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Atopic disease in relation to *Staphylococcus aureus* carriage and spa type distribution in a subarctic adult population

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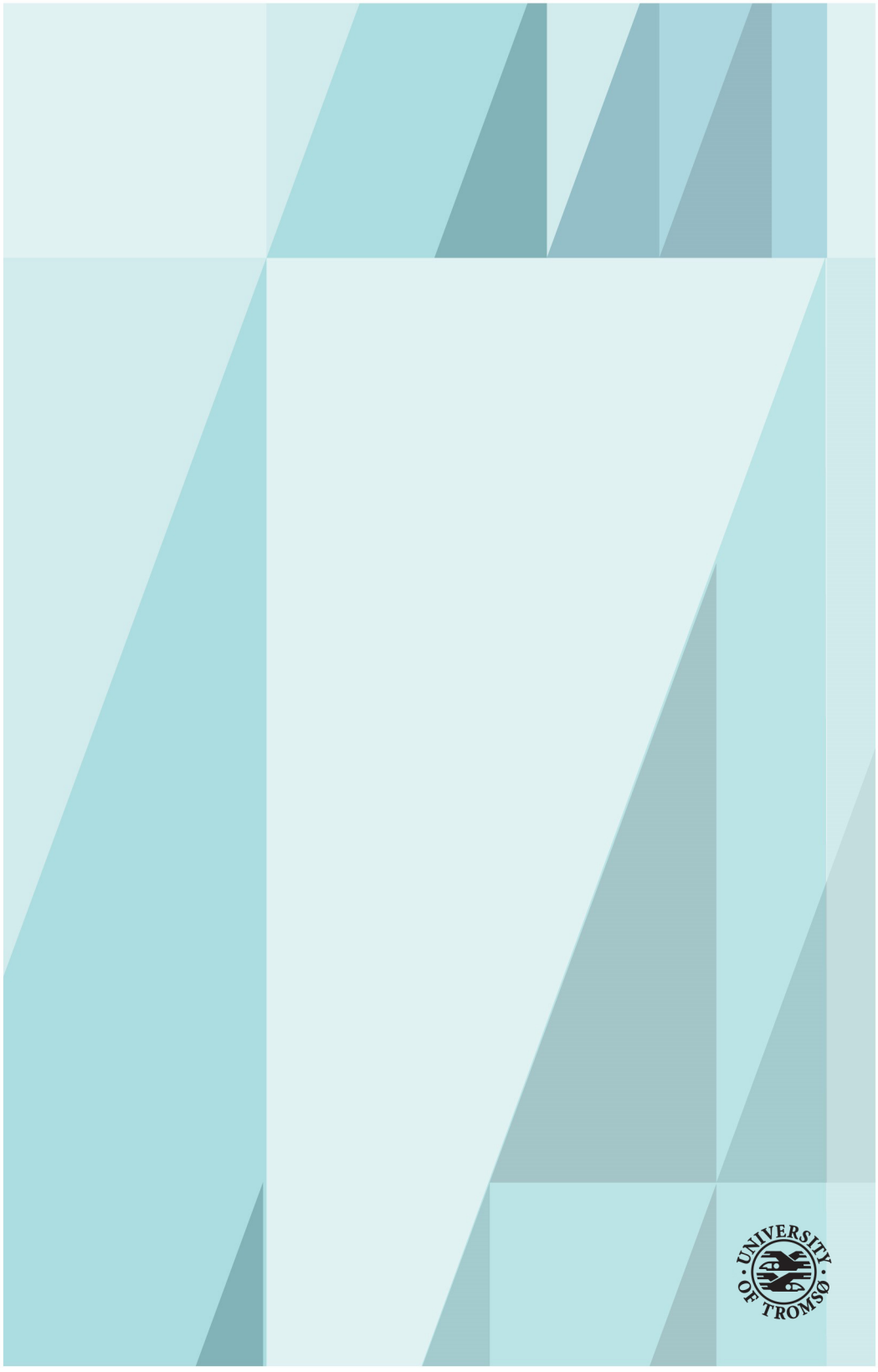

Preface

The exact mechanisms and aetiologies in development of allergy remains poorly understood, but the interplay between heritage and environment is in the lead. One of the many environmental factors implicated is staphylococcal carriage and sensitization to staphylococcal enterotoxins. The relationship between *Staphylococcus aureus* and atopic disease has long been appreciated with many small studies but few properly sized studies have been conducted on the matter.

The main focus of this thesis is to study atopic disease in an adult population and more specifically, the association between the bacterium *S. aureus* and atopic disease. This association will be studied in a colonized (culturing result of one single sample) and a carrier (culturing result of two repeated samples) population separately and findings will be reported accordingly. Furthermore, in the colonized population we will study whether having atopic history is associated with certain *S. aureus* strains through *spa* typing. This study is the result of a progress of developing and improve my methodological skills and the ability to summarize the literature and in light of this, explain new observations according to the scientific biological model of the human.

In the making of this master thesis it seemed suitable to provide a historical backdrop for allergic disease, resulting in a review and the present understanding of the allergic epidemic and thus, illustrating our efforts to further elucidate and expand this field.

Takk til mine fantastiske veiledere, Anne-Sofie Furberg, Karina Olsen og Martin Sørensen.

Jonas A Devold

Jonas Arvola Devold *02.06.2019*

Table of contents

Abbreviations

IgE; Immunoglobulin E

AD; atopic dermatitis

AR; allergic rhinitis (or pollen allergy)

RHE; recurrent hand eczema

SE; Staphylococcal enterotoxin

MRSA; Multi-resistant *Staphylococcus Aureus*

CI; confidence interval

OR; odds ratio

MeDALL; Mechanisms of the Development of Allergy

TSSS; Tromsø Staph and Skin Study

FF2; FitFutures2 study

ISAAC; International Study of Asthma and Allergies in Childhood

WHO; World Health Organization

WHS; World Health Survey

Th1; T helper cell type 1

Th2; T helper cell type 2

Th17; T helper cell type 17

LC; Langerhans cell

mRNA; Messenger ribonucleic acid

GWAS; Genome wide association study

WGS; Whole Genome Sequencing

MLST; Multilocus sequence typing

CC; Clonal complex

Summary

Introduction: Atopic diseases are common, and the atopic epidemic has ravaged through the westernized countries and is heading for the developing countries too. Despite being a common disease, however, treatment and diagnostic options are still lacking. The immune system and development of atopic disease are interconnected with the environment through exposures or lack thereof. The microbiota of the mucosal membranes and skin throughout the body have been purposed serving a sentinel role in this interplay. In this study, we investigated if atopic disease is associated with *Staphylococcus aureus* nasal colonization and carriage in adults. Furthermore, we wanted to test whether the distribution of *S. aureus* spa types differs in adults with atopic disease compared to adults without atopic disease in a general population of men and women.

***Material and methods*:** The Tromsø Staph and Skin is a sub-study of Tromsø 6, a population-based cohort with high attendance rate (65.7%). Out of a total of 4,026 participants who had nasal swab sample taken, 3,367 and 2,485 participants were enrolled in our study of *S. aureus* colonization and carriage, respectively. The study included questionnaires on lifestyle and disease, clinical examination, and blood samples. In order to investigate the association between atopic disease and *S. aureus* independently of known risk-factors we used multivariable logistic regression analysis.

Results: In the Tromsø Staph and Skin study, the lifetime prevalence for asthma, atopic dermatitis, recurrent hand eczema and pollen allergy were 9.4%, 12.5%, 13.8% and 19.7% respectively. In the total population, *S. aureus* colonization and carrier rate was 29.5% and 26.1%, respectively. *S. aureus* colonization and carriage was most frequent in males; colonization and carrier rates were 38.0% and 34.7%, accordingly. In females the corresponding rates of colonization and carrier were 19.6% and 22.9%. We found pollen allergy to be associated with 45% [odds ratio OR= 1.45, 95% CI, 1.11 to 2.52] and 55% [OR=1.55, 95% CI, 1.06 to 2.24] higher risk of *S. aureus* colonization and carriage in males, respectively. Furthermore, among males, having one atopic disease was associated with a 67% [OR=1.67, 95% CI, 1.11 to 2.52] higher risk of *S. aureus* carriage.

Conclusion*: S. aureus* colonization and carriage is associated with pollen allergy in males. This cross-sectional study cannot define causality, but our data provide some evidence that support the hypothesis of *S. aureus*' role in modulation and maintenance of atopic disease and pollen allergy, even in adults.

1 Background

1.1 Abstract

Atopic diseases are a group of clinical syndromes linked by shared pathology in the immune system. These diseases are highly prevalent around the world with high potential of impairing the patient's health, socioeconomic status and quality of life considerably. An American overview article published in 2004 concluded that asthma in children and adults had a total estimated cost at 14 billion US$ annually.(1) In Europe, asthma and allergic rhinitis alone are estimated to result in more than 100 million lost workdays and missed school days and an indirect cost between 55 and 140 billion euro per annum, according to the European Academy of Allergy and Clinical Immunology.(2) As of 2019 there is no known cure for atopic diseases; through extensive research we may shed light on the mechanisms by which these diseases occur and potentially unveil novel treatment strategies. Thus, the purpose of this study is to investigate the association between atopic disease and nasal *Staphylococcus aureus* colonization and carriage, and test whether there is a predominant genotype of the bacteria in patients with atopic diseases.

1.2 Atopic disease

The term atopy, derived from Greek *atopia* translated to "the state of being out of place", was first introduced in 1923 by Arthur Coca and Robert Cooke attempting to describe a type of hypersensitivity towards environmental substances with tendency to occur in families. The first documented example of an atopic family is the Julio-Claudian family of emperors, namely emperor Augustus who allegedly suffered from extremely itchy skin, seasonal rhinitis and bronchial asthma. (3)

Atopic diseases are especially common in industrial or westernized countries and observational studies have found a 30% prevalence in a general adult population.(4) Today, the term atopy implies a tendency towards developing allergic asthma, rhinitis or atopic dermatitis associated with a genetic predisposition to increased production of sensitized and specific immunoglobulin E (IgE) against allergens.(5) Allergic rhinitis, atopic dermatitis, asthma and food allergy form the atopic march and they are thought to be linked, to some extent, by a common pathogenesis mediated through IgE. Due to this, observing several atopic diseases simultaneously in a patient is not seldom. (6)

In fact, a genome wide association study (GWAS, n=360,818)(7) of a broad allergic phenotype discovered 136 risk variants of genes closely related with atopic diseases. Disease-

specific effects were detected in only six of the risk variants. Furthermore, more detailed analysis of the risk variants shared by the atopic diseases, indicates that the risk variants influence lymphocyte-mediated immunity including helper T cells (Th1, Th2 and Th17), regulatory T cells, CD4+ and CD8+ memory T cell, CD56+ NK cells, and CD19+ B cells. Interestingly, 27% of these risk variants had nearby CpG-sites with methylation levels significantly correlated with mRNA in blood suggesting that a substantial fraction of the risk variants is susceptible to environmental epigenetic regulation.

