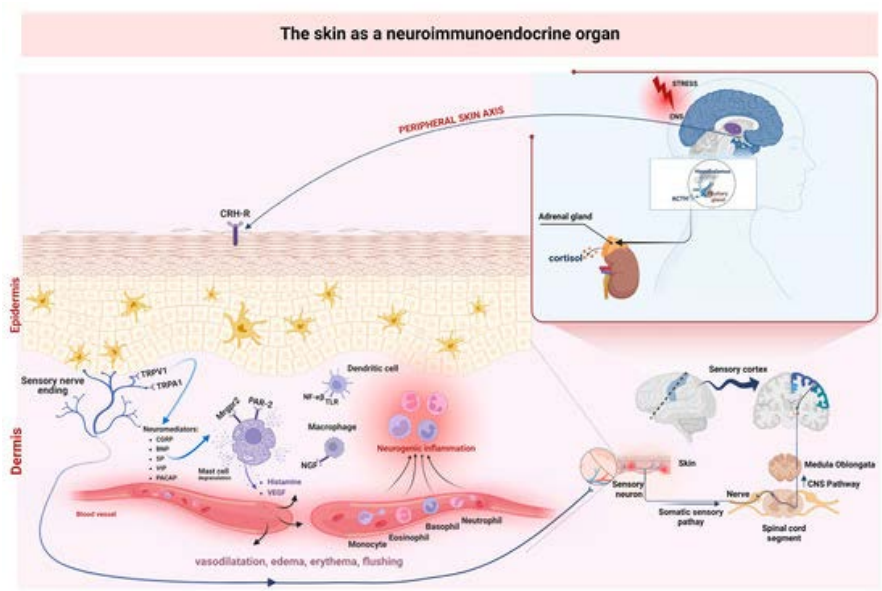
Furthermore, stress responses differ by sex and gender, presumably as a result of sex hormones and gender socialization, respectively, according to numerous studies of larger populations [24]. According to a Korean population-based study, tension levels were found to be higher among females than among males [19].

Case studies [20] have shown that insight-oriented psychotherapy yielded positive outcomes in individuals with persistent AD, leading to improvements in both skin condition and psychological well-being. The use of psychological interventions in individuals diagnosed with AD has revealed significant enhancements in anxiety reduction, coping with frustration, and mitigating itch-scratch behaviors. Cognitive behavioral therapy has been used as a means

to provide young patients and their parents with a deeper understanding of the difficulties associated with AD and to reconfigure cognitive processes [20].

1.6 Bidirectional interaction between the neuroendocrine system and the immune system

The neuroendocrine system and the immune system, including the skin, exhibit a bidirectional connection (Fig 2). The communication between these entities occurs through three pathways: the hypothalamic-pituitary-adrenal (HPA) axis, which is involved in the release of cortisol; the release of neuropeptides, including substance P and calcitonin gene-related peptide (CGRP); and the activation of the sympathetic system, which controls the release of acetylcholine and monoamines [25]. The neuromediators synthesized by the neuroendocrine system are involved in the immune system, as are their receptors. The vast majority of the cells in the skin have receptors for neuromediators, enabling them to receive signals from the neuroendocrine system [26].



Marek-Jozefowicz et al. Molecular Mechanisms of Neurogenic Inflammation of the Skin. *Int. J. Mol. Sci.* **2023**

Figure 2. The hypothalamus, together with its central and peripheral axis, controls skin barrier function and inflammatory responses. Under stress, the hypothalamus produces neuromediators (such as corticotropin-releasing factor), which may prompt the adrenal hypothalamic-pituitary-adrenal axis to increase the production of cortisol and other stress hormones. Circulating leukocytes are crucial for controlling immune responses during periods of inflammation. Immune cell-produced cytokines and neuropeptides may regulate skin inflammation. CGRP and tachykinins are released from sensory nerve terminals in response to tryptase from degranulated mast cells, which activate the protease-activated receptor 2. In response to mediators released by mast cells and other inflammatory cells, vasoactive sensory nerve peptides are produced.

1.7 AD and stress and physiological mechanisms

The activation of the HPA axis is initiated by stress, resulting in the secretion of glucocorticoids, notably cortisol. The disruption of the axis caused by chronic stress might lead to long-term raised cortisol levels. There is a documented correlation between heightened levels of cortisol and compromised skin barrier function, increased epidermal water loss, and diminished skin hydration. These factors all contribute to the exacerbation of symptoms associated with AD [27, 28].

The stimulation of the sympathetic nervous system and subsequent release of neuropeptides in response to stress might result in the occurrence of neuroinflammation. In the context of AD, neuroinflammation has the potential to disturb the integrity of the skin barrier, intensify pruritus, and facilitate the infiltration of immune cells, thus worsening the inflammatory response characteristics of this illness [29].

It has been shown that stress can influence immunological responses, perhaps causing them to shift towards a condition that promotes inflammation. The deregulation of the immune system can result in heightened infiltration of immune cells, such as T lymphocytes and mast cells, into the skin, exacerbating the inflammatory cascade observed in AD [29].

1.8 Methods for analyzing neuro-dermatological symptoms

Neuro-dermatological symptoms and pathogenic mechanisms in inflammatory diseases, such as AD, are difficult to evaluate and measure. In this project, multiple methods have been used to capture and identify the psychological contribution to the disease burden in AD.

1.8.1 Focus group research in dermatology and AD

Morgan [30] defines focus groups as a research methodology in which data are gathered via group interactions pertaining to a subject matter predetermined by the investigator. Focus group research consists primarily of the following phases: sample selection, facilitation of group member contributions, transcription, and text analysis. The belief that a group dynamic can aid individuals in articulating and elucidating their perspectives in ways that are less common in one-on-one interviews is fundamental to the concept of focus groups [31]. Focus groups have the ability to elicit information in a spontaneous manner, which would be unattainable through formal, in-depth interviews. Focus groups are frequently used ahead of health surveys to gather unfiltered insights from the study population, facilitating the formulation of questionnaires [30]. Focus group discussions have demonstrated considerable

promise in dermatology, particularly in studies of patients' perspectives and attitudes toward the treatment and etiology of skin diseases [32] [33]. For example, focus groups were used in a study examining the perspectives of parents of children with AD concerning the disease's etiology, precipitating elements, and affective consequences [33]. An area that has been investigated in prior focus group research in the field of dermatology is the potential exacerbating influence of psychological stress [32] [33].

1.8.2 Survey methods in dermatology

Collecting data on specific issues from a wide population through interviews or focus groups would be a time-consuming endeavor. Therefore, surveys are an important method used by researchers to assess and quantify several aspects of a sample, including beliefs, perspectives, behaviors, experiences, and expectations. The survey questions should be relevant to the research hypothesis and the intended participants, and should be constructed in a way that guarantees clarity and understanding [34]. A research approach could involve employing a survey with a set of identical questions being distributed to a large cohort of individuals. This methodology would yield quantitative data, with a lesser emphasis on qualitative outcomes, due to the predetermined nature of the questions used. Subsequently, researchers could summarize and analyze the gathered materials in order to address research questions or hypotheses. The method is characterized by its systematic nature, necessitating meticulous planning and execution to guarantee precise and dependable outcomes. This is because the questions should be interpreted uniformly by all participants [34].

In dermatology, surveys can be used to study various topics, such as the effectiveness of treatments, the impact of skin disease on quality of life, and the psychological dynamics of skin disease. Previous research in AD has used survey methods to investigate psychosocial aspects related to the disorder. For example, the survey approach was used to evaluate the association between work-related stress and hand eczema in the Korean population [19]. According to the findings, higher levels of work-related stress related to an increased risk of developing hand eczema, emphasizing the need to address psychological variables in the prevention and management of this skin condition [19].

Another study that used survey techniques looked at factors that aggravated itch in AD. A comprehensive review of the literature was used in that investigation. It was shown that environmental variables, including dry air, allergens, and psychological stress, were significant itch triggers in AD [35].

1.8.3 Functional brain imaging and AD

In order to gain insight into the fundamental brain processes that influence systemic and dermatological disorders like AD, a limited number of studies have used functional magnetic resonance imaging (fMRI) to examine neurological pathways in patients and healthy controls (HCs). Some investigations have employed block-designed experiments in which an itch stimulus is generated by a visually evocative experimental video or histamine [36] [37]. Others have utilized resting-state fMRI, for more intricate analysis of the connectivity between functional brain networks [38] [39]. A common characteristic among these studies is the relatively small sample sizes, occasionally consisting of fewer than ten subjects per group, which substantially diminishes statistical power. Collectively, the results of these investigations indicate that variations in the response of brain activity within the salience network, which regulates attention and facilitates sensorimotor readiness for scratching, may correspond to disparities in the perception of itching. However, much remains unknown regarding the functional circuitry of the brain, which is impacted by the incapacitating symptoms of AD.