1.2.1 Allergic rhinitis

Allergic rhinitis (AR, also known as allergic rhino-conjunctivitis or hay fever) is an allergic disease where a collective of symptoms appears in reaction to allergen-exposure in a susceptible individual. AR is characterized by inflammation in the nasal mucosa with the Th2-type immune response pivoting in the centre of the process as suggested by numerous in vitro studies. (8, 9) Exposed to an allergen, the patient develops rhinorrhoea, sneezing, obstruction of nasal airways as well as pharyngeal and nasal pruritus. Some may present with conjunctival injection, epiphora and pruritus.(5)

Allergens are either temporary (e.g. pollen due to its seasonal distribution) or in more chronic forms such as mite or dander. AR shares, to some extent, the same pathogenesis as asthma, and they are highly associated with up to 40% of AR patients also developing asthma. Similar epidemiology, the interrelated pathophysiological, and functional traits of AR and asthma suggest that they can be thought as two different features of the same disease. (5, 10) In North-America AR have an annual incidence of 7%, total prevalence of approximately 20% peaking at 40% in childhood and adolescence.

1.2.2 Asthma

Asthma is a heterogeneous disease characterized by acute and chronic mucosal inflammation in the airways leading to narrowing of the airways and subsequent airflow obstruction. The narrowing occurs mainly due to bronchoconstriction, but also mucosal oedema, vascular congestion and exudates narrows or occludes the airway lumen. The inflammation can spread throughout the trachea and to the terminal bronchioles, but the predominant site is in the bronchi. Underlying pathology in the different phenotypes of asthma is uniform, and although defined as a chronic inflammatory lung disease it is characterised by intermittent reversible exacerbations. Exacerbations are usually triggered by viral infections, cold air, air pollutants, allergens, psychological stress or occupational factors.

The pathogenesis is incompletely understood but involves genetic and environmental risk factors, the strongest predictor being atopy. Inappropriate immune response in the

presence of a trigger is thought to be orchestrated by mast-cell, eosinophilic granulocyte and T-lymphocyte activation causing an inflammatory process in the lower airways. As a result, the basal membrane thickens and mucus-congestion develops giving symptoms such as wheezing, chest tightness, dyspnoea and cough. In addition, approximately 80% of patients with allergic asthma will have symptoms reconcilable with allergic rhinitis. (5)

1.2.3 Atopic dermatitis

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by dry, red erythematous scaly skin and intense pruritus, usually in flexures and bends of the lower and upper extremities. Patients with AD usually have a positive family history of atopic diseases such as asthma, AR or eczema. Concordant twin studies have shown that if one twin is affected there's a 85% chance that the other twin also have the condition. (11) In children where a parent or both have AD the prevalence is 50% and 80%, respectively.(5)

Although the aetiology is only partially described, a mutation in the filaggrin-gene, an important protein constituent of stratum corneum, causes a defect in the barrier-function of the skin and subsequent outbreak of AD in some patients. Immunological studies in the lesioned skin of AD patients, have shown that a Th2-driven inflammatory process is predominant. Supporting this concept, Dupilomab, a human-recombinant monoclonal antibody, ameliorate AD by inhibiting the IL-4-receptor – an important receptor protein in the Th2-signalling cascade.(12)

Elevated serum IgE levels have also been associated with development and severity of AD. As with other atopic diseases, AD patients have high risk of allergic comorbidities with asthma and AR with up to 80% of AD patients having either asthma or AR. (13) In adulthood, the disease manifests as chronic eczema affecting hands or head/neck regions. The disease-form affecting hands is otherwise known as recurrent hand eczema (RHE). Tendency to hypersensitive skin type and relapsing AD has been documented in patients despite durational asymptomatic intervals. (14, 15)

1.2.4 The allergy epidemic - description

Seasonal allergic rhinitis (AR) was first described in the US in 1872 (Autumnal catarrh or ragweed hay fever) (13) and in England in 1873 (Catarrhus aestivus)(16). By the start of the 20th century the disease was well known throughout England and Germany, and in the US by 1920. Summer refuges were established e.g. the treeless island of Heligoland in the North Sea where the famous physicist Werner Heisenberg, plagued by allergic rhino-conjunctivitis, retreated in the 1920s. (14) Sure enough, at that time hay fever was considered a disease of the wealthy and was rarely observed in the working-class.

The prevalence of AR continued to increase from the 1870s through the 1950s with 10% of New Yorkers suffering from AR.(17) Since the 1960s the prevalence has remained relatively stable at approximately 15% throughout the 1990s.(18)

A slight worldwide increase in prevalence of AR was observed between 1994 and 2004 in the International Study of Asthma and Allergies in Childhood(ISAAC).(19) No consistent global pattern could be found, but interestingly some of the low-prevalent countries in the mid-1990s had increased rates in the early 2000. Contrasting, the countries with the highest prevalence in the mid-1990s generally decreased over the same period. Since then prevalence rates in most westernized countries appear to be approximately 15 to 20%.

The first increase of **asthma** was reported in 1970 among schoolchildren in Birmingham, England(20) and over the next 10 years a rise in asthma prevalence was reported in Australia, Japan and New Zealand marking the start of the so-called "asthma epidemic". (21-23) There was a strong association between having asthma and sensitization to dust mites in each of these countries. Interestingly, in Finland where dust mites as an allergen is of less importance, a six-fold increase in asthma rates was seen between 1966-1989.(24) This finding in Finland is seen in other westernized countries with at least 10-fold increase in asthma rate from 1960 to 2000.

Recently, the rates might appear to have peaked, particularly in English speaking countries, Denmark, and Netherland. (25-27) Also, the international differences are decreasing as the asthma rate in low-income countries in Latin America, Africa and parts of Asia increase. (28) Nevertheless, the asthma rates in some westernized countries continue to increase, e.g. Sweden and Italy. (27, 29) The data is conflicting, as illustrated by cross-sectional surveys in Swedish schoolchildren aged 7-8 years; despite the asthma rate apparently were levelling-off, the rate of sensitization to aeroallergens continued to increase from 21% in 1996 to 30% in 2006. (30) Also, in Swedish adults, the pollen sensitization rate increased from 17.1% in 1992 to 29.0% in 2012. (31) Norwegian cohort study from 2006 reported a lifetime prevalence of asthma at approximately 20% by the age of 10 (32) and in North Norway a three-survey cohort during the period 1985-2008 found evidence of increasing asthma rates in schoolchildren. (33)

Atopic dermatitis (AD) origins from the word atopy and was coined by Coca and Sulzberger in 1933 to isolate AD from a heterogenic group of pruritic skin disorders and distinguish the aetiology from a neurologic origin. Their definition encompassed infantile eczema localized to face or flexures and occurring in an individual with a family history of allergic diseases. Later, AD was added to the group of genetically predisposed allergic

diseases such as AR and asthma. Unfortunately, numerous different diagnostic definitions and terms have since been used and complicated the epidemiological studies; even as late as in the 1970s, 14 synonyms for AD were in widespread use.(34) Also, the instruments and procedures used for diagnosing AD have differed, and therefore prevalence rates are hardly comparable across time and populations. Although the quality of the data from the earliest reports on AD is limited, there is evidence of a steady increase in the prevalence of AD since the first reports in the 1950s when prevalence estimates ranged from 1.1 to 4.6% in UK and USA.(35, 36)

Schäfer et al. report three-fold increase in Denmark (3.2% to 10.2%) during the period 1964 to 1974. In the 1990s some studies report rates as high as 20%. The trend is summarized in Table 6 in the article (37). In ISAAC, the only study with a global perspective, they confirm the increasing trend but find worldwide variation with low-income countries reporting lower rates compared to westernized countries(28). Recently, a nationwide Norwegian cohort based on data from the national prescription registry found evidence of an increasing incidence rate from 0.028 to 0.034 per person-year in children younger than 6 years between 2009 and 2014. For children under 1 year the incidence rate increased from 0.052 to 0.074 in the same period.(38) In contrast, studies from Sweden and Denmark suggests a levelling-off to a plateau as seen in other allergic diseases.

1.2.5 The allergy epidemic- hypothesis

The epidemiological trend seen over the last two centuries in westernized societies and now happening in developing countries invoke the question: What change occurred in this time-period, when the rise of atopic diseases was seen?

Gerrard et al. in 1976(39) observed higher prevalence of atopic diseases among ethnic whites compared to Métis Canadians in Saskatchewan. Contrasted with the increased prevalence of helminth, viral and bacterial infections in the Métis community the authors proposed that *atopic disease is the price paid by some members of the white community for their relative freedom from diseases due to viruses, bacteria and helminths*.

In 1989 D. Strachan(40) introduced the hygiene hypothesis emerging from a British birth cohort study, following 17,414 children until the age of 23. He reported that growing up in larger families in the UK, was protective against atopic eczema and hay fever compared to the children growing up in smaller families. He suggested that atopic diseases were prevented by infections in the early childhood, induced by unhygienic contact with other siblings or transmitted prenatally from a mother infected through contact with her older children.

However, not all infections are protective for allergy; frequently reoccurring lower respiratory tract infections caused by rhinovirus or respiratory syncytial virus in children younger than three years, increase the risk of wheezing and asthma in adulthood.(41) Another inconsistency is that the major changes in hygiene in some northern European cities occurred long before the increasing trend of asthma, AD and food allergy.

The biodiversity hypothesis, or "old friends" hypothesis extends on the hygiene hypothesis, disregarding the role of infectious pressure and the lack of important microbes, or "old friends". Through human evolution microbes have co-evolved and developed mechanisms to modulate and evade the host immune system. The idea is that some of these "old friends" were essential to the normal functioning of the human immune system. (41) Changes in lifestyle, diet, activity and weight greatly affect the microbiome diversity in new-born infants suggesting that allergy is a drawback or a necessary evil of a modern westernized lifestyle. Furthermore, there is some evidence that a farm-based life and chronic helminth-infections in childhood offer a protective effect against allergy.(42, 43)

Thus, according to the biodiversity hypothesis, the increase in allergies are due to loss of beneficial symbiotic relationships and subsequently tendency towards an imbalance in the immune system and immune-related diseases. There is a substantial body of evidence of increased risk of allergy among children with a microbiota dysbiosis. Among numerous studies of the gut-, nose- or skin-flora a common finding is not the lack of one single microbe but an overall reduction of microbiome diversity.(44) Other studies observe a protective feature of chronic helminth infections and a Ugandan trial (45)with 2507 pregnant women showed that anthelmintic therapy during pregnancy increased the risk of eczema in infancy, and especially in infants whose mother were infected by *Schistosoma Mansoni.*

But what, exactly, in the westernized lifestyle is causing the microbiome dysbiosis? A westernized lifestyle is characterized by reduced physical activity, increased time spent indoors, a diet with low amounts of fibre and high amounts of carbohydrates and saturated fats all leading to obesity. Contact with microbial diversity in early childhood from the natural environment is crucial. As of now, we know that family size, presence of pets or farm animals, mode of infant feeding and childbirth, and use of antibiotics all have strong effects on the development of allergy during childhood. (41)

The MeDALL-project was initiated by the European Institute for Systems Medicine & Biology aimed to elucidate the mechanisms underlying the development of allergy. (46) During pregnancy, the foetus is dependent of a maternal immunologic shift from Th1- to Th2-predominance and increased T_{reg}-interaction. The MeDALL-authors hypothesized that

persistence or re-occurrence of foetal type 2 signalling genes plays an important role in the development of allergic comorbidity and polysensitization throughout infancy and childhood.

Further, the MeDALL-project postulated that the allergy epidemic may have resulted from recent environmental changes that in turn interfered with central immunomodulating genes through epigenetic mechanisms. One of the many environmental factors implicated is staphylococcal carriage and sensitization to staphylococcal enterotoxins (SE).