1.9 Neurogenic inflammation in AD

1.9.1 Neuromediators and skin inflammation in AD

Keratinocytes in the epidermis are stimulated to generate proinflammatory cytokines, including IL-2, IL-6, and IL-8, by neuropeptides released from sensory nerve fibers [40] [41]. Additionally, the antigen presentation capability and migration of Langerhans cells (LCs) in the epidermis may be impacted by neuropeptides [42]. Sensory nerve fibers coexist with noradrenergic and acetylcholinergic nerve fibers, which also comprise neuropeptides, including vasoactive intestinal peptide and neuropeptide Y, in the dermis.

In the dermis, sensory nerve fibers frequently come into proximity with mast cells and blood vessels, even in the presence of neurogenic inflammation. The release of substance P and CGRP from sensory nerve endings triggers the degranulation of mast cells, which in turn leads to the activation of pro-inflammatory mediators, including histamine [43]. Mastocyte-secreted histamine stimulates the secretion of neuropeptides via receptors located on sensory nerve endings.

Additionally, neuropeptides have the potential to impact the proliferation and vascularization of endothelial cells, thereby augmenting an inflammatory response [44]. Fibroblasts located in the dermis are capable of producing neuropeptides and expressing receptors for them [44].

These neuropeptides may exacerbate an acute inflammatory response and subsequently contribute to fibrosis in the chronic phase of skin inflammation.

Besides substance P and CGRP, other neuropeptides have been shown to be involved in AD, and play a role in pruritus and inflammation. They include vasoactive intestinal peptide, neuropeptide Y, and gastrin-releasing peptide [45].

Additionally, a disruption in acetylcholine responsiveness results in atypical vascular responsiveness and a reduction in sweating, causing elevated skin temperature, inflammation, and dehydrated skin[46]. Thus, acetylcholine may modulate itch and inflammation in the skin of AD patients [47].

1.9.2 Neurogenic inflammation

The term “neurogenic inflammation” refers to the contribution of sensory nerves to inflammation through the action of neuropeptides, such as substance P and CGRP, on vascular endothelial and smooth muscle cells [44]. This contribution is characterized by vasodilation and protein extravasation.

Skin nociceptors convert harmful stimuli into electrical activity. Nociceptors have the capability to transmit action potentials in both an axon reflex (antidromic to the peripheral nervous system or orthodromic to the central nervous system) [48]. Neuropeptides with biological activity are secreted in order to transmit these signals.

Currently, the notion of neurogenic inflammation encompasses the involvement of a multitude of inflammatory cells as well.

1.9.3 Calcitonin gene-related peptide

CGRP is a peptide belonging to the calcitonin family and consists of 37 amino acids. CGRP exists in two forms, α-CGRP (CGRP I) and β-CGRP (CGRP II) [48]. [49]**.** In humans, they are from two different genes, differ by three amino acids and share >90% homology, exerting biological effects, including vasodilation through similar mechanisms . α-CGRP is more abundant, located in specific regions in the central and peripheral nervous system. β-CGRP is found primarily in the gut, enteric nerves and pituitary gland CGRP acts on calcitonin receptor-like receptors (CLRs) associated with receptor activity modifying proteins (RAMPs), needed for full functionality. CGRP is found in unmyelinated C and myelinated A delta sensory nerve fibers in the periphery. CGRP is produced primarily in sensory nerves together with neuropeptides such as substance P (SP), as well as in the

central nervous system. CGRP expression is also abundant in trigeminal ganglion neurons [49].

CGRP expression by other cells is also known, including Langerhans cells (LCs), endothelial cells, keratinocytes, fibroblasts, T lymphocytes, B lymphocytes, and monocytes (see ref. 47). Its production is regulated by nerve growth factor (NGF) and can be secreted together with SP (Park et al., 2010) Signaling pathways that mediate nerve growth factor-induced increase in expression and release of calcitonin gene-related peptide from sensory neurons).

1.9.4 CGRP and AD

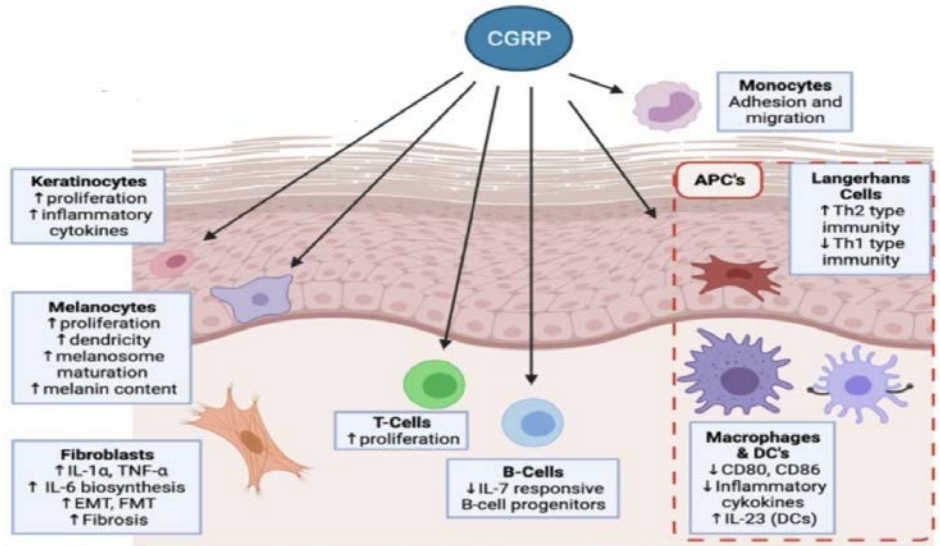
CGRP exhibits robust vasodilatory properties and seems to direct immune responses in the direction of the T helper 17 cell pool. By increasing the expression of IL-23 receptors, it directly increases IL-17 production from T helper 17 cells [50]. CGRP-positive nerve fibers and epidermal LCs occur in close proximity [51]. CGRP has been found to promote LCs' antigen presentation during Th2 responses,but inhibit presentation during type 1 helper T cell responses. Thus, CGRP facilitates the transition of LCs to Th2 respons [42]. CGRP increases the ratio of ILs to interferon gamma, IL-13, and human cutaneous lymphocyte-associated antigen-positive T cells in AD (for references, see Choi and Di Nardo, 2018)[26]. These results suggest that CGRP plays a unique role in AD.

It has been documented that AD lesional skin is hyper-innervated compared with non-lesional skin, as evidenced by an increase in CGRP-positive nerves in the epidermis and papillary dermis and mast cell-nerve fiber contacts [26, 52]. In contrast [53] another study reported that CGRP-positive nerve fibers in the epidermis of AD patients were identical to those in HCs.

It has been documented that CGRP causes less pruritus than substance P [54]. It is worth noting that AD patients who experienced intense pruritus have been found to have substantially elevated plasma levels of CGRP in comparison to those who did not have pruritus [26]. Furthermore, a correlation has also been found between more severe disease and higher plasma levels of CGRP [52].

Certain moods, including tension, melancholy, and anxiety, are intricately intertwined with CGRP from a neurobiological standpoint. CGRP is increased in dorsal root ganglion cells and cutaneous sensory nerves in rodents and humans exposed to stressors [55]. Acute and chronic stress both regulate distinct neuro-immunological events[56]. One study found that CGRP expression in the epidermis of psoriasis patients was decreased in response to a transient

moderate stressor, whereas chronic stress increased CGRP expression. It has been reported that CGRP expression in the hippocampus of mice is associated with depression-like behavior [57]. A significant correlation was observed between CGRP expression in the frontal cortex and the hippocampus of an anxiety-related behavioral rat strain and an anxiety-like behavior. Anxiety could be induced in rodents via CGRP infusion into the lateral ventricle [57]. Additionally, CGRP levels may be elevated in the spinal fluid [58], plasma, and sweat [52] of depressed patients.



Modified from: Kim et al. Roles of calcitonin gene-related peptide in the skin, and other physiological and pathophysiological functions. *Brain Behav Immun Health.* **2021**

Figure 3. The skin's reaction to CGRP. Epidermal cells such keratinocytes, melanocytes, and fibroblasts, as well as other immune cells like monocytes and macrophages, LCs, dendritic cells, and neutrophils, are all affected by CGRP, which likely contributes to a multitude of illnesses.

2 Research aims

Studies I–II: To examine and investigate the relation between AD and distress using focus groups and a population study.

Study III: To investigate brain activity in AD patients during acute stress, induced by an arithmetic test, using fMRI.

Study IV: To investigate expression of CGRP in the skin of AD patients.

3 Materials and methods

3.1 Focus groups in AD (Study I)

3.1.1 Patients and methods

Potential participants, diagnosed with AD, were selected from the outpatient clinical records of the Department of Dermatology at Karolinska University Hospital in Solna, Sweden. All participants involved in the research had received a diagnosis of AD from a dermatologist. The research was carried out in compliance with the principles outlined in the Declaration of Helsinki, after getting the necessary ethical approval and informed consent from the participants.