1.3 *Staphylococcus aureus*

1.3.1 Bacterial description

Staphylococcus aureus (*S. aureus*) is a gram-positive coccus belonging to the genus staphylococcus which currently consists of 53 species and 28 subspecies. (47) Most of these are harmless and act as commensal bacteria on the skin and mucous membrane of the host. Staphylococci are facultative anaerobic which implies capability of growing under both aerobic and anaerobic conditions. An important phenotypic trait used in staphylococci-classification is the production of coagulase, enabling the bacteria to form blood clots. *S. aureus* is by far the most important coagulase-positive staphylococci. *S. aureus*' virulence factors include various enzymes (protease, lipase and hyaluronidase), surface proteins such as protein A, enterotoxins (SE), and a protective capsule.(48)

1.3.2 *S. aureus* colonization

S. aureus can act as a commensal on human skin or mucosa, in fact 20-30% of the adult population are persistent carriers of *S. aureus*. (49, 50) In humans the anterior nasal vestibules are regarded as the most constituent site of *S. aureus* colonization from where the bacteria spread to other parts of the body. *S. aureus* resides in the nasal vestibule close to the mucocutaneous junction.(51) Patients colonized with *S. aureus* are at increased risk for autoinfection. An estimated 80% of all nosocomial *S. aureus* infections are caused by cultures already present prior to hospitalization. (52)

Colonization rate is higher among patients with diabetes requiring insulin-injections, IV- drug users, patients undergoing haemodialysis, surgical patients and immuno-compromised patients.(48, 53) Risk factors for *S. aureus* colonization include hormonal contraceptive use, smoking, obesity, circulating vitamin D, males, and age. Furthermore, in the Tromsø Staph and Skin study a 54% higher risk of nasal carriage was observed in female healthcare workers compared to non-healthcare workers. (50)

1.3.3 Clinical significance

S. aureus is the second most found bacteria in blood cultures from septic patients. Staphylococcal bacteraemia may lead to endocarditis, metastatic infection and systemic inflammatory response syndrome. Other infections encompass skin and soft-tissue infections, necrotizing fasciitis, osteomyelitis, septic arthritis, pneumonia, meningitis, toxin-mediated diseases (toxic shock syndrome, staphylococcal scalded skin syndrome) and food poisoning. Another aspect is the increasing danger of methicillin-resistant *S. aureus* (MRSA). A total of 2567 (approximately five-fold increase since 2005) new cases of MRSA-carriage or infection were reported to the Norwegian Surveillance System for Communicable Diseases in 2018 (48, 54).

1.3.4 *S. aureus* colonization and atopic disease

S. aureus colonization and secretion of enterotoxins has been proposed as a promising aetiology and modulator of allergic disease.(55, 56) This hypothesis was reinforced by an overview article with meta-analysis published in 2016 where a clear-cut association between prevalence and severity of AD and colonization of *S. aureus* was found. (57)

The risk of developing atopic disease is determined by genetic and environmental factors. Having atopic disease in the family and/or having one atopic disease increases the risk of co-morbidity with other atopic diseases. *S. aureus* has been proposed as a potential culprit and Iwamoto et al. have found that AD-strains induced altered T-cell response skewing the immune response towards Th2. Moreover, *S. aureus* strains in AD-lesioned skin activated Langerhans cells (LC) more compared to strains in healthy skin. (58, 59) Thus, *S. aureus* colonization possibly induce and modulate AD, but the causal question remains unanswered to this day.

An article published in 2016 showed a possible association between *S. aureus* carriage and some allergic diseases in young adults. Although there was no statistically significant difference in prevalence of allergic asthma in *S. aureus* carriers and non-carriers, the study showed that SE produced by *S. aureus* could in fact lead to IgE-sensitization which is strongly associated with allergic co-morbidity including allergic asthma.(56, 60) Adults with allergic rhino-conjunctivitis is four times more likely to be sensitized to SE compared to healthy controls.(61) Moreover, rhinitis severity increases in SE-sensitized patients.(62)

According to current literature approximately 20-30% in a healthy adult population are persistent carriers of *S. aureus* in contrast to 90% of AD patients. Available research mainly focus on AD and to some extent allergic rhinitis and asthma. The populations mainly consist

of children, adolescents and young adults; only a few studies shed light on hand eczema, allergic rhinitis and asthma in an adult population.

1.4 spa type

Protein A (=Staphylococcal protein A; spa) is a vastly conserved surface protein of *S. aureus.* It serves a multifunctional role in the bacteria through both pro-inflammatory and numerous immunosuppressive traits. Thus, strains of *S. aureus* lacking protein A on its surface show reduced bacterial virulence. Because of its high genetic conservation sequencing variable-number tandem-repeat regions in the spa gene provides an efficient and reliable method of distinguishing *S. aureus* genotypes.

It has been hypothesized that spa contributes to the pathogenesis of AD. (63, 64) A study by Do-Won Kim et al. among 35 AD patients observed large heterogeneity of spa types in *S. aureus* colonizing the skin. Importantly, there was no predominant spa type and no difference in disease severity by spa type.(63) Another study by Yeung et al. including 119 paediatric and 40 adult AD patients, found the same major groups of spa types in *S. aureus* from these patients as in nosocomial *S. aureus* isolated from patients in intensive care units. (64) However, AD was associated with two toxic subtypes of *S. aureus* indicating the role of endotoxins in the development of allergic diseases through IgE-sensitization. Moreover, Yeung et al. did find overrepresentation of a specific *S. aureus* strain - specifically CC1, using multi-locus sequence typing (MLST) clonal complexes (CC). Both studies have relatively small populations and include controls from hospitals only. In comparison, Rojo et al. investigated MLST-CC of colonizing *S. aureus* from 32 AD patients and 30 healthy controls, without finding any statistically significant difference in MLST-CC distribution. (65) The studies available on the *S. aureus* genotype distribution in atopic disease is conflicting and consist of small populations. Larger population-based studies with adequate number of observations in different age groups are required to gain insight into the epidemiology of spa type of *S. aureus* in atopic disease.(65)

1.5 Aims and objectives

Despite an immense increase of atopic diseases in westernized countries, there are few studies investigating the interplay between *S. aureus* and allergic disease in a population-based adult population. The aim of this study is to contribute to our understanding of the allergic diseases and elucidate *S. aureus'* role in the complicated interplay between heritage and environment in the development of expressive atopy. Based on the Tromsø Staph and Skin study, we identify *S. aureus* colonized and carrier subjects to establish if a correlation exist between the

bacteria and allergic disease. Additionally, identifying predominant strains of *S. aureus* in atopic subjects and non-atopic subjects might unveil strains with more allergic potential.
Specific objectives:

- To study associations between atopic diseases and nasal *S. aureus* colonization and carriage.
- To identify and compare predominant strains of atopic subjects and non-atopic subjects using *spa* types.

2 Material and methods

2.1 Population and study design

The Tromsø study is a longitudinal population-based cohort study with seven studies undertaken between 1974 and 2016. The studies obtained information through clinical examination and measurements, blood samples and biological material and self-reporting questionnaire focusing on lifestyle-related diseases.
The sixth study (Tromsø 6) took place from October 2007 to December 2008 and based on the official population registry, residents of the municipality of Tromsø were invited to take part in the survey. All residents aged 40-42 and 60-87 years, a 10% and 40% randomized sample aged 30-39 and 43-59 years accordingly were invited. In addition, 295 participants were invited because they attended the second visit in the fourth version of the Tromsø Study in 1994. Invitations were sent out randomly to minimize selection bias during the sampling period. A total of 12984 men and women aged 30-87 attended the sixth study. (attendance rate 65.7%)(66) (The questionnaires can be accessed from URL: https://uit.no/om/enhet/artikkel?p_document_id=104991&p_dimension_id=88111)

In the Tromsø 6 sub-study Tromsø Staph and Skin Study (TSSS), the aim was to map *S. aureus* nasal colonization and carriage in the general population, and study host, environment and microbe factors associated with colonization. During the first months of Tromsø 6 (October 2007-July 2008), all attendants in the age group 30-49 (n=1730) and random samples from older attendees aged 50-89 (n=2629) were invited to take part in TSSS. The invitations were evenly distributed across age groups relative to the municipality. This study included a nasal swab at first visit and a second nasal swab a few weeks later. A total of 4026 participants had at least a single nasal swab, and of these 2997 subjects repeated the nasal swab within a few weeks. (67)

For the present study of *S. aureus* colonization and carriage a total of 659 and 1541 subjects were excluded, respectively; 28 subjects who had taken antibiotics within 24 hours of the first visit were excluded; 105 subjects had no growth in swab culture. 519 subjects aged above 70 years were excluded. 7 subjects lacking data on all the questions regarding atopic disease were also excluded. In total 3367 participants with at least one valid nasal swab was included for analysis of *S. aureus* colonization in atopic disease. 873 of these did not attend a second visit and were excluded. 9 subjects who had taken antibiotics within 24 hours of the second visit were excluded. In total 2485 participants were included with two valid nasal swabs were included for the analysis of *S. aureus* carriage in atopic disease. (Figure 1; flowchart)

Variables used in the present study included participant characteristics; age (recoded into four categories: 30-39, 40-49, 50-59, 60-69 years), sex, diabetes, psoriasis, BMI (calculated by weight divided by weight squared and recoded into four categories: <20.0, 20.0-<25.0, 25.0-<30.0, ≥30.0 kg/m^2), smoking status (current, former, never; category former and never were merged into one category), alcohol consumption(calculated by $\frac{Alcohol\ units}{week} * 8\frac{gram}{unit}$, expressed as gram/week), recreational physical activity, healthcare worker status (defined as working in hospital, nursing home, senior care services, general practitioner's office, public health centre), healthcare worker in household; residing with children, recent hospitalization, and circulating levels of glycated haemoglobin (HbA1c) and serum 25-hydroxyvitamin D (se-25(OH)D).

2.2 Assessment of atopic disease

In this study, atopic disease is defined as every individual with a life-time history of either asthma, allergic rhino-conjunctivitis, AD or RHE. In Tromsø 6, information about the prevalence of atopic disease was collected by the self-administered questionnaire and restricted to asthma, allergic rhino-conjunctivitis, AD or RHE. Asthma was determined from the question "Do you have, or have you had asthma (YES/NO)? ". AD and RHE were determined from the question "Have you or have you ever had the following skin disorders: atopic eczema (children's eczema) or recurrent hand eczema (YES/NO)?" Allergic rhino-conjunctivitis was determined by "Do you have, or have you ever had pollen allergy (NEVER/SOME/MUCH)? Pollen allergy was dichotomized defining "NEVER" as no and "SOME" or "MUCH" as yes. An atopic score was computed where participants gain one score for each of the atopic diseases. The score ranged from 0 to 4; 0 indicating none of the

specified atopic diseases and 4 indicating presence of all specified atopic diseases. Due to small numbers the two upper levels (3-4) of atopic score were merged.

2.3 Assessment of nasal *S. aureus* colonization and carriage

The methods for assessment of S. aureus colonization and carriage in TSSS have been described.(68) Both anterior nares were sampled by a sterile NaCl-moistened rayon-tipped swab and transported to Amies charcoal transport medium. Within 3 days, all samples were cultured on blood agar. The swabs were plated on ChromID *S. aureus* and ChromID MRSA agar incubated in 48 hours at 35 degrees C. Colony morphology was the basis for *S. aureus* and MRSA identification. Suspected *S. aureus* colonies were confirmed by the Staphaurex Plus agglutination test. *S. aureus* colonization state was defined as positive or negative for *S. aureus* in the first sampling. *S. aureus* carrier state was defined by two consecutive samplings; carriage was defined as two positive nasal swab cultures and non-carriage as none or one positive nasal swab culture according to the definition by van Belkum et al.(69)

2.4 spa typing

The *S. aureus* positive cultures were spa typed using primers spa-1113f and spa-1514r. PCR products were sequenced by Macrogen Europe or Korea. Spa types were identified using Ridom StaphType software and Ridom SpaServer. The BURP algorithm was applied as described previously (70). For *S. aureus* samples with negative spa PCR the procedure was repeated. Samples that tested negative twice for spa PCR were checked twice with a Staphaurex plus agglutination test and coagulase test. If both tests were positive, the sample was regarded as not typeable for spa; if not the sample was excluded. Spa types were obtained from 99% of the isolates and 364 unique spa types were assigned in the first visit of TSSS. Recently, Sollid et al. have done a whole-genome sequencing (WGS) analysis on five common spa types (unpublished data). The WGS-analysis suggests that spa types t015 and t065 can be regarded as one subspecies, and spa type t012 and t021 as one subspecies. Thus, in our analysis we grouped the spa types accordingly (denoted in text and tables as spa type t065&t015 and t012&t021)

2.5 Statistical analysis

Baseline characteristics of *S. aureus* colonized, and non-colonized individuals were compared by two-sampled t-test for continuous variables and Pearson's chi-square for categorical variables. Multivariable logistic regression models were used to analyse the association between atopic disease and *S. aureus* colonization and carriage estimating odds ratios (ORs) using 95% confidence intervals (CI) setting the significance p level to 0.05. After evaluating

model fit and plausibility of confounders our final model included the covariates age, sex, BMI, serum 25(OH)D, and HbA1c. Smokers were excluded from the multivariable analysis due to the 15-20% higher serum 25(OH)D in smokers compared to non-smokers caused by the assay. Analysis for spa types were restricted to *S. aureus* detected by the first swab sample in Tromsø 6. Normality was assessed by visual inspection of histograms.

2.6 Ethical approval

The sixth Tromsø Study was approved by the Regional Committee of Medical and Research Ethics (REK) and followed the ethical standards of the Helsinki Declaration. Written consent was obtained from all participants.

3 Results

3.1 Baseline characteristics

Table 1 shows baseline characteristics stratified by *S. aureus* colonization (n=3,367) and carriage (n=2,485). In women, 23% were colonized (434/1,894) and in men, 38% were colonized (560/1,473). The mean age of colonized and non-colonized individuals was 50 and 51 years, respectively. The prevalence of *S. aureus* colonization was lower throughout the age groups from 36.2% at 30-39 years to 26.5% at 60-69 years. Prevalence of *S. aureus* colonization was higher in overweight and obese compared to normal weight, and mean BMI in colonized was 27.2 compared to 26.7 in the non-colonized individuals. Among current smokers, 24.3% were colonized, while 31.2% of never or former smokers were colonized. The mean alcohol consumption (in gram per week) in colonized participants was 38.2 compared to 37.2 in non-colonized participants. In addition, colonized participants had significantly lower serum 25(OH)D and higher HbA1c compared to non-colonized; mean serum 25(OH)D 54.4 versus 57.5 nmol/L, and mean HbA1c 5.57 versus 5.52%.

In women, 19.6% (276/1409) were persistent carriers of *S. aureus*, and in men 34.7% (373/1076) were carriers. Variation in prevalence of *S. aureus* carriage across categories of age, BMI, and smoking, and correlations with serum 25(OH)D and HbA1c were similar to findings for *S. aureus* colonization.

Table 1 shows that *S. aureus* colonized individuals are significantly more likely to be younger, non-smokers, males, and to have low serum 25(OH)D and higher HbA1c (all P-values <0.05). Also, *S. aureus* carriers are more likely to be non-smoking males with lower serum 25(OH)D and higher HbA1c compared to non-carriers. No statistically significant difference in *S. aureus* colonization and carriage was found with respect to diabetes, psoriasis,

recent hospitalization (within 12 months), physical activity, healthcare employment status and residing with children.

3.2 Prevalence of atopic diseases

Table 2 shows the prevalence of atopic diseases stratified by *S. aureus* colonization or carriage status. Total *n* may vary due to missing data as denoted in the corresponding tables. In the study population with at least one valid nasal swab the lifetime prevalence rate for asthma, AD, RHE, and pollen allergy was 9.4%, 12.5%, 13.8% and 19.7% respectively. Furthermore, 12.0% and 3.7% had atopic multimorbidity, defined as an atopic score of two or more and three or more, respectively.

Among participants with two valid swabs the lifetime prevalence rate for asthma, AD, RHE, and pollen allergy was 8.9%, 12.4%, 12.8% and 19.22%, respectively. Atopic multimorbidity was found in 11.8%, and 3.4% had an atopic score of three or more.

3.3 Risk of *S. aureus* colonization in atopic disease

In the total population of participants with atopic disease, the *S. aureus* nasal colonization rate varied from 26.8% among those with RHE to 34.3% among those with pollen allergy. Pollen allergy and having several allergic diseases were associated with higher prevalence of *S. aureus* nasal colonization in men and women combined, while RHE was associated with lower *S. aureus* nasal carrier rate.

Data on *S. aureus* nasal colonization and carriage by atopic disease status was further stratified with respect to sex (see Table 3-1 and 3-2). The sex-specific colonization rates in females with asthma, AD, RHE and pollen allergy were 22.2%, 19.9%, 20.0% and 22.9%, respectively (Table 3-1). Age-adjusted logistic regression analysis found no statistically significant association between *S. aureus* colonization and atopic disease or atopic score. However, when looking at sex-specific nasal carriage rates, RHE was associated with lower carriage rates (14.2% in females with RHE versus 20.5% in non-RHE females (p=0.033).

The sex-specific colonization and carrier rate in males with asthma, AD, RHE and pollen allergy were 44.6%, 47.6%, 42.7%, 49.6% and 39.2%, 46.2%, 40.2%, 45.9%, respectively (Table 3-2). With respect to atopic score, the colonization and carrier rates were higher with higher atopic multimorbidity. Among males with no atopic disease, 36.6% was colonized, conversely, among males with an atopic score of 2 or more 53.3% was colonized. Moreover, in men, AD, pollen allergy and atopic multimorbidity were associated with higher prevalence of *S. aureus* nasal colonization (all p-values <0.05), and the same trend was also seen in males for persistent *S. aureus* carriage.

In the first multivariable analysis, adjusted for age, gender, BMI, serum 25(OH)D, and HbA1c, none of the atopic diseases were associated with *S. aureus* colonization or carriage. Significant sex-interaction was found for all atopic diseases except asthma in both populations with significant effect-modification in pollen allergy, atopic score and RHE. Thus, we decided to carry out subgroup analysis among men and women separately.

In the age-adjusted logistic regression analysis restricted to women, an atopic score of one was associated with lower risk of *S. aureus* colonization [odds ratio, 0.71; 95% CI, 0.52 to 0.95; *n*=1,513]. Also, RHE was associated with lower risk of *S. aureus* carriage [odds ratio, 0.65; 95% CI, 0.42 to 0.97; n=1,210]. In the multivariable model including age, BMI, serum 25(OH)D, and HbA1c however, no association was found between atopic disease and colonization or carriage among women. (table 4-1) No effect modification was seen when excluding females using hormonal contraceptives or hormonal postmenopausal treatment (data not shown)

For males, pollen allergy [odds ratio, 1.64; 95% CI, 1.24 to 2.17; n=1,347], an atopic score of 2 [odds ratio, 1.60 95% CI, 1.04 to 2.46; *n*=1,194] and 3 or more [odds ratio, 2.86; 95% CI, 1.30 to 6.30; *n*=1,194] were all associated with higher risk for *S. aureus* colonization. Furthermore, among male participants with two valid nasal swabs, pollen allergy [odds ratio, 1.64; 95% CI, 1.17 to 2.30; n=995] and an atopic score of 3 or more [odds ratio, 2.90; 95% CI, 1.16 to 7.25; n=885] were associated with higher risk of *S. aureus* carriage. (Table 4-1)

In the multivariable analysis adjusted for age, BMI, serum 25(OH)D, and HbA1c, however, only pollen allergy remained statistically significantly associated with higher risk of *S. aureus* colonization in men [odds ratio, 1.45; 95% CI, 1.06 to 1.97, n=1,058]. Among men with two valid nasal swabs, pollen allergy [odds ratio, 1.54; 95% CI, 1.06 to 2.24; n=786] and an atopic score of 1 [odds ratio, 1.67; 95% CI, 1.11 to 2.51; n=701] was associated with higher risk for *S. aureus* carriage. (table 4-1)

3.4 Spa type distribution

From each participant with a positive nasal culture at the baseline screening in Tromsø 6 (n=987), one *S. aureus* isolate was frozen for spa typing. Among the *S. aureus* colonized participants, a total of 26, 33, 36, 49, 74 and 89 participants were colonized with spa types t002, t015, t021, t065, t084 and t012, respectively, comprising the six predominant spa types. As described previously (see statistical analysis), based on the Sollid et al. WGS-analysis of spa types t012, t021, t015, t065 and t084, we decided to group together spa type t015 and t012 with spa types t065 and t021, respectively. In this manner, the four most common spa types

were t002 (2.6%), t084 (7.5%), t012 & t021 (8.3%), t065 & t015 (12.7%). Figure 3 demonstrates the distribution of the 38 most common spa types (excluding all spa types with less than 3 observations) stacked by presence of atopic disease (defined as at least one of the specified atopic diseases). None of the most common spa types were found in participants with atopic disease only. Spa types t050 (n=9) and t127 (n=4) were restricted to participants with atopic history.

3.5 Risk of atopic disease by spa type

The prevalence of t012 & t021 among males with a history of atopic disease was 6.4 % compared to 3.3% in the total male population. (Table 5-2) The corresponding rates among females are 2.9% and 4.1%. (Table 5-1) Thus, the sex-specific rates of t012 & t021 illustrate a gender difference for t012 & t021. For t065 & t015, the prevalence rate among males was 3.9% compared to 2.2.% in the total male population. The corresponding rates among females were 2.7% and 1.4%, respectively.

In the logistic regression model adjusted for sex, smoking and age, a history of atopic disease was associated with higher risk of having *S. aureus* spa type t065&t015 [odds ratio, 1.99; 95% CI, 1.19to 3.34; n=987] both when restricting the analysis to the colonized population and when looking at the total study population [odds ratio, 1.86; 95% CI, 1.14 to 3.06; n=2,805]. However, including sex*t065&t012 interaction term in the model suggested effect modification between sex and spa type t065&t015, however, not statistically significant (p for interaction 0.22). When restricting the regression model to females, a history of atopic disease was associated with higher risk of spa type t065&t015 [odds ratio, 2.57; 95% CI, 1.19 to 5.57; n=353] in the colonized subgroup but not in the total population. Among males, a history of atopic disease was associated with higher risk of spa t012&t021 [odds ratio, 1.96; 95% CI, 1.13 to 3.43; n=1,248] in the total study population.

4 Discussion

4.1 Discussion of results

In this cross-sectional study, we have investigated associations between atopic disease and *S. aureus* nasal colonization or carriage. The data illustrate higher colonization and carrier rates in males (colonized, 38.0%; carriers, 34.7%) compared to females (colonized, 22.9%; carriers, 19.6%). This is in concordance with other studies, confirming the male pre-dominance in *S. aureus* colonization and carriage. We observed a lifetime prevalence rate for asthma, AD, RHE and pollen allergy at 9.4%, 12.5%, 13.8% and 19.7% respectively.