The study encompassed a total of 28 patients, with detailed patient characteristics provided in Table 1 of Paper I. The individuals in question had been sent to the Department of Dermatology by their primary care physicians or a dermatological outpatient clinic, for the purpose of receiving specialist evaluation for systemic therapy or enhanced topical treatment, or for further investigations, such as epicutaneous patch testing. The majority of the patients had been diagnosed with eczema in youth. The study participants consisted of 23 females and 5 males, ranging in age from 18 to 67 years. They were organized into 12 groups, each including 2–3 patients, in order to ensure appropriate interaction levels between participants.

The sessions were held at the Department of Dermatology, located at Karolinska University Hospital in Solna, Sweden. Each session had a duration of roughly 45 minutes. The designated space offered a neutral environment; the participants were seated in comfortable chairs arranged around a table and offered refreshments. Prior to the commencement of the conversation, it was stated that the perspectives of the collective were of utmost importance, with the objective being to foster dialogue between the participants.

At the start of each session, the moderators, who were medical professionals, initiated the discussion by introducing the subject matter and encouraging the participants to express their perspectives and personal experiences. This was followed by an enumeration of thematic areas, which were deliberated upon in each respective focus group. The study examined the influence of psychological stress as a contributing element in the exacerbation of eczema. It investigated the effects of stress on the affected region and the characteristics of eczema and associated itching. Additionally, it explored significant psychological triggers and various treatment approaches, as outlined in Table 2 (Paper I).

The initial pace of discussions was quite slow, but improved over time, aided by the moderators' active facilitation. The moderators intervened if a conversation went beyond the predetermined topics.

The researchers used audio recording devices to capture the discussions held in each focus group. They also made written records of the proceedings. The recorded audio materials were subsequently transcribed and translated into English.

3.2 Survey (Study II)

3.2.1 Patients and methods

A total of 3,395 individuals on specialist dermatological treatment for AD in Stockholm county, at the Departments of Dermatology at Karolinska University Hospital Solna, Södersjukhuset, and Älvsjö Hudmottagning, were sent an invitation letter to participate in an anonymous online survey. The correspondence was sent in late fall 2022, and the process of data collecting was completed within the span of two months. The survey was conducted using the survey system of Karolinska Institutet. Exclusion criteria included having a protected identity or an unknown address. Moreover, a total of 22 letters were deemed undeliverable, resulting in the exclusion of these patients from the overall count. Out of the total population, 17.9% of the sample, or 609 individuals, responded to the survey.

The participants were provided with information on the objective of the research, which was to examine the correlation between stress and AD. Additionally, they were told about the estimated duration of the study, which was roughly 25 minutes. The correspondence included a hyperlink to the survey website. All participants provided electronic informed consent. Prior to the commencement of the study, it received approval from the Swedish Ethical Review Authority.

In order to formulate the questions, a series of focus groups were conducted[59]. Following the identification of areas of interest, questions were deliberated upon with the involvement of the patient organization, Atopikerna, within the Asthma and Allergy Association in Sweden. Additionally, the questions underwent validation via individual interviews conducted with patients in order to ascertain their comprehension of the questions. Following a few minor adjustments, the survey was sent to a sample of 20 patients as part of a

preliminary pilot study, before the final version of the survey was subsequently developed.

The subjects that were addressed are outlined in Table 1 (Paper II) (for a comprehensive presentation, please refer to Supplementary Material 1 (Paper II)). Previously established measurements to assess the extent of disease, including the Subjective-SCORing Atopic Dermatitis (S-SCORAD)[60] for subjective evaluation, the Dermatological Life Quality Index (DLQI) [61] for assessing quality of life [61], the Hospital Anxiety and Depression Scale (HADS) for measuring depression and anxiety, the Perceived Stress Scale (PSS) [62] for evaluating perceived stress, and the Karolinska Exhaustion Disorder Scale (KEDS) [63] for chronic stress assessment, were also included. In order to assess the extent of disease, a modified iteration of the S-SCORAD method was used. This approach required patients to indicate the specific regions of their body affected by eczema.

3.2.2 Statistics

The numerical values were calculated using Student's *t*-test or the Mann-Whitney test (depending on the distribution of the values). Correlations between the different parameters were measured using Spearman's or Pearson's tests (depending on the distribution of the values and if they were absolute numbers). Differences were considered to be statistically significant when $p < .05$.

3.3 Functional magnetic resonance imaging (Study III)

3.3.1 Patients

Ethical approval of the study was obtained from the local ethical committee (Dnr: 2013/1684-31/1). A total of 29 adult female subjects (age = 32.3 ± 7.7 years) with a clinical diagnosis of AD and a S-SCORAD score of over 30 were enrolled from the Department of Dermatology, Karolinska University Hospital, Solna, Sweden. Twenty-three age-matched healthy female controls (age = 32.7 ± 7.3 years) were also included. An array of 13 different clinical and laboratory measurements was used to assess the neuropsychological traits of the study subjects, including objective and subjective degrees of eczema using SCORAD, level of itch (estimating the degree of itch for the preceding 3 days on a visual analogue scale 0–10, where 10 is the highest and 0 the lowest degree of itch), sleep disturbance (using a visual analogue scale 0–10), salivary cortisol, and a questionnaire on exhaustion symptoms of chronic stress

(Karolinska Exhaustion Disorder Scale, (KEDS), depression, stress susceptibility (SS), somatic trait anxiety (STA), physical trait anxiety (PsTA), lack of assertiveness, transient irritability (TI), and impulsivity (IM). The clinical examination was performed by a dermato-venereologist (KN) immediately after the fMRI session, and the questionnaires filled out in connection therewith. One HC and one AD subject did not complete some of the core clinical measurements and were excluded from the statistical analysis. Salivary cortisol samples were obtained from 16 AD patients and 23 HCs. The saliva samples were collected in plastic vials at 8 AM on 3 consecutive days after the visit to the department. The samples were stored at -20 °C until analysis. The cortisol concentrations were measured using a radioimmunoassay (RIA) with a rabbit polyclonal antibody "Cortisol 3," catalog number MBS535414 (MyBioSource, San Diego, USA). Changes in heart rate between active and resting epochs were estimated to assess the induced acute psychological stress.

3.3.2 Imaging data processing procedures

The T1-weighted MPRAGE imaging data were pre-processed with the CAT12 Toolbox (http://www.neuro.uni-jena.de/cat/) in Statistical Parametric Mapping (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The processing pipeline, as detailed elsewhere[64, 65] was segmented into grey matter, white matter, and cerebrospinal fluid probability maps, registered to the Montreal Neurological Institute standard space. Smoothing was performed with an 8 mm full width at half-maximum Gaussian kernel before statistical assessment. Voxel-wise two-sample t-test analysis was conducted, with age and total intracranial volume as covariates. In addition, a ROI-based voxel-based morphometry analysis was performed for the paraventricular nucleus of the hypothalamus, as previously described [66].. Accordingly, the mean value of grey matter density 6 was estimated for a 10 mm sphere centered at the Montreal Neurological Institute coordinate (x, y, z) = (2, –1, –12), and its correlations versus SS and other clinical metrics were also evaluated. The fMRI datasets underwent an updated preprocessing procedure with a shell wrapper based on the AFNI (http://afni.nimh.nih.gov/afni) software package. Briefly, the pipeline included motion correction, spatial registration to Montreal Neurological Institute template space, bandpass filtering, and detrending. The preprocessed fMRI data were smoothed using a local Gaussian smoothing up to full width at half-maximum = 4 mm. A brain activation map at an individual level was generated by modelling the time series data using regression based on the general linear theory with the hemodynamic response function convolved and the block-designed paradigm used as the reference function. The model also included 12 additional auxiliary

regressors related to involuntary head motion. From the general linear theory regression analysis, voxel-wise activation maps (the b-parameter) were extracted for each subject.

3.3.3 Statistics

The experimental results at the group level were analyzed using a multivariate approach. As the study focused on testing if the blood-oxygen-level-dependent (BOLD) activation responses were significantly different between the HCs and the AD subjects, after excluding potential confounding contributions from the covariate variables, such as psycho-demographic and clinical scores. The study also focused on which covariate variables might be correlated with brain activation and whether such correlations were different between HCs and AD subjects. Multivariate analysis was carried out using the AFNI program 3dttest++ with a covariance matrix including all relevant clinical scores for the participants. The statistical significance of the t-test results at the group level was assessed using a two-step approach. First, a voxel-wise threshold $p < 0.001$ was used to identify the initial cluster candidates. For multiple comparison correction, permutation simulations were performed to identify the brain regions of interest (ROI) out of the initially detected clusters at least with a family-wise error rate (FWER) $p<0.05$. For thorough understanding of the data structure, an additional t-test without covariates and multiple linear regression were also conducted.