In combined analysis of men and women, we found that RHE was associated with lower risk of *S. aureus* carriage whereas an atopic score of 1compared to an atopic score of 0, was associated with lower risk of *S. aureus* colonization. An interaction between sex and atopic disease was found, and we therefore continued with subgroup analysis separating by sex.

Stratifying the multivariable model by sex, we found pollen allergy to be associated with both higher risk of *S. aureus* colonization and carriage in males, whereas atopic score of 1 compared to an atopic score of 0, was also associated with higher risk of *S. aureus* carriage in males. Moreover, there was a trend towards higher risk of *S. aureus* colonization with higher allergic multimorbidity, although not statistically significant in the multivariable model. The low numbers in subcategories of the atopic score might lead to type II error due to loss of statistical power. In females, we found no association between atopic disease and *S. aureus* colonization or carriage and adjusting for use of hormonal contraceptives and hormonal postmenopausal treatment did not show significant changes of the risk estimates. (data not shown)

Atopic history was associated with higher risk of spa type t065&t015 compared to no atopic history. However, including an interaction term with sex by t065&015 showed effect modification. Interestingly, we found significantly higher risk of spa type t012&t021 among males with atopic history. In the study of spa types distribution by Sangvik et al., also based on TSSS-data, male sex was associated with lower risk of spa type t012 and higher risk of spa type t021. (70) In this study, combining spa t012 and t021 demonstrates an overall preference for males.

In females, we found significant association between atopic history and spa type t015 & t065 in the colonized population. However, atopic history was not related to colonization with specific spa types when the total female population were included in the analysis. Thus, our finding in the colonized subgroup of females should be interpreted with caution as removing non-colonized participants from the model potentially omits participants with atopic disease also. Furthermore, validity might be compromised due to small numbers in the male and female group, and the lack of statistically significant interaction between sex and t065 & 015 despite seeing an effect modification also makes the finding uncertain.

Interestingly, when comparing the predominant spa types in this study population to the literature in Europe, only spa type t002 is found in common. Conversely, spa type t084, t002, and t012 is among the five most common spa types in America. Thus, the spa type

distribution in TSSS has more in common with the distribution in America compared to Europe. (71)

In our study, as described above, pollen allergy was associated with *S. aureus* colonization and carriage in adult males. The reason is not necessarily apparent, and the temporal relationship between cause and effect can't be elucidated because of the cross-sectional study design. However, *S. aureus* is known to establish a Th2 immunity. Th2 immune response is involved in IgE production through the isotype-switch, cellular inflammation of asthma, AD and pollen allergy and the regulation of the epithelial skin barrier.

Although an overall effect isn't found, our findings in males are in line with other studies on the relationship between allergic rhinitis and *S. aureus* carriage. Adults with AR is four times more likely to be sensitized to SE compared to healthy controls.(61) A case-control study including 65 patients(mean age 25.5 yr) with perennial allergic rhinitis(PAR) and 45 non-allergic controls(mean age 27.6 yr) found significantly higher carrier rates in the PAR-patients (45%) compared to the controls (20%). (72)Recently, a study conducted in Seoul including 32 participants found that dysbiosis of the nasal conchae is linked to higher total IgE levels, a known risk factor for atopic disease. The dysbiosis was characterized by high relative abundance of *S. aureus* and lower abundance of *Cutibacterium Acnes* (previously *Propionibacterium acnes*).(73) Furthermore, a systematic review meta-analysis of asthma in adults published primo 2019 provides modest evidence that *S. aureus* carriage is associated with higher risk of asthma prevalence and asthma severity.(74) Concordantly, in older children and adolescents *S. aureus* nasal carriage is associated with higher risk of asthma and asthma exacerbations. (55) Also, higher *S. aureus* nasal carrier rates in patients with AD is thoroughly documented, as illustrated by Totte et al. meta-analysis, reporting odds ratios of 4.50 [95% CI, 3.00-6.75]. In lesioned skin AD was associated with 20-fold higher *S. aureus* carriage rates compared to health controls. Difference was smaller in non-lesioned skin, with eight-fold higher S. aureus skin colonization rates compared to healthy controls (57)

Contrasting the available research in the first multivariable model including both females and males we found carriers to be associated with lower risk of RHE. As current consensus understands it, eczema is associated with higher rates of *S. aureus* colonization and carriage. (57, 75)Currently, the reason for this observed difference is unclear. However, confounders not taken into account by this study might partly explain our findings; the use of topical treatments such as ointments, creams, foams and gels in combination with superior

cleaning routines in participants with RHE can have an explanatory value for the lower rates of *S. aureus* carriage.

Although sparse, available literature find no association between spa type and atopic disease. However, these studies are flawed consisting of small-sample studies, with a case-control study design, focusing on AD and the participants recruited from intensive care units, and from dermatology centres, potentially giving a biased population structure. (63, 64)Thus, our population-based study is the first of its' size studying spa types in an atopic setting.

The sex-difference suggests a biological mechanism where reproductive hormones and behavioural or environmental differences between men and women play a role. Studies suggest that reproductive hormones might have immuno-modulative traits and thus mediate or counter the colonization or carriage of *S. aureus.* Androgens appear to be immunosuppressive and oestrogens pro-inflammatory. In The Tromsø Study FitFutures 2(FF2) Stensen et al.(76) observed that the use of combinational hormonal contraceptives is associated with higher risk(OR) of *S. aureus* colonization. From other studies, it is well-known that sex, age, HbA1c, serum 25(OH)D, and smoking status inflict *S. aureus* colonization and carriage status.

From the MeDALL-project, the data suggested that SE-sensitization was associated with allergic disease. Furthermore, Sørensen et al.(56) found AD, severe asthma, severe allergic rhinitis and allergic multimorbidity to be associated with higher risk of nasal *S. aureus* carriage. Exposure to *S. aureus* in early childhood might thus lead to development of allergic diseases, especially in atopic individuals.

Pollen allergy or allergic rhinitis might facilitate favourable growing conditions in the nasal mucosa and introduction of *S. aureus* to the mucosa can promote extensive inflammation, giving rise to a hostile environment for other less virulent bacteria. On the other hand, early exposure to *S. aureus* induce the Th2- inflammation, may initialize pollen allergy and consequently affect the mucosa to be more susceptible to *S. aureus* throughout life. Although causation can't be proved, overall, our data shows that *S. aureus* colonization might contribute to pollen allergy by maintaining or initializing the atopic cascade. There's a possibility that the association can be attributed to confounders. In our model-selection phase we considered including physical activity and alcohol intake, but these covariates were of less importance in the model. Moreover, diabetes is a known risk factor for *S. aureus* colonization and carriage. To adjust for diabetes, we included HbA1c (%) instead, as the Chi-squared x^2 value was non-significant for diabetes in Table 1.

4.2 Internal and external validity

4.2.1 Study design

The study is based on the TSSS-data with a relatively large number of participants, including 4,026 from the first and 2,997 from the second visit. TSSS is population-based cross-sectional study indicating that exposure and outcome are measured simultaneously. A limitation of cross-sectional studies is that they fail to express the relationship between cause and exposure, thus, the study design can find associations and hypotheses but can't elucidate the causal relation between exposure and outcome.

In this study, the significance level is set to 0.05 which means that a p-value under 0.05 rejects the null hypothesis. Thus, if enough scientists study a phenomenon one in 20 insignificant findings will turn significant by chance. Furthermore, positive findings are more appealing to the editors, publishers and readers leading to positive results bias, a type of publication bias.

4.2.2 Selection bias

In general, the attendance rate was 65.7% in Tromsø 6. Although efforts were made to ensure a randomly distributed population, attendance rate varied throughout the age groups leading to an under-representation in age group 30 to 39 years (47% attendance rate) and age group 80-87 years (40% attendance rate). Lack of motivation, socio-economic aspects and obstacles might produce a participant-bias among certain subgroups and lead to a population of healthier and more health-focused participants. For TSSS, however, a more evenly distributed sampling across age groups were considered suitable for its purpose. Furthermore, in our study we excluded all participants over 70 years. Thus, TSSS sub-study remarks itself with a relatively large number of enrolled participants, including 4,026 from the first and 2,997 from the second visit, and an attendance rate of 92.7% of 4359 eligible invited participants.

For this study, the enrolled population included 3,367 for the analysis of *S. aureus colonization* and 2,485 for *S. aureus* carriage, increasing statistical power and thus minimizing the risk of type II errors. A type II error implies failure to disprove the null hypothesis, when in fact it is false. However, the relatively small subpopulations studied during stratified analysis might be prone to type II errors. Due to the need of stratifying the multivariable logistic model by smoking-status the risk of committing a type II error increases in this population. Analysis of spa types is also exposed to small samples, 65% of all the spa types were only observed in single participants and when stratifying by sex the predominant spa types comprised 112 females and 148 males of the total population of 2,835 with valid data on atopic diseases.

4.2.3 Information bias

The sampling procedure for the assessment of nasal colonization and carriage status is exposed to potential misclassification bias. Nasal swabs were collected and analysed by technicians, mostly nurses and lab-technicians that ahead of the screening were trained in sampling technique and the standard procedures as specified in a protocol. Despite of this, some control nasal cultures had poor or non-existent bacterial growth on the control blood agar. If the nasal swabs showed no bacterial growth on both the control blood agar and the chromId^{TM} *S. aureus* plates, the samplings were excluded from the analyses. The lack of bacterial growth may be attributed to inadequate sampling, faulty sampling-equipment or due to lack of enrichment broth.

Enrichment broth was omitted from the laboratory method based on a sensitivity analysis in the FitFutures survey including 990 high-school students where they found an acceptable sensitivity of 77.2% when omitting the enrichment broth step (unpublished data). In contrary, a Swedish study concluded in 2013 that the use of enrichment broth has a high additive effect on the colonization rate of all sites, with at least a doubling of positive samples in the anterior nares. (77) In the present study, the colonization rate was 29.52% and carriage rate was 26.11% in concordance with other reports. (49, 50)Still, the culturing method may have led to sensitivity-loss and consequently more false-negatives and misclassification of *S. aureus* colonization or carriage status. Furthermore, all participants that had used antibiotics within 24 hours prior to the first or second visit were excluded in the present study of *S. aureus* colonization and carriage, respectively.

The exposure variables regarding atopic disease is based on self-reported information and the lack of clinical examination of the participants may reduce the precision of the exposure variables. Furthermore, the variables were based on lifetime prevalence and not present disease, which can lead to information bias. The actual presence of atopic disease varies throughout life and answering yes to e.g. ever having AD does not imply current presence of the disease. Thus, the generalizability of the present study may be restricted to the diseases present in the adult population. On the other hand, atopy is a genetic pre-disposition and atopic individuals with prior allergic multimorbidity might be associated with *S. aureus*.

In the literature, lifetime prevalence rates vary for asthma. Data from World Health Survey (78) range from 7.58% in Germany to 20.09% in Sweden. In Norway, the WHO report found a lifetime prevalence of 11.05% contrasting the Norwegian birth cohort with lifetime prevalence of 20.2%.(32) For AD, prevalence ranges from 0.6% to 10% in Europe and the US. (79) Furthermore, a Swedish cross-sectional study observed a lifetime prevalence

rate of 13.7% for childhood eczema.(80) For RHE, the literature reports one-year prevalence of up to 10%. Prevalence rates for allergic rhinitis in most westernized countries range from 15% to 20%. The lifetime prevalence rate observed for RHE in this study is marginally higher than what the literature reports. An overrepresentation of healthcare workers, an occupation exposed to hand eczema, may partly explain this phenomenon. Overall, our findings supplement the literature available on atopic disease in adults.