3.4 CGRP in AD (Study IV)

3.4.1 Patients

Twenty-seven adult AD patients, 18 female and 9 male, mean age 29.5 years (range 18–48 years), were included. These patients, in whom serotonergic [23] and tachykinergic [24] mechanisms had previously been studied, were recruited from among patients referred to the aforementioned clinical department. The patients had to have ongoing AD in accordance with the criteria of Williams et al. [25] and could not be on systemic therapy (including phototherapy and antihistamines) during the study or within one month prior to inclusion. The patients were clinically investigated, answered questionnaires, and left biopsy samples during their visit to the department. Two of the patients did not complete the questionnaires. Ethical permission was obtained from the local ethical board and patients gave written informed consent to participate in the study.

The extent of the disease was assessed using SCORAD [26]. Both objective and subjective SCORAD score were recorded. The intensity of pruritus was assessed using a visual analogue scale, where patients rated their pruritus over the preceding three days on a scale 0–10 (0 = no pruritus, 10 = maximum pruritus). The Swedish Universities Scales of Personality [27], a 91-item questionnaire, was used to evaluate the patients' personality traits. The questionnaires were analyzed regarding STA, PsTA, and SS. Absolute scale values were calculated. The Montgomery-Åsberg Depression Rating Scale - Self-assessment [28] was used to assess the level of depressive symptoms.

3.4.2 Salivary cortisol and immunohistochemistry

Salivary cortisol samples were obtained from 23 of the patients. The saliva samples were collected in plastic vials at 8 AM on three consecutive days after the visit to our department. At 10 PM on the third day, 0.25 mg of dexamethasone was administered orally, with a new cortisol test taken on the following morning. The cortisol concentrations were measured using a RIA with a rabbit polyclonal antibody "Cortisol 3," catalog number MBS535414 (MyBioSource, San Diego, USA). Compared with cortisol, its affinity for prednisolone is 37%, that for 11-deoxycortisol 5%, for corticosterone 3%, and for cortisone < 1%. The intra-assay coefficient of variation for the RIA was 7% at 10 nmol/L. The ratio of the mean of the first three values to the last cortisol value was determined, with a low ratio being an indicator of chronic stress [29].

Biopsies, 3 mm in diameter, were taken from lesional skin on the cubital fossa, and from non-lesional skin in the sacral region. Only emollients were to have been used on these areas for at least 14 days prior to inclusion in the study. Lana's fix (phosphate buffered 4% formaldehyde containing 0.2% picric acid) was used for fixation of the biopsies for 2 h at 4 °C. The samples were then rinsed in 0.1 M Sörensen's phosphate buffered saline supplemented with 10% sucrose for 24 h and frozen and stored at -70 °C until being cut into 14 µm thick sections for immunohistochemical staining. The sections were incubated overnight with a rabbit polyclonal antibody against CGRP (1:10,000) from Bachem (St Helens, UK) at 4 °C. Thereafter, biotin-labeled goat anti-rabbit (BA-1000, 1:200, Vector Laboratories, Burlingame, CA, USA) was added as the secondary antibody, followed by the fluorochrome Alexa Fluor [6]488 streptavidin (1:1,000, Life Technologies, Stockholm, Sweden). To estimate background staining, the primary antibody was omitted from the negative control. Immunoreactivity for CGRP was studied using epifluorescence (Zeiss Axioskop 2 MOT microscope, Carl Zeiss, Stockholm, Sweden). Nerve-like fibers were defined as linear

structures with a slight vesiculated appearance at a high magnification and were found either in the dermis, epidermis, or crossing the dermal/epidermal boarder. Dendritic cells observed in the epidermis were defined as round cell bodies with dendrites. Acanthosis and degree of inflammation (infiltration of inflammatory cells in the dermis) were graded semi-quantitatively, 0–3 (0 = noninflammatory, 1 = mild, 2 = moderate, and 3 = severe). The sections were counted manually by one observer (KN). Four sections per biopsy were analyzed at a magnification of ×400. The absolute numbers of CGRP-positive epidermal inflammatory cells and nerve-like fibers were determined; a mean value was calculated for the sections, and standardized to 2.75 fields of vision. The percentage of CGRP-positive dermal inflammatory cells out of the total number of dermal inflammatory cells was calculated. A semi-quantitative evaluation for keratinocyte CGRP expression was performed by two observers (LL and KN) and rated 0 (0 positive cells per standard vision field), 1 (1–10 positive cells per standard vision field), 2 (11–20 positive cells per standard vision field), or 3 (21–30 positive cells per standard vision field).

3.4.3 Statistics

The numerical values for CGRP expression were calculated using Student's t-test or the Mann-Whitney test (depending on the distribution of the values). For semi-quantitative evaluations, the chi-squared test was used. Correlations between the different parameters were measured using Spearman's or Pearson's tests (depending on the distribution of the values and if they were absolute numbers). Differences were considered to be statistically significant if $p < 0.05$.

4 Results

4.1 Focus group study and survey study (Studies I and II)

4.1.1 General findings

In total, 28 patients participated in the focus groups, 23 females and 5 males, age range 18–67 years. The survey study involved 629 patients with AD, with 32.9% male and 66.5% female. In the survey study, the median age was 39 years, with 45% having education at university level or higher. Most patients (54%) got their diagnosis at a dermatological clinic, whereas 20.5% got it at a family doctor clinic. Most patients (78.9%) reported symptom debut at 0–9 years. Ongoing eczema was reported by 87.2% of the patients, whereas 12.8% had no symptoms at the time of the questionnaire. Regarding treatment, 86.6% of the patients in the survey used emollients daily, with 63.5% using glucocorticoids or other anti-inflammatory topical treatment. A proportion of 14.3% were on systemic treatment, whereas 85.7% had topical treatment. Among systemically treated patients, 44.9% were taking dupilumab. A proportion of 12.3% had received UVB treatment during the preceding year.

Regarding pruritus, 72.5% of the patients had pruritus in the preceding 24 hours, with a burning sensation. The largest proportion (45.7%) reported both superficial and deep type localization. The itch was most often localized in the arm region, followed by the head, trunk, and legs.

Patients reported affected quality of life with a mean value of 7.55 points (a moderate effect on quality of life), and a moderate correlation was found between the extent of disease measured with SCORAD and DLQI ($r = 0.64$; $p < 0.0001$).

4.1.2 Stress-worsening and relation between psychological stress and other worsening factors

All the patients in the focus groups agreed with the hypothesis that psychological stress might influence eczema and pruritus. Some patients indicated that the eczema itself might induce psychological stress. It was stated that the magnitude of psychological stress was significant. Year-round psychological stress could have an impact. The correlation between chronic psychological tension and depression was examined.

In each of the focus groups, the difficulty of differentiating psychological stress from environmental and infectious stimuli, as well as allergic factors, was acknowledged. Some

patients stated that infections and climate conditions were the most significant determinants of deterioration. Nonetheless, psychological stress was rated as important. It was acknowledged that exacerbating factors might operate in concert.

When asking a larger patient material through the survey, the majority (82.5%) of the patients reported stress worsening of their eczema, with work-/study-related stress being rated as most important, followed by family problems and economic stress. Among different worsening triggers, psychological stress was rated the highest, followed by climate, infections, chemicals, pollen, and food.

4.1.3 Acute and chronic psychological stress

In the focus groups, the majority of patients believed that chronic stress was a more significant deteriorating factor than acute stress; for instance, chronic stress could cause sleep disturbances. Generally, acute stress induces a transient, immediate pruritus that is frequently manifested through scratching. Conversely, chronic tension results in increased persistence of pruritus and scratching.

Through the survey questions on chronic stress (KEDS) and more acute stress (PSS), a weak correlation could be observed between SCORAD score and chronic stress ($p < 0.0001$, $r = 0.38$), and SCORAD score and anxiety ($p < 0.0001$, $r = 0.31$) and depression ($p < 0.0001$, $r = 0.29$), respectively. The correlation between SCORAD score and acute stress was statistically significant, but weak ($p < 0.05$, $r = 0.14$).

4.1.4 Effect of psychological stress on eczema/pruritus

Both eczema and pruritus were impacted by psychological tension, according to the patients. Some patients indicated that psychological stress occurred prior to the development of eczema, with the stress manifesting on the skin. Conversely, other patients reported that psychological stress was associated with the progression, persistence, and severity of the condition.

The majority of patients (85.1%) in the survey reported that psychological stress worsened the itch. Further, the majority (53.5%) stated that the stress preceded the itch, but many (40.7%) indicated that this could vary.

4.1.5 Psychological distress and other triggers

In contrast to environmental triggers, such as the Nordic winter climate, psychological tension was associated with increased erythema and sporadic dermatitis, according to the patients. Eczema exacerbated by environmental factors was more amenable to amelioration with emollients than eczema exacerbated by psychological stress.

According to patient reports, psychological tension was associated with more severe pruritus than other aggravating factors. Some patients reported experiencing pruritus in particular anatomical regions, including on the scalp. Conversely, climate stress induced more generalized pruritus. Unexpectedly, a subset of the patients indicated that climate stress induced more superficial pruritus, whereas psychological stress was associated with more subjectively intense pruritus. The majority of patients indicated that psychological stress-induced pruritus was more challenging to manage than climate stress.

As previously stated, patients have reported that psychological stress induces scratching. Thus, in the absence of any stress, the patients reported a greater propensity to refrain from scratching.

It should be stated that the issue may have been the act of itching, independent of pruritus precipitation. Self-discipline to refrain from scratching could be considered a potential strategy for managing psychological stress and its detrimental impacts on the epidermis. The majority of patients reported that fatigue caused increased pruritus and itching.

It is significant that patients indicated a higher propensity for pruritus to manifest in response to unanticipated events that induced psychological stress. An illustrative instance was provided by a patient employed at a Swedish convenience store specializing in the sale of newspapers and periodicals. The patient encountered anxious clients on a daily basis; upon returning home, the patient could sometimes think about the customers' facial expressions and develop pruritus. However, the majority of patients reported that their eczema/pruritis was less affected if they could anticipate psychological stress.

This was explored further in the survey and psychological stress was rated as the most common trigger, followed by climate factors, chemicals, food, pollen, and infections.

Also, 55.7% of the patients reported that they had consulted a doctor for stress, anxiety, depression, or exhaustion, with the majority (62.4%) experiencing stress. Further, 50.2% had ongoing stress, anxiety or depression/exhaustion, with the majority (82.4%) being troubled by stress.

4.1.6 Psychological triggers

The patients in the focus groups indicated that their work environment, including overworking, a negative mood, excessive task stacking, and/or working at a high pace, could exacerbate AD. A lack of structure in the workplace could also exacerbate the disease. Anxiety brought on by an excessive workload, in addition to sleep disruptions, could contribute to deterioration. Remarkably, loneliness and retirement could also function as catalysts. It has also been reported that flare-ups occur during school exam periods, which served as an exacerbating factor. Concerns regarding future life events and finances, as well as family issues (e.g., separation), were significant stimuli. Generally, circumstances involving decision-making or uncertainty were associated with adverse effects on AD. A summary of reported triggers is provided in Table 3 (Paper I).

Work-related psychological stress was rated as the most influential psychological stressor, with family- and relationship-related psychological stress as the second most important. Economic stress was also rated as important in the survey. Another factor mentioned in the survey was stress related to one's own or a relative's psychiatric diagnoses (anxiety disorder, obsessive-compulsive disorder, etc.). Worries about the outside world were mentioned several times and were not covered by any other option.

4.1.7 Treatment of stress-worsened eczema

In the focus groups, patients indicated that fat emollients provided significant alleviation during the winter season, but not during the summer (several patients recommended increasing the water content of the emollient in summer). Additionally, patients favored emollients with a higher water content during the day and those with fat at night. Topical tacrolimus and topical corticosteroids were considered beneficial. Despite reports that ultraviolet light treatment induces stress in itself, it continues to be considered beneficial due to the limited time required to attend treatments, which is also the case with conventional topical treatments. As per the patients' accounts, antihistamines could induce sedation. One patient's pruritus was exacerbated by the administration of a selective serotonin reuptake inhibitor. It was reported that cognitive behavioral therapy, acceptance and commitment therapy, and talking therapy/psychotherapy were beneficial, as the patients learned to recognize and prepare for the triggers. Still, only a minority of the patients had undergone such treatment modalities. According to the patients, climate therapy or travelling abroad could be extremely beneficial. Some patients had attempted to treat their conditions with

topical alternatives, but were unsuccessful. A number of the patients longed for an anti-stress lotion.

According to the survey results, 11.2% of the patients had been offered treatment for stress, anxiety, depression, or exhaustion, with most (67.2%) treated with cognitive behavioral therapy or mindfulness.

4.1.8 Physical activity

Patients in the focus groups reported that physical activity did not exacerbate their eczema. In and of itself, the eczema did not appear to be a barrier to exercise. Physical activity was proposed as a potential method to alleviate psychological tension and was also generally acknowledged for its positive impact on health.

In the survey, 56.4% of the patients reported that they performed regular exercise, most commonly 1–2 times a week.

4.2 fMRI study (Study III)

4.2.1 Clinical characterization of the study subjects

All subjects, 29 AD patients and 23 HCs, completed a set of psycho-demographic and laboratory measurements of 9 different traits and scorings. The AD patients completed 4 additional clinical scorings specifically relevant for AD symptoms (Table 1S, Paper III). Except for salivary cortisol levels, there were no significant differences regarding clinical and neuropsychological results between the HCs and AD subjects. A two-sample t-test of the salivary cortisol data revealed that the AD group has a significantly higher mean value ($p < 0.038$) than the HCs. For the AD subjects, a statistically significant correlation was found between the objective SCORAD score and sleep disturbance ($r = 0.447$; $p < 0.017$) and with KEDS score ($r = 0.419$; $p < 0.027$). Furthermore, in the AD patient group, IM correlated with STA ($r = 0.508$; $p < 0.006$), PsTA ($r = 0.382$; $p < 0.045$), and TI ($r = 0.623$; $p < 0.001$) (for values please see paper III)

4.2.2 The BOLD activation response to induced acute stress

Each participant was monitored with a pulse oximeter to record changes in heart rate during the arithmetic task experiment, to indicate the induced acute stress. Both AD patients and HCs showed an increase in mean heart rate values between rest and arithmetic task activity

(AD = 11.2 ± 6.0%, HC = 8.7 ± 6.8%), but no statistically significant difference between the two groups was seen ($p < 0.013$).

Both groups showed similar BOLD activation patterns for the arithmetic task, as illustrated in Figure 4. The brain areas which are involved in the arithmetic task are listed in Tables 1 and 2 in Paper III, and include the motor sensory system, the default mode network, and the supramarginal and angular gyri. To assess differences in BOLD response between HCs and AD subjects, the contrast images at the second level were compared between HCs and AD patients, with the contrast images defined at the first level (task versus rest segments). No clusters survived the voxel-wise two-sample t-test between the groups, with or without confounding covariates. These clusters failed to withstand the multiple comparison correction.

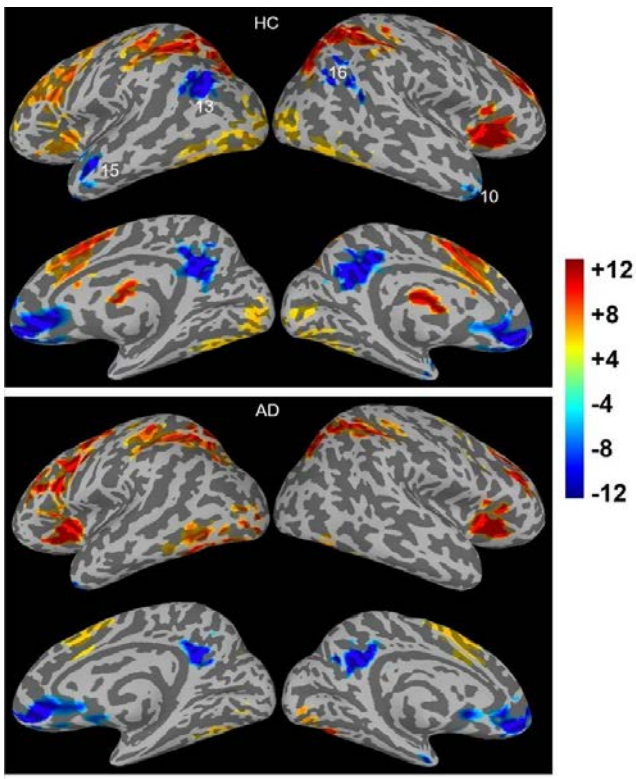


Figure 4. The group averaged BOLD responses to the block-designed arithmetic paradigm as obtained by one-sample t-test for each subject group. The annotated numerical numbers are the numbering of the ROIs (see Table 1, Paper III) depicting ROIs with negative responses that are unique for the HCs and missing in AD subjects. The color bar indicates the t-score scalar.

Notably, four ROIs (10, 13, 15, and 16; marked in Figure 4) in the HC group had negative BOLD responses (ranging from -3.78% to -4.01%). The ROIs were situated bilaterally in the temporal lobe, as indicated in Table 1 (Paper III) and Figure 4. The corresponding deactivations did not exhibit statistical significance in patients with AD. The BOLD responses of the two groups in other regions stimulated by the arithmetic task were identical.