Pollen allergy has a seasonal and geographical variation (pollination normally last from March to August) and *S. aureus* carriage has also been found to vary by season. Thus, taking season into account seems to be necessary to adjust for fluctuation throughout the year. Both nasal swabs from TSSS was collected during October 2007 to June 2008, and thus participants with pollen allergy sampled in June will potentially present with higher carrier rates compared to participants sampled in December.

Overall, this is a cross-sectional study based on TSSS with high attendance rate (92.2%) in a subarctic geographic location. The study population is randomly selected and represent the Troms County across age groups, the general high living standards and relatively small differences in socio-economic status. The external validity might thus be held true despite limitations discussed above.

5 Conclusion

In summary, this population-based cross-sectional study reports that pollen allergy is associated with higher risk of both *S. aureus* nasal colonization and carriage in males whereas having one allergic disease is associated with higher risk of *S. aureus* carriage. Although the trend is not significant, patients with allergic comorbidity present with higher colonization and carrier rates. Moreover, spa type distribution of patients with atopic disease closely follows that of the general population.

6 References

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7 Appendix

7.1 Tables

Table 1 – Baseline characteristic by nasal *S. aureus* colonization or carriage. The Tromsø Staph and Skin Study

Variable	Colonization (*n*=3,367) Total N	Yes	No	*p*†	Carriage (*n*=2,485) Total N	Yes	No	*p*†
Sex	3,367				2,485			
Female	1,894	434 (22.9%)	1460(77.1%)	<0.001	1,409	276 (19.6%)	1,133 (80.4%)	<0.001
Male	1,473	560 (38.0%)	913 (62.0%)		1,076	373 (34.7%)	703 (65.3%)	
Age, yr	3,367				2,485			
30 – 39	257	93 (36.2%)	164 (63.8%)	0.024	171	49 (28.7%)	122 (71.3%)	0.631
40 – 59	1,286	381 (29.6%)	905 (70.4%)		908	246 (27.1%)	662 (72.9%)	
50 – 59	1,000	302 (30.2%)	698 (69.8%)		770	196 (25.5%)	574 (74.5%)	
60 – 69	824	218 (26.5%)	606 (73.5%)		636	158 (24.8%)	478 (75.2%)	
Mean (SD)		50 (±10)	51 (±10)			51 (±10)	51 (±10)	
BMI, *kg/m²*	3,366				2,485			
<20	93	19 (20.4%)	74 (79.6%)	0.055	65	14 (21.5%)	51 (78.5%)	0.113
20-<25	1,125	314 (27.9%)	811 (72.1%)		816	202 (24.8%)	614(75.2%)	
25-<30	1,457	439 (30.1%)	1,018 (69.9%)		1,075	274 (25.5%)	801 (74.5%)	
>30	691	222 (32.1%)	469 (67.9%)		529	159 (30.1%)	370 (69.9%)	
Mean (SD)		27.20 (±4.49)	26.74 (±4.35)			27.36 (±4.64)	26.84 (±4.32)	
HbA1c, % (SD)	3,287	5.57 (±0.67)	5.52 (±0.51)	0.037	2,423	5.60 (±0.74)	5.53 (±0.51)	0.023
Serum 25(OH)D nmol/L	3,315	54.42(±18.83)	57.47 (±19.48)	<0.001	2,443	53.71 (±18.47)	57.23 (±19.49)	<0.001
Psoriasis	2,898				2,171			
Yes	330	107(32.4%)	223(67.6%)	0.277	242	71(29.3%)	171 (70.7%)	0.247
No	2,568	758(29.5%)	1810(70.5%)		1,929	499(25.9%)	1,430 (74.1%)	
Diabetes	3,299				2,433			
Yes	93	31 (33.3%)	62 (66.7%)	0.399	68	24 (35.3%)	44(64.7%)	0.077
No	3,205	939 (29.3%)	2,267 (70.7%)		2365	609 (25.8%)	1,756 (74.2%)	
Smoking	3,330				2,456			
Current	721	175 (24.3%)	546 (75.7%)	<0.0001	505	104 (20.6%)	401 (79.4%)	0.001
Former/Never	2,609	808 (31.0%)	1801 (69.0%)		1,951	538 (27.6%)	1,413 (72.4%)	
Alcohol, *gram/week*	3,079	38.21 (±21.56)	37.15 (±20.88)	0.194	2,295	37.57 (±20.89)	36.73 (±20.56)	0.392
Recreational phys. act.	3,233				2,400			
Sedentary	603	198 (32.8%)	405 (67.2%)	0.259	444	124(27.9%)	320 (72.1%)	0.638
Moderately active	1,962	564 (28.7%)	1,398 (71.3%)		1,467	380 (25.9%)	1,087 (74.1%)	
Active	601	175 (29.1%)	426 (70.9%)		442	110 (24.9%)	332 (75.1%)	
Highly active	67	18 (26.9%)	49 (73.1%)		47	10 (21.3%)	37 (78.7%)	
Recent hospitalization[Ω]	3,192				2,353			
Yes	330	105(31.8%)	225(68.2%)	0.332	246	68 (27,6%)	178 (72.4%)	0.548
No	2,862	837(29.2%)	2,025(70.8%)		2,107	545 (25.9%)	1,562 (74.1%)	
HCW[§]	2,322				2,270			
Yes	414	127 (30.7%)	287 (69.3%)	0.715	405	105 (25.9%)	300 (74.1%)	0.956
No	1,908	568 (29.8%)	1,340 (70.2%)		1,865	486 (26.1%)	1,379 (73.9%)	
Residing with children	2,945				2,177			
Yes	1,370	425 (31.0%)	945 (69.0%)	0.109	984	268 (27.2%)	716 (72.8%)	0.332
No	1,575	446 (28.3%)	1,129 (71.7%)		1,193	303 (25.4%)	890 (74.6%)	
Household/HCW[§]	3,184				2,353			
Yes	645	184 (28.5%)	461 (71.5%)	0.522	482	122 (25.3%)	360 (74.7%)	0.678
No	2,539	760 (29.8%)	1,789 (70.2%)		1,871	491 (26.2%)	1,380 (73.8%)	

Values are expressed as n (percent) or mean ± standard deviation (SD). *p,* p-value*; n,* numbers; HCW, Healthcare worker;
§ Healthcare worker; Hospital, nursing home, senior care services, GP's office. public health centre
† Pearson chi-square for categorical variables. Independent two-sample t-test for continuous variables.
[Ω] Recent hospitalization; last 12 months.

Table 2 – Prevalence rates of nasal *S. aureus* colonization (n=3,367) and carriage (n=2,485) by categories of atopic disease. The Tromsø Staph and Skin Study

	Colonized (n=3,367)				Carriage (n=2,485)			
	Total *n*	Yes	No	*p*	Total *n*	Yes	No	*p*
Asthma	3,098				2435			
Yes	310	96 (31.0%)	214 (69.0%)	0.536	216	60 (27.8%)	156 (72.2%)	0.549
No	2,988	875 (29.3%)	2,113 (70.7%)		2219	577 (25.9%)	1,642 (74.1%)	
Atopic Dermatitis	2,838				2,095			
Yes	355	105 (29.6%)	250 (70.4%)	0.809	264	73 (27.7%)	191 (72.3%)	0.654
No	2,483	750 (30.2%)	1,733 (69.8%)		1,831	492 (26.4%)	1,375 (73.6%)	
Recurrent hand eczema	2,848				2,129			
Yes	392	105 (26.8%)	287 (73.2%)	0.141	294	63 (21.4%)	231 (78.6%)	**0.046**
No	2,456	748 (30.5%)	1,708 (69.5%)		1,835	496 (27.0%)	1,339 (73.0%)	
Pollen allergy	2,975				2,232			
Yes	586	201(34.3%)	385(65.7%)	**0.023**	429	131 (30.5%)	298 (69.5%)	0.051
No	2,389	704(29.5%)	1685(70.5%)		1,803	467 (25.9%)	1,336 (74.1%)	
Atopic Score	2,707				2,026			
None	1,782	539 (30.2%)	1,243 (69.8%)	**0.07**	1,358	354 (26.1%)	1,004 (73.9%)	0.643
1	609	163 (26.8%)	446 (73.2%)	·	428	111 (25.9%)	317 (74.1%)	
2	226	80 (35.4%)	146 (64.6%)		170	52 (30.6%)	118 (69.4%)	
3-4	90	31 (34.4%)	59 (65.6%)		70	19 (27.1%)	51 (72.9%)	

Values are expressed as n (percent). *n, numbers; p,* p-value. *n* might vary due to missing data.
† Pearson chi-square for categorical variables. Significant X^2-values in bold.

Table 3-1. Prevalence of nasal *S. aureus* colonization and carriage by categories of atopic disease in females. The Tromsø Staph and Skin Study.

	Colonized (*n*=1894)			Carriage (*n*=1409)		
	Yes	No	*p*†	Yes	No	*p*†
Asthma						
Yes	42 (22.2%)	147 (77.8%)	0.810	29 (21.0%)	109 (79.0%)	0.651
No	382 (23.0%)	1,279 (77.0%)		240 (19.4%)	997 (80.6%)	
Atopic dermatitis						
Yes	46 (19.9%)	185 (80.1%)	0.205	31 (17.9%)	142 (82.1%)	0.509
No	324 (23.7%)	1,042 (76.3%)		208(20.1%)	828 (79.9%)	
Recurrent hand eczema						
Yes	55 (20.0%)	220 (80.0%)	0.191	30 (14.2%)	182 (85.8%)	**0.033**
No	314 (23.6%)	1,014 (76.4%)		205 (20.5%)	793 (79.5%)	
Pollen allergy						
Yes	77(22.9%)	259(77.1%)	0.790	53(20.5%)	206(79.5%)	0.822
No	305(23.6%)	987(76.4%)		194(19.8%)	784(80.2%)	
Atopic score						
None	232 (24.6%)	711(75.4%)	0.145	147 (20.6%)	568 (79.4%)	0.410
1	71 (18.9%)	305(81.1%)		47 (17.2%)	226 (82.8%)	
2	33 (25.0%)	99(75.0%)		23 (22.3%)	80 (77.7%)	
3-4	13 (21.0%)	49(79.0%)		7 (14.0%)	43 (86.0%)	

Values are expressed as n (percent). *n, numbers; p,* p-value.
† Pearson chi-square for categorical variables.

Table 3-2 Prevalence of nasal *S. aureus* colonization and carriage by atopic disease categories. The Tromsø Staph and Skin Study.

	Colonization(*n*=1473)			Carriage(*n*=1076)		
	Yes	No	*p*†	Yes	No	*p*†
Asthma						
Yes	54 (44.6%)	67 (55.4%)	0.104	31 (39.7%)	47 (60.3%)	0.353
No	493 (37.2%)	834 (62.8%)		334 (34.1%)	645 (65.9%)	
Atopic dermatitis						
Yes	59 (47.6%)	65 (52.4%)		42 (46.2%)	49 (53.8%)	**0.022**
No	426 (38.1%)	691 (61.9%)	**0.041**	284 (34.2%)	547 (65.8%)	
Recurrent hand eczema						
Yes	50 (42.7%)	67 (57.3%)	0.368	33 (40.2%)	49 (59.8%)	0.314
No	434 (38.5%)	694 (61.5%)		291 (34.8%)	546 (65.2%)	
Pollen allergy						
Yes	124(49.6%)	126 (50.4%)	**<0.001**	78(45.9%)	92(54.1%)	**0.001**
No	399(36.4%)	698 (63.6%)		273(33.1%)	552(66.9%)	
Atopic score						
None	307 (36.6%)	532 (63.4%)	**0.002**	207(32.2%)	436 (67.8%)	**0.006**
1	92 (39.5%)	141 (60.5%)		64(41.3%)	91 (58.7%)	
2	47 (50.0%)	47 (50.0%)		29(43.3%)	38 (56.7%)	
3-4	18 (64.3%)	10 (35.7%)		12(60.0%)	8 (40.0%)	

Values are expressed as n (percent). *n, numbers; p,* p-value.
† Pearson chi-square for categorical variables.