4.2.3 Association between BOLD response and covariates

A summary of the results of the linear regression is provided in Table 3 (Paper III) and Figure 2 (Paper III). SS scores were positively correlated with the amplitudes of the BOLD response in the right postcentral gyrus and inferior parietal lobe in AD patients ($r = 0.44$, $p < 0.050$). A contrasting trend was observed in the HCs, as illustrated in Figure 3a (Paper III) ($r = -0.47$, $p < 0.030$). The correlation between the PsTA scores of individuals with AD and the BOLD activation in the left cerebellum and fusiform cortex (ROI 2 in Table 3 (Paper III)) was also negative ($r = -0.61$, $p < 0.010$), as illustrated in Figure 3b (Paper III). Similarly, the correlations between the IM scores and the BOLD response in the bilateral occipital cortices, right angular gyrus, and left inferior parietal lobe were negative (ROIs 3–5 in Table 3 (Paper III) and Figure 2 (Paper III)). No correlations among the HCs exhibited statistical significance (Figure 4 (Paper III)). The correlations between the itch scores of AD patients and the brain activations of the right post-central gyrus, the supramarginal gyrus, and the inferior parietal lobe (ROI1 in Table 4 (Paper III)) are illustrated in Figure 5 (Paper III). Additionally, significant correlations were observed between objective scores and the BOLD response of the left insula lobe, the superior temporal gyrus (Table 4 (Paper III)), and the Rolandic operculum (ROI2 in Table 4 (Paper III)).

4.3 CGRP study (Study IV)

4.3.1 Clinical, laboratory and psycho-demographic characteristics

The mean SCORAD score for the patients was 42.1 ± standard deviation 11.7, while the subjective SCORAD score was 51.4 ± 13.7. The average intensity of pruritus reported by the patients was 5.2 ± 2.5. The average cortisol concentration measured 84.1 ± 84.8 nmol/L, while the average cortisol ratio was determined to be 1.7 ± 1.4. The patients achieved mean scores of 15.2 ± 4.3 for STA, 15.3 ± 3.8 for PsTA, and 16.4 ± 4.2 for SS. The average score for melancholy was 8.0 ± 6.6.

4.3.2 General histopathological findings

Lesional skin exhibited a greater degree of acanthosis (2.2 ± 0.6) than non-lesional skin (1.2 ± 0.5; $p < 0.05$). There was a significant increase in the degree of inflammation (2.1 ± 0.6) in lesional skin as compared with non-lesional skin (1.1 ± 0.4; $p < 0.05$).

4.3.3 CGRP expression

Lesional skin contained a greater quantity of nerve-like fibers positive for CGRP (5.1 ± 3.7) than non-lesional skin (2.6 ± 1.8, $p < 0.05$). Additionally, lesional skin exhibited intraepidermal nerve-like fibers (Figure 1a (Paper IV); 2.6 ± 2.7) and indications of nerve-like fiber proliferation (Figure 1b (Paper IV)). There was a significant increase in the overall count of intraepidermal inflammatory cells that were positive for CGRP in lesional skin (3.6 ± 4.6) relative to non-lesional skin (0.9 ± 1.7, $p < 0.05$). There was no significant difference in the number of intraepidermal CGRP-positive round inflammatory cells between lesional and non-lesional skin (0.7 ± 0.9 vs. 0.3 ± 0.9). However, lesional skin contained a greater number of intraepidermal CGRP-positive dendritic cells (Figure 1(c) (Paper IV)) than non-lesional skin (2.9 ± 4.4 vs. 0.6 ± 1.6; $p < 0.05$). In lesional skin, keratinocyte CGRP expression was greater than in non-lesional skin (1.1 ± 1.1 vs. 0.1 ± 0.4; $p < 0.001$) (Figure 1a (Paper IV)). No significant difference was observed in the percentage of CGRP-positive dermal inflammatory cells between lesional and non-lesional epidermis (24.0 ± 8.6 vs. 22.0 ± 7.8).

4.3.4 Correlations

The depressive score was correlated with the total ($r = 0.58$; $p < 0.01$), epidermal ($r = 0.42$; $p < 0.05$), and dermal ($r = 0.46$; $p < 0.05$) counts of CGRP-positive nerve-like fibers in lesional skin (Figure 2). According to Spearman's test, the total ($r = 0.40$; $p < 0.05$) and dermal ($r = 0.42$; $p < 0.05$) counts of CGRP-positive nerve-like fibers both correlated with the degree of apprehension.

Depressive score was correlated with the number of epidermal round CGRP-positive inflammatory cells ($r = 0.40$; $p < 0.05$). Additionally, salivary cortisol levels were negatively correlated with the number of epidermal inflammatory round cells ($r = -0.55$; $p < 0.01$).

A strong tendency correlation ($r = 0.39$; $p = 0.06$) was observed between keratinocyte CGRP expression and PsTA score.

5 Discussion

5.1 General considerations regarding skin-brain bidirectional communication

The skin and the brain interact via several different pathways. It has been shown that inflammation in the skin can result in depressive symptoms, and that cytokines like IL-6 may be involved in this [67]. Further, psychological symptoms like stress and anxiety can result in skin inflammation with one responsible mechanism being neurogenic inflammation, which causes nerve fibers to release neuropeptides that bind to cells mediating inflammatory response, such as mast cells. The role of the endocrine system in the context of stress-worsening of dermatological symptoms must also be considered, potentially acting through the HPA axis and resulting in prolonged raised cortisol levels, and over time possibly exhausting the system and thus lowering cortisol levels [68].

This complex interaction is of interest, as treatment of AD in clinical practice is far from simple and cannot be limited to pharmacological interventions. An increasing number of options for treatment of AD is available today, including systemic therapies. However, most patients with AD do not have severe enough skin symptoms that systemic treatment is motivated, and patients might also want to avoid such treatment options due to the fear of side effects. In the case of patients with milder disease activity, topical treatment is of greater importance and with this comes the question of adherence to the recommended therapy. The psychological burden and worsening of disease are important factors to highlight and the mechanisms for stress-worsening are still largely unexplored. These were the focus of the first two manuscripts in this thesis (Papers I and II), which explored the clinical features of AD in relation to the patients' experiences of their psychological status.

Among potential mediators for the connection between skin and brain, one interesting neuropeptide is CGRP, which has been described in neurogenic inflammation and is also thought to be of importance in depression. This makes it especially interesting in regard to a possible interaction between skin inflammation and psychological symptoms. This was investigated and discussed in Paper IV.

Another factor that is interesting to consider is how chronic inflammatory skin diseases might impact the brain and the central signaling pathways, especially as regards coping during stress. Could patients with chronic skin inflammation, and possible sleep deprivation due to itch, be more susceptible to stress and/or have a different processing of this type of

psychological stimulus? This was explored through fMRI in Paper III, where support for a different activation during stress processing was found, see below.

5.2 Studies I–II

5.2.1 AD, stress, and itch

The first part of the project used focus groups to investigate the relations between AD and psychological stress, anxiety, and depressive symptoms, respectively, in order to get a deeper understanding of psychological factors underlying worsening of eczema or pruritus and to identify new patient perspectives. A goal was to present questions to a large patient population, also outside of hospitals, using a survey, in collaboration with the patients' organization Atopikerna, to investigate how different psychological symptoms in AD were perceived by patients.

The hypothesis that psychological stress may have an impact on eczema and pruritus was supported by all of the patients. In addition, stress was reported to lead to more scratching. Most of the patients thought that chronic stress worsened eczema or pruritus more than acute stress, though opinions on what came first – eczema or psychological stress – varied. Other aspects were also discussed, for example the nature of pruritus in relation to psychological stress, with some patients reporting feeling a deep pruritus differing from their "normal" pruritus. In the larger survey population, patients reported that psychological stress was an important worsening factor, rated higher than climate factors. Stress is a well-known trigger of AD, and more than half of the patients answering our survey had consulted a doctor at least once for stress, anxiety or depression, something my co-authors and I considered remarkable.

Another observation from the focus groups was that stress was reported to lead to more scratching. This was also supported by the survey, where the majority (85%) answered that itch was worsened by stress.

There are several ways that stress may theoretically affect eczema. One is through behavioral mechanisms. A common reaction to pruritus is to scratch the skin. This results in mechanical injuries to the skin barrier and an increased inflammatory response [69]. Patients with AD tend to scratch more when experiencing anxiety or depressive symptoms[70]. In addition, stress can have direct proinflammatory effects through activation of mast cells, via release of neuropeptides and decreased epidermal lipid synthesis [71]. Studies have shown that patients with AD have higher anxiety levels than controls and that this has a direct correlation with

pruritus [72]. Not surprisingly, a majority (72.5%) of patients participating in Study II reported pruritus and a majority of them (85.1%) reported that this was worsened by stress. Most patients reported that the stress preceded pruritus, but that this might also vary. This was further investigated in the survey, where the greater part (53.5%) answered that stress resulted in itch, whereas 40.7% stated that it varied.

One important factor that was observed in the survey study was that disease extent correlated more strongly with chronic stress than with acute stress. This underlines the impact of chronic stress on the immune system and its function.

The problem of distinguishing psychological stress from other triggers, such as infection and climate, and even allergic factors, was recognized in the focus groups. A possible synergy of these different factors was suggested in the focus group sessions. This highlights the complexity of the disease mechanisms and the importance of a holistic view on AD.

5.2.2 Treatment of AD and stress

In the focus groups, conventional treatment with topical corticosteroids and emollients, ultraviolet light treatment, cognitive behavioral therapy/psychotherapy, climate therapy, and exercise were commonly preferred by the patients for AD worsened by stress.