Table 4-1. Estimated risk of *S. aureus* nasal colonization and carriage in females. Logistic regression analysis. The Tromsø Staph and Skin Study

	Colonized							**Carriers**						
		OR*			OR§				OR*			OR§		
	Total n†	(n=1,894)	95% CI	*p*	(n=1,442)	95% CI	*p*	Total *n*†	(*n*=1,409)	95% CI	*p*	(*n*=1,094)	95% CI	*p*
Asthma	1,850/1,376	0.958	(0.667-1.375)	0.816	1.006	(0.662-1.531)	0.976	1,375/1,040	1.105	(0.717-1.704)	0.650	1.242	(0.764-2.019)	0.382
Atopic Dermatitis	1,597/1,193	0.779	(0.550-1.103)	0.159	0.977	(0.663-1.438)	0.905	1,209/918	0.864	(0.567-1.315)	0.495	1.206	(0.764-1.902)	0.422
Recurrent hand eczema	1,603/1,199	0.806	(0.585-1.112)	0.19	0.867	(0.589-1.278)	0.362	1,210/919	0.638	(0.421-0.966)	**0.034**	0.726	(0.458-1.150)	0.173
Pollen Allergy	1,628/1,213	0.943	(0.708-1.256)	0.688	0.861	(0.622-1.191)	0.366	1,237/939	1.039	(0.738-1.464)	0.827	0.998	(0.680-1.064)	0.991
Atopic Score	1,513/1,136							1141/871						
None	943/712	1.0	Ref.	0.132	1.0	Ref.	0.235	715/547	1.0	Ref.	0.414	1.0	Ref.	0.391
1	376/285	0.707	(0.524-0.952)	**0.029**	0.757	(0.542-1.056)	0.101	273/215	0.803	(0.559-1.155)	0.237	0.838	(0.550-1.255)	0.392
2	132/94	0.993	(0.650-1.516)	0.974	1.175	(0.728-1.896)	0.508	103/72	1.109	(0.672-1.831)	0.686	1.404	(0.801-2.463)	0.236
3-4	62/45	0.782	(0.416-1.472)	0.447	0.706	(0.331-1.507)	0.369	50/37	0.628	(0.276-1.428)	0.267	0.754	(0.305-1.868)	0.542

n, numbers; OR, odds ratio; CI, confidence interval; *p*, p-value. Significant values($p<0.05$) in bold.
* age-adjusted logistic regression model.
§ multivariable logistic regression model including: age, BMI, HbA1c, serum 25(OH)D in non-smokers.
† *n* might vary due to missing data. Total *n* denotes cases included in analysis for age-adjusted and multivariate logistic regression, respectively.

Table 4-2. Estimated risk of *S. aureus* nasal colonization and carriage in males. Logistic regression analysis. The Tromsø Staph and Skin Study.

	Colonized							**Carriers**						
		OR*			OR§				OR*			OR§		
	Total *n*†	(n=1,473)	95% CI	*p*	(n=1,167)	95% CI	*p*	Total *n*†	(*n*=1,076)	95% CI	*p*	(n=857)	95% CI	*p*
Asthma	1,448/1,122	1.344	(0.923-1.958)	0.123	1.139	(0.733-1.771)	0.563	1,057/825	1.265	(0.788-2.030)	0.331	1.036	(0.593-1.810)	0.901
Atopic Dermatitis	1,241/978	1.377	(0.945-2.005)	0.096	1.359	(0.895-2.065)	0.150	1,076/730	1.541	(0.991-2.396)	0.055	1.447	(0.881-2.376)	0.144
Recurrent hand eczema	1,245/986	1.182	(0.803-1.740)	0.398	1.065	(0.699-1.621)	0.770	919/732	1.243	(0.781-1.980)	0.359	1.080	(0.649-1.795)	0.768
Pollen Allergy	1,347/1,058	1.641	(1.239-2.174)	**0.001**	1.447	(1.062-1.973)	**0.002**	1,076/786	1.644	(1.171-2.309)	**0.004**	1.545	(1.063-2.244)	**0.022**
Atopic Score	1,194/941							885/701						
None	839/651	1.0	Ref.	0.014	1.0	Ref.	0.244	643/504	1.0	Ref.	0.24	1.0	Ref.	0.056
1	233/185	1.079	(0.799-1.457)	0.621	1.104	(0.787-1.549)	0.566	155/122	1.424	(0.991-2.047)	0.056	1.672	(1.111-2.516)	**0.014**
2	94/81	1.598	(1.036-2.464)	**0.034**	1.273	(0.791-2.048)	0.320	67/59	1.503	(0.897-2.518)	0.122	1.226	(0.668-2.154)	0.479
3-4	28/24	2.864	(1.300-6.307)	**0.009**	2.227	(0.948-5.229)	0.066	20/16	2.904	(1.163-7.248)	**0.022**	2.116	(0.730-5.887)	0.151

n, numbers; OR, odds ratio; CI, confidence interval; *p*, p-value. Significant values($p<0.05$) in bold.

* age-adjusted logistic regression model.

§ multivariable logistic regression model including: age, BMI, HbA1c, serum 25(OH)D in non-smokers.

† *n* might vary due to missing data. Total *n* denotes cases included in analysis for atopic diseases in age-adjusted and multivariate logistic regression, respectively.

Table 5-1 Estimated risk of the four most common nasal *S. aureus* spa types by atopic history in females*;* estimates from analysis of the sub-population of *S. aureus* colonized females versus the total female population. The Tromsø Staph and Skin Study, first visit.

	Colonized population (n=353)					Total population (n=1,587).				
	Atopic history					Atopic history				
	Yes (n=141) N%	No (n=212) N%	OR§	95% CI	*p*	Yes (n=711) N%	No (n=876) N%	OR§	95% CI	*p*
t012 & t021(n=57)	21(14.9%)	36(17.0%)	0.908	(0.496-1.664)	0.756	21(2.9%)	36(4.1%)	0.688	(0.397-1.193)	0.183
t065 & t015(n=31)	19(13.5%)	12(5.7%)	2.573	(1.189-5.571)	**0.016**	19(2.7%)	12(1.4%)	1.991	(0.958-4.139)	0.065
t002(n=6)	2(1.4%)	4(1.9%)	0.798	(0.143-4.459)	0.798	2(0.3%)	4(0.5%)	0.612	(0.112-3.357)	0.572
t084(n= 18)	7(5.0%)	11(5.2%)	1.087	(0.398-2.968)	0.871	7 (1.0%)	11(1.3%)	0.859	(0.324-2.273)	0.759

n, Numbers; *p*, p-value; OR, odds ratio; CI, confidence interval. Significant values (p-value<0,05) in bold.
§ Logistic regression model; age and smoking-adjusted.

Table 5-2 Estimated risk of the four most common nasal *S. aureus* spa types by atopic history in males; estimates from analysis of the sub-population of *S. aureus* colonized males and the total male population. The Tromsø Staph and Skin Study, first visit.

	Colonized (n=483)					Total (n=1,248)				
	Atopic history					Atopic history				
	Yes (n=189)	No (n=294)				Yes (n=435)	No (n=813)			
	N%	N%	OR§	95% CI	*p*	N%	N%	OR§	95% CI	*p*
t012 & t021(n=55)	28(14.8%)	27(9.2%)	1.730	(0.962-3.111)	0.067	28(6.4%)	27(3.3%)	1.968	(1.128-3.433)	**0.017**
t065 & t015(n=35)	17(9.0%)	18(6.1%)	1.500	(0.739-3.043)	0.261	17(3.9%)	18(2.2%)	1.720	(0.869-3.403)	0.120
t002(n=18)	4(2.1%)	14(4.8%)	0.430	(0.137-1.346)	0.147	4(0.9%)	14(1.7%)	0.490	(0.159-1.511)	0.214
t084(n=40)	14 (7.4%)	26 (8.8%)	0.779	(0.383-1.586)	0.491	14 (3.3%)	26(3.2%)	0.886	(0.446-1.761)	0.731

n, Numbers; *p*, p-value; OR, odds ratio; CI, confidence interval. Significant values(p-value<0,05) in bold.
§ Logistic regression model; age and smoking-adjusted.

7.2 Figures

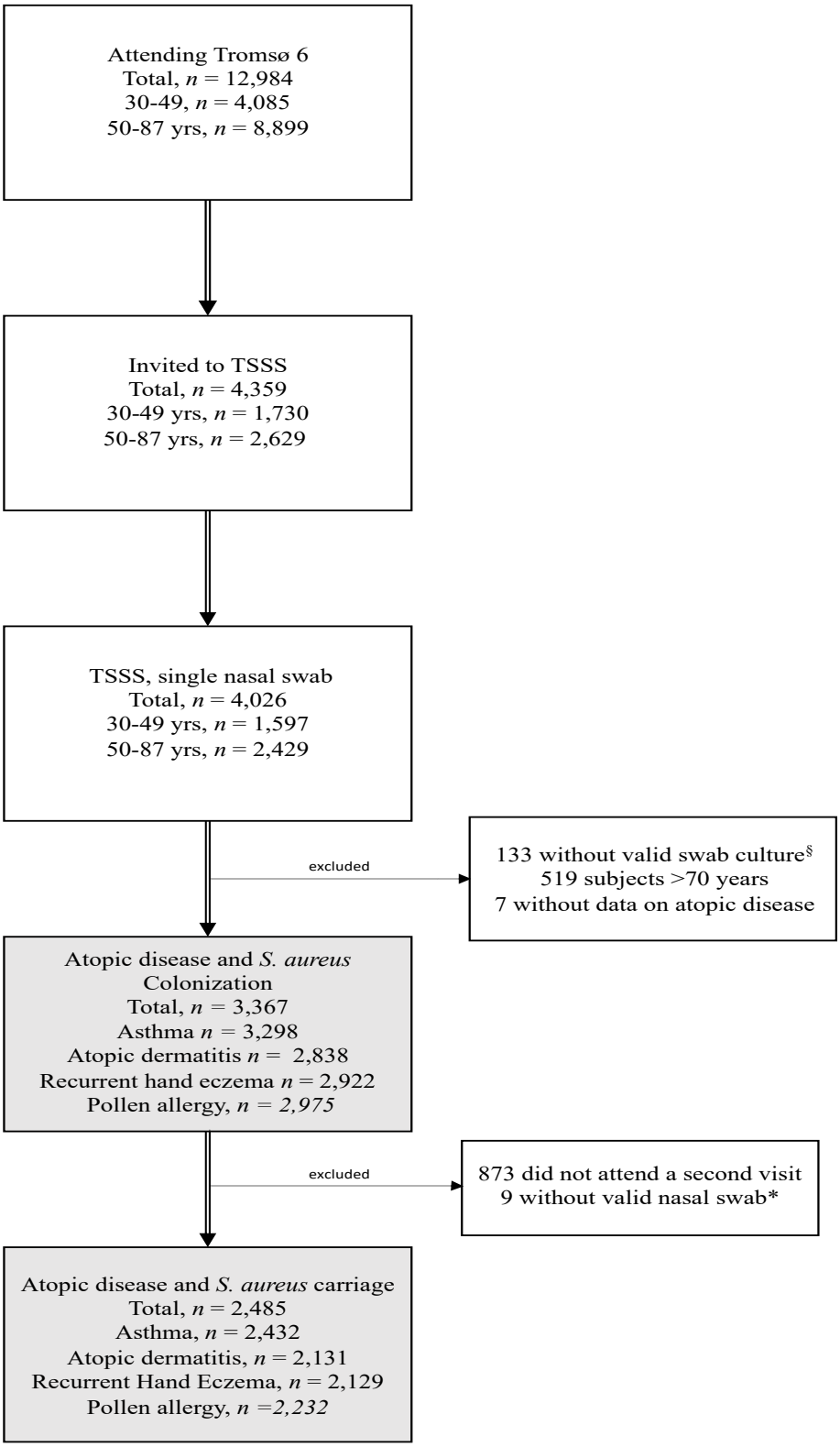

Figure 1. The study population as included and excluded from The Tromsø 6 and the Tromsø Staph and Skin
§ without valid swab culture: 105 had no growth in swab culture, 28 had taken antibiotics last 24 hours
* without valid swab culture second visit: 9 had taken antibiotics last 24 hours.