One of the patients reported improvement from psychotherapy, another from conversational therapy. Early case studies on the use of insight-oriented psychotherapy in adults with recalcitrant AD showed skin clearing and psychiatric improvement. Psychological treatment in patients with AD has been shown to improve anxiety levels, response to frustration, and itch-scratch patterns. Cognitive behavioral therapy has been used to give young patients and their parents insight into AD-related problems and to restructure thought patterns.

AD is known to have a strong impact on patients' quality of life and the disease is associated with increased risk of anxiety and depression including suicidal behavior [73]. Long-term stress and exhaustion may result in, and is not always easy to differentiate from, anxiety and depression. Treatments targeting stress for its role in worsening both AD and pruritus are not commonly available to patients today. However, a recent clinical study investigating the effects of cognitive behavioral therapy has shown an effect on symptoms of AD [74].

The survey responses regarding preferred treatment for treatment of AD and stress showed that emollients were the first choice, while a small number of patients answered that they preferred psychological treatment.

Knowledge about patients' perspectives on the relation between psychological stress and AD is essential for a better understanding of their condition, including in relation to available treatment options.

5.2.3 Physical activity, stress, and AD

Observations from the focus group study showed that exercise was generally well-tolerated, was not affected by the disease, and even decreased symptoms of stress. In previous studies, physical exercise with sweating caused worsening of AD symptoms [22]. This has been suggested to be an obstacle, possible decreasing physical activity in AD patients. However, in a previous questionnaire study, it was found that Swedish patients with AD had the same levels of physical exercise and attitudes towards physical exercise as the general population, and skin symptoms of AD did not appear to be an obstacle to moderate physical exercise [75]. Further, a recent study showed that stress levels of Korean youths with AD were lowered by physical exercise [23]. In a study of atopic mice (NC/Nga), mild exercise was shown to decrease symptoms of dermatitis [76].

It seems that even though sweating could be a possible AD trigger, the beneficial effects experienced by the patients outweighed the negative ones.

5.2.4 Patient selection and methodological considerations

The participants selected for the focus groups were recruited from among patients at the outpatient clinic at Karolinska University Hospital. Limitations of the focus group study were the relatively small number of individuals, with a majority of female patients. Previous studies have shown that stress responses differ by sex and gender due to sex hormones and gender socialization, respectively [24]. In a population-based study from Korea, females with AD were reported to be more stressed than males [19].

The survey was sent to patients who had been seen and diagnosed in specialist dermatological clinics (at either Karolinska University Hospital, Södersjukhuset, or Älvsjö Hudmottagning, all in Stockholm). In total, the response rate was 17.9% among the patients who received the survey. As expected, most had a mild to moderate extent of eczema, though a substantial share (14.3%) was on systemic treatment. The selection of patients diagnosed with AD in specialist care minimized the risk of responses from patients with an incorrect diagnosis, though this automatically included patients who had somewhat more severe AD, as most patients with mild eczema are treated by general practitioners, without the involvement of a dermatologist.

There are always risks related to inviting patients to answering surveys digitally or by letter, such as low response rates or poor understanding of the questions. Patients seen in a hospital environment, not answering digitally, might give more valid responses as well as higher response rates [72] However, the ability to reach a large number of patients makes online surveys interesting. Also, giving participants the option to submit an anonymous response via an online survey might increase the chances of getting truthful information about sensitive matters such as stress, anxiety, and depressive symptoms. However, there is a risk of selection bias.

It is possible that patients who paid more attention to their skin due to more itch and skin complaints and those with high amounts of perceived stress were more likely to participate in the online survey.

Like in the focus groups, with more females participating than males, more females than males answered the survey. This might, to some extent, reflect the gender distribution of AD in the general population, where more women are affected that men[77]. Lastly, there might be a higher interest among women in stress and stress-related diseases like anxiety and depression.

5.3 Study III

Based on the hypothesis that there might be some differences in the central processing of stress in patients with AD, my co-authors and I designed a study investigating the activity of areas and circuits important in stress and compared the results to the activity in HCs. Possible differences could be related to pruritus and severity of inflammation, as well as levels of stress and other personality traits. Therefore, clinical data were also investigated.

5.3.1 Scientific context and clinical significance

Study III failed to show correlation between activity in ROIs and pruritus. The degree of pruritus was reported for the three days preceding the examination, which may explain the lack of correlation.

A correlation for impulsivity with both stress, anxiety, and depression has been reported in AD patients [78]. In Study III, HCs showed a correlation between impulsivity and KEDS score, whereas AD patients showed a correlation between KEDS score and both STA and PsTA scores.

A voxel-wise two-sample t-test did not detect any cluster with statistically significant difference for the HCs. A significant negative BOLD response (ranging from -3.78% to -4.01%) in the temporal pole, inferior, middle, and superior temporal gyri was observed bilaterally (Figure 5, see Study III). The ROI-averaged results for the AD subjects were overall less negative (-1.0% to -0.5%) and a two-sample t-test based on the ROI-averaged results showed a significant difference between the HC and AD groups. However, the ROIs defined in this way lacked statistical independence. Nevertheless, there was an evident qualitative difference in BOLD activation between the two groups. This seemed to be consistent with previously reported findings that mathematics anxiety reduces default mode network deactivation in response to a numerical task.

A recent voxel-based morphometry study [79] based on 14 AD patients (male/female = 5/9) and 11 healthy volunteers found that the grey matter density in the paraventricular nucleus was highly correlated with the perceived stress in AD patients, but not in healthy subjects. The imaging acquisition protocols and voxel-based morphometry analysis methodologies employed were quite similar to those used in Study III. As discussed above, the voxel-based morphometry results of Study III failed to confirm this finding. In Study III, every effort was made to dissociate potential confounding factors such as gender, age, and total intracranial volume.

Regarding pruritus, past fMRI studies [80, 81] on AD have been focused on studying the mechanisms for itch by using an itch-inducing/intervention technique, injecting histamine or other pruritogens, or letting patients watch video clips of other patients with itch. Most of the literature in chronic itch research has focused on the skin and the peripheral nervous system (bottom-up approach). In recent years, there is a growing interest in using neuroimaging approaches [82, 83] to gain insights into the central mechanism of itch (top-down approach). Neuroimaging studies in both animal model and human subjects have demonstrated that the itch perception is associated with brain activity responses in the salience network for attention control, sensorimotor preparation for scratching, and the reward system. The itch sensation is processed by a network of these different brain regions contributing to the encoding of sensory, emotional, attention-dependent, cognitive-evaluative, and motivational patterns [84].

There is also a handful of neuroimaging studies [84, 85] focused on the abnormalities in central mechanisms for itch processing in the brains of AD patients. A consistent finding of these studies is that subjects with AD show different activation patterns and kinetics compared with HCs. A H215O-PET study [86] showed significant differences in regional cerebral blood flow between AD and HC. Histamine-induced itch can lead to a significant

increase in regional cerebral blood flow, which was found in the contralateral somatosensory, motor cortex, midcingulate gyrus, and ipsilateral prefrontal cortex. Data from the AD subjects showed regional cerebral blood flow increase in the contralateral somatosensory and motor cortex, midcingulate gyrus, and ipsilateral prefrontal cortex. For the AD subjects, on the other hand, the regional cerebral blood flow increase was in the contralateral thalamus, somatosensory, motor, and prefrontal cortex, and cerebellum. Overall, more brain regions were activated in AD patients than in HCs. In AD patients, activation was also significantly higher in the contralateral thalamus, ipsilateral caudate, and pallidum. These observations were confirmed later through a magnetic resonance imaging study [87] based on arterial spin-labeling measurements. Moreover, that study [87] also detected a significant correlation between percentage changes of brain activation in the anterior cingulate cortex and contralateral insula, and histamine-induced itch intensity and disease severity in patients with AD. A resting-state fMRI study [85] reported decreased functional connectivity from baseline resting state to the evoked itch state between several itch-related brain regions, particularly the insular and cingulate cortices and the basal ganglia. There, decreased connectivity was significantly correlated with increased levels of perceived itch. In contrast, evoked itch increased connectivity between key nodes of the frontoparietal control network (superior parietal lobule and dorsolateral prefrontal cortex), and a higher increase in connectivity was correlated with a lesser increase in perceived itch. A more recent resting-state fMRI study [88] found that the right default mode network was associated with the severity of chronic itch.

To summarize, earlier studies investigating itch have found changes in brain regions of the first itch matrix; other brain regions involved include the cingulate gyrus, prefrontal cortex [89], and striatum [90]. These regions are probably responsible for the affective and cognitive aspects of itch. Detailed knowledge regarding the interaction between the cognitive state of mind (such as stress, emotions, and personality traits like impulsivity) and the perception of itch is still lacking. Like pain, itch is probably processed in multiple networks in the brain [89, 90].

In conclusion, the reduced deactivation in the default mode network in response to the arithmetic stimuli indicated that there was likely a cognitive functional variability in AD patients manifested as lowered inhibition ability under psychological stress compared with HCs. This was also supported by their differences in correlations between brain activities and various psycho-demographic data. BOLD fMRI measurements based on arithmetic paradigms can provide useful insights into the altered central processing and its association

with psychological traits in subjects with AD. Findings from Study III supported the notion that psychological stress affects brain activities in AD subjects in the motor and somatosensory association cortex, and perception and sensory integration processing.

5.3.2 Patients and methodological considerations

Study III focused on females. Previous studies have shown that stress responses differ by sex and gender due to differences in sex hormones and gender socialization factors [91]. In a population-based study from Korea, females with AD were reported to be more stressed than males [92]. This should be taken into consideration in interpreting the results.

An important issue was whether the AD patients perceived sufficient stress during the block-designed arithmetic paradigm. The physiological data recorded through fMRI indicated that there was at least a tendency for the AD patients to have a higher heart rate than the HCs during the task periods.

Cortisol has been suggested to be a useful biomarker to evaluate stress in AD patients; it has also been reported to be higher in AD patients than in control subjects [93]. In the fMRI study, salivary samples were obtained from only 16 of the AD patients, an obvious limitation. Still, there was a significant difference between the AD patients and controls. Addressing the role of stress in the pathology of AD is of clinical importance. It is known that cortisol release is not only increased by acute stress, but also blunted in AD patients, as observed in Study III. The cortisol response to stress is believed to facilitate the Th2 dominance and hyperproduction of IgE and to affect skin barrier functions.

5.4 Study IV

5.4.1 CGRP and its role

An increased number of nerve-like fibers positive for CGRP was found in lesional compared with non-lesional AD skin. There was also an increase in intraepidermal nerve-like fibers and intraepidermal inflammatory cells, both round and dendritic.

The findings of increased numbers of CGRP-positive nerve fibers in lesional compared with non-lesional AD skin were in line with the majority of other human studies [26, 79]. However, in earlier studies from our group, we found no difference in the number of CGRP-positive nerve fibers between contact allergic and control human skin [79].

Moreover, there was a lower concentration of CGRP in the inflamed ears of mice with a contact allergic reaction compared with controls [94]. In addition, we have previously reported a decreased concentration of CGRP in *Leishmania major* murine leishmaniasis skin compared with in controls [95]. Thus, in summary, varying results have been found for CGRP in inflamed skin.

There was a striking difference in that intraepidermal CGRP-positive nerve-like fibers were present in AD lesional skin, but a significantly smaller number in non-lesional skin. Hyperinnervation of CGRP-positive nerve fibers has been reported in the epidermis of humans with AD and in an NC/Nga AD mouse model [96]. Regarding the finding of sprouting of CGRP-positive nerve fibers, this has been reported in inflamed rat molar pulp and periodontium [97]. In addition, dense CGRP-positive nerve bundles and dividing nerves have been found in the dermis of patients with the highly pruritic skin disorder prurigo nodularis [98]. These findings might indicate a role for CGRP in both inflammation and pruritus. One mechanism whereby CGRP could, albeit indirectly, induce pruritus would be an interaction between CGRP and other mediators, such as the neuropoietic cytokine IL-6, which is also present in AD skin [99]. However, no correlation between CGRP expression in the different structures and extent of the disease or degree of pruritus. While intradermal injection of CGRP did not directly induce a skin itch response in humans, an antagonist to CGRP has been shown to reduce itching caused by, e.g., histamine [100]. A higher CGRP plasma level has been found in AD patients with more severe disease [101] and also in AD patients with intense pruritus compared with AD patients without pruritus, though the values were lower than those in HCs [101].

In this investigation, keratinocytes in AD lesional skin were CGRP-positive. Keratinocytes have previously been reported to be able to synthesize CGRPβ [102]. Furthermore, atopic keratinocytes have been shown to mediate CGRP-positive neurite outgrowth in a co-culture model of human skin cells and porcine dorsal root ganglion cells [103]. In AD, CGRP might be released from keratinocytes during scratching, mediating neurite outgrowth, which might in turn increase pruritus, thus causing a vicious circle. However, the role of CGRP for pruritus in AD needs further study.

The CGRP immunoreactivity in epidermal dendritic cells in lesional skin indicates a role for CGRP in the antigen presentation in AD and for its development [104]. Epidermal dendritic cells have previously been reported to express CGRP in psoriatic skin [105].

To gain a better understanding of a possible role of CGRP in AD and its psychological mechanisms, as well as neuro-immune interaction, psycho-demographic data were collected from the patients. It has previously been reported that CGRP expression in the murine hippocampus is associated with a depression-like behavior [57]. CGRP levels may be increased in the spinal fluid [40], plasma, and sweat [52] of depressed patients. CGRP is also involved in various behaviors suggestive of anxiety [106]. Study IV showed a correlation between the number of nerve-like fibers in AD lesional skin and both depressive and anxiety scores, as well as between intraepidermal inflammatory cells and depressive scores. In addition to the signaling of CGRP itself, the potential interaction with other mediators and the proinflammatory and vasodilating action of CGRP may be possible mechanisms through which CGRP could interact with the central nervous system via the circulation and possibly increase symptoms of distress in patients. Interestingly, a study of another important sensory neuropeptide in the skin, substance P, which is often colocalized with CGRP, showed that neurokinin-1 receptor-positive cells in AD were correlated with depression [107].

Intraepidermal CGRP-positive round inflammatory cells showed a negative correlation with cortisol levels, indicating a role for CGRP along the HPA axis, and thus a role for CGRP in stress [57].

In summary, CGRP may have a role in both the inflammatory process and distress in AD.

5.4.2 Patients and methodological considerations

Regarding the distribution of CGRP in skin, it is of importance to consider that there might be a difference in nerve density depending on the site of the body from which biopsies are taken. In this study, different areas were chosen for the biopsies; the cubital fossa for lesional skin as an area with substantial inflammation, whereas non-lesional skin was taken from the lower back, which is seldom clinically involved in AD patients. This anatomical difference should be taken into account when interpreting the results. Yet another explanation for diverging results in different studies might be that CGRP is quickly degraded by proteases [108].

Also, when comparing clinical and psycho-demographic data, having a larger number of patients would be beneficial.

6 Conclusions

The focus group study and the survey study underline the importance of stress as a trigger and worsening factor for patients with AD. In both studies, it was emphasized that stress, especially chronic stress, could be an important worsening factor. Regarding triggers of stress, decision-making and unforeseen events were often mentioned. In both the focus groups and the survey, patients rated stress as of greater importance than climate factors. Itch was reported to be a result of stress and the type of stress could possibly affect the nature of the pruritus experienced by the patients. Furthermore, physical exercise was reported to have beneficial effects.

Differences were found in possible mechanisms for stress processing in AD patients compared with controls. The reduced deactivation in the default mode network in response to stress indicated that there was likely a cognitive functional variability in AD patients compared with HCs, manifested as lowered inhibition ability during psychological stress. This was also supported by the differences in correlations between brain activities and various psycho-demographic measures. BOLD fMRI measurements during arithmetic tests can provide useful insights into the altered central processing and its association with neurophysiological traits in patients with AD. Findings from the fMRI study supported the notion that psychological stress affected brain activity in the motor and somatosensory association cortex, perception and sensory integration processing in AD patients.

Increase of CGRP in nerve-like fibers and inflammatory cells in inflamed skin of AD patients compared with non-lesional skin suggested one possible peripheral mechanism for stress-worsening of AD. CGRP also has functions in the central nervous system and in Study IV, the increase of CGRP-positive nerve-like fibers in skin correlated with depressive and anxiety scores in patients.

In conclusion, the results of the studies showed that psychological stress is an important trigger factor in AD and emphasized the importance of a holistic approach to treatment of AD for healthcare to offer more individualized treatment depending on each patient's challenges and needs.

7 Points of perspective

Regarding investigating patient perspectives in AD, especially in relation to stress, it would be of interest to try to differentiate between positive and negative stress. Investigations regarding other medical conditions and general health have indicated differences between positive and negative stress regarding the impact on health. This would be important to investigate further in relation to AD. There might be an argument for including moderate physical exercise in the treatment options for AD.

Using fMRI to further investigate the central processing of itch and possible differences in stress management in AD patients is a very interesting approach. For example, by using fMRI to map the resting state networks in the brain, the effect of chronic stress can be studied rather than that of acute stress. If differences can be found, can pharmacological or non-pharmacological interventions, like mindfulness or different psychological therapies, have different effects and can this be seen through fMRI? Also, it would be interesting to study both males and females, to identify any sex differences.

In addition, it would be of interest to further investigate CGRP, its different isoforms, and their respective receptors *in situ* in AD, especially during stress, to explore the role of CGRP as a marker of anxiety and depression. Could an antagonist to CGRP be used to treat AD, either topically or systemically? In the possible comorbidity of AD and migraine, CGRP could be a target molecule.

In summary, it would be interesting to continue to study the roles of acute and chronic stress in AD and potentially find new, individualized, and optimized treatments for AD and distress.

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