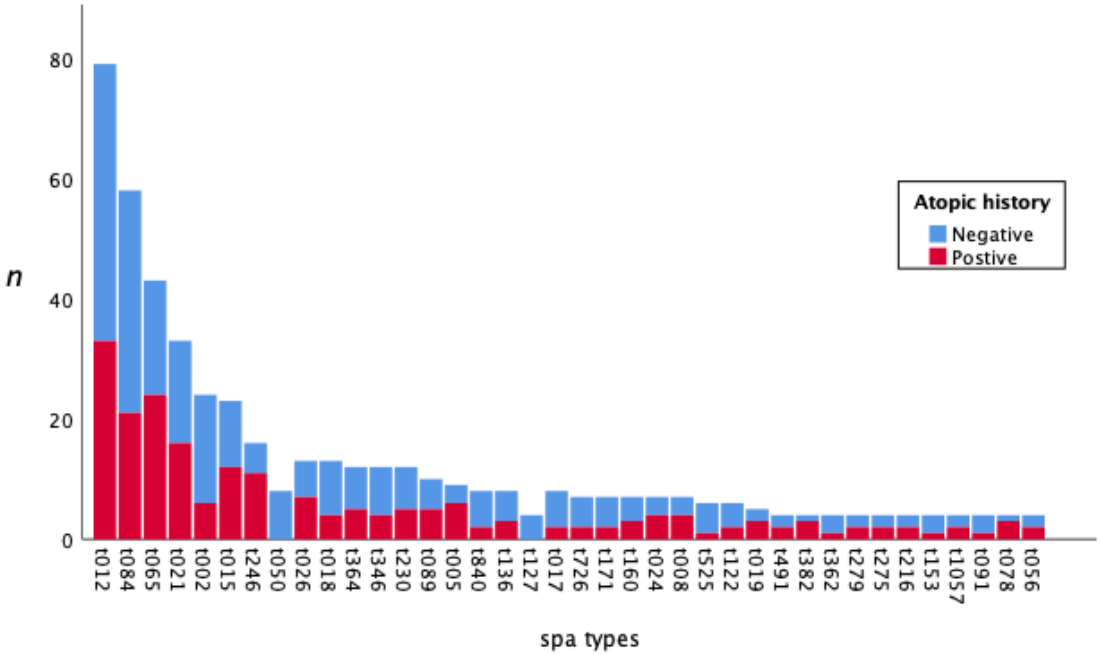

Figure 2. Distribution of *S. aureus* spa types in participants with negative and positive atopic history at first visit. The Tromsø Staph and Skin Study. Spa types with =< 3 are not shown. Atopic history defined as either asthma, atopic eczema, recurrent hand eczema or pollen allergy.

7.3 Grade evaluation

Referanse: Davis MF, Peng RD, McCormack MC, Matsui EC. Staphylococcus aureus colonization is associated with wheeze and asthma among US children and young adults. Journal of Allergy & Clinical Immunology. 2015;135(3):811-3 e5.			**Studiedesign: Kohortestudie** Grade - kvalitet: 2C B
Formål	Materiale og metode	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
Evaluate S aureus nasal colonization as a risk factor for a range of asthma-associated outcomes, including diagnosis, symptoms, and exacerbations, among the US population using data from NHANES 2001–2004. In this analysis of NHANES data, representative of the US population, *S aureus* nasal colonization was associated with increased risk of asthma prevalence, symptoms, and exacerbations in children and young adults. **Land** US **År data innsamling** 2001-2004	**Populasjon:** NHANES, a nationally representative survey, includes data on demographic characteristics, health status, and nutrition of noninstitutionalized US residents ages 6 to 85 years old with respiratory outcomes. The total population consist of 16,234 **Hoved utfall:** **S. aureus colonization associated with wheezing or asthma.** **Viktige konfunderende faktorer** Age, sex, family size, social network, Vitamin D **Statistiske metoder** Population-standardized prevalence rates were calculated, and unadjusted and adjusted associations between *S aureus* nasal colonization and asthma and wheeze outcomes were examined using logistic regression modeling to estimate odds ratios (OR) using Stata 13.1 (Stata, College Station, Tex)	**Hovedfunn** *S. aureus* colonization in the younger group was associated with increased population prevalence rates for respiratory outcomes. Odds ratio 1.35 95% CI [1.06, 1.73] Negative associations was observed among older individuals (31–85-year-olds) odds ratio, 0.64 95% CI [0.48, 0.84] **Bifunn** S. Aureus colonization rate is 28.4% and age predict colonization.	**Strengths** **Their data is based on a big demographic representable population. Robust findings to adjustments for the numbe of ported health care. They excluded participants with comorbidities that can imitate asthma and lead to misclassification bias to check without seeing an effect modification in the older population.** **Weaknesses** **Cross-sectional design, can't prove causality. The NHANE questionnaire had no information on atopic status, a well-known risk factor for asthma. Furthermore, the study wasn't stratified by S. aureus carriage and the authors tha the biased and incomplete measurement of Staphylococcus enterotoxins limited their ability to analys the respiratory outcome by SE status in the colonized population.**

Referanse: Tomassen P JD, Newson R, Van Ree R, Forsberg B, Howarth P, Janson C, Kowalski ML, Krämer U, Matricardi PM, Middelveld RJ, Todo-Bom A, Toskala E, Thilsing T, Brożek G, Van Drunen C, Burney P, Bachert C. Staphylococcus aureus enterotoxin-specific IgE is associated with asthma in the general population: A GA2LEN study. Allergy Eur J Allergy Clin Immunol Journal Translated Name Allergy: European Journal of Allergy and Clinical Immunology. 2013;68(10):1289-97.			**Studiedesign: Kohortestudie** Grade - kvalitet: 2b B
Formål	Materiale og metode	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
Aimed to determine the prevalence of and risk factors for serum SE-IgE and to examine the association with asthma. **Konklusjon** SE-IgE is common in the general population throughout Europe. Its risk factors differ from those of IgE against aeroallergens. SE-IgE is associated with asthma in the general population **Land** International. Europe. **År data innsamling** 2008.	**Populasjon:** Based on a postal survey in the 18 centres participating in Europe, 113,938 were invited to take part in clinical survey, 56936 responded. Of these 12461 was invited for further testing and 3505 responded(28.1% attendance rate) 2 centres were excluded due to low response rate and blood sampling. 1 centre sampled no serum and 270bject sin 15 centres were excluded by providing no serum-samples. For the analysis 2908 participants were included. **Hoved utfall:** Asthma; reporting 'ever' had asthma and they had experienced at least one of the following in the preceding 12 months: wheezing, waking up with chest tightness, waking up with shortness of breath, or waking up with cough Skin prick test for common aeroallergens Total IgE and specific IgE **Statistiske metoder** All analyses were carried out within centres. Because of small sample sizes ($n < 5$) in some case groups in the two UK and three Polish centres, data were pooled to country level for these centres. For estimating overall effects, within-centre estimates were combined using fixed-effects meta-analysis, and heterogeneity was assessed with the chi-square and I^2 statistic Estimates were mutually adjusted for confounders, which included gender, age-group, smoking pack-years, sibship size, and parental history of allergy. Only subjects with serum sampled were included in the analyses. Other missing data were deleted pairwise.	Table 1- Overall prevalence of SE-IgE was 29.3%, wide confidence intervals around estimates. No geographic variation is seen for SE-IgE. For house dust mite skin prick test, however, there was a considerable geographical variation in prevalence, ranging from 8.6% in Stockholm to 28.2% in Ghent. Asthma prevalence range from 4.6% in Germany to 17.9% in the UK with a mean of 10.6%. Table 2 – Having a positive skin prick test was more common in families with history of allergy, in older age groups, in larger families, in smokers with >15 pack years. Positive skin prick test of house dust mite as less common in older subjects, more common in those with family history of allergy, and was not associated with family size or smoking. SE-sensitization was more common in those with familial history of allergy and more common in smokers, both current smokers and ex-smokers. OR current smokers = 2.02 [1.42–2.88] OR ex-smokers = 1.41 [1.06–1.89] There was no evidence ($P > 0.05$) that SE-IgE was associated with parental smoking during pregnancy or childhood, birth order, history of severe childhood respiratory infections, day care attendance, bedroom sharing with siblings, and rural *vs* urban living during childhood. Table 3 - SE-IgE-positive subjects were more likely than SE-IgE-negative subjects to have positive skin prick test for house dust mite or any of the aeroallergens (age-/sex-adjusted OR: 1.97 [1.47–2.65] and 2.95 [2.23–3.90], respectively) The prevalence of SE-IgE was higher in asthmatics than in nonasthmatics (OR 2.10 [1.60–2.76], $P = 0.001$).	**Strengths** **- Large-scale population study. Multicentre and international.** **- analysis of the non-respondents demonstrate that disease was not related to response.** **- Weighted analysis using appropriate statistical methods were carried through to derive estimates that reflect epidemiological trends in the general population.** **- Standardized and validated method of predicting asthma outcome through questionnaires.** **Weakness** **- Low attendance rate.** **- Cross-sectional design, disallowing causal clarity.** **- Participants were recruited to the clinical interview and thus the analysis on the basis of symptoms reported in the primary postal survey.** **- Lack of symptom-based definition for nasal polyposis, a known risk factor for staphylococcus aureus carriage, potentially confounding.**

Referanse: Mpairwe H, E W, L M, J N, D A, M N, et al. Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: Randomised-controlled trial results. Pediatr Allergy Immunol Journal Translated Name Pediatric Allergy and Immunology. 2011;22(3):305-12.			Studiedesign: **RCT** **Grade - kvalitet** 2a - B
Formål	**Materiale og metode**	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
To determine whether anthelminthic treatment during pregnancy increases the risk of allergy in infancy. **Konklusjon** Maternal albendazole treatment was associated with a significantly increased risk of eczema **Land** Uganda **År data innsamling** April 2003 and November 2005	**Rekruttering deltakere** **Pregnant women attending** Entebbe Hospital in their first antenatal visit **Inclusion:** **If** they were well, in their second or third trimester, resident in the study area, planning delivery at Entebbe Hospital, willing to participate and willing to know their HIV status Exclusion: - Hb <8 - Clinical apparent liver disease - Bloody stool with diarrhoea - Abnormal pregnancy - History of adverse reactions to anthelminthic drugs - Earlier participation in the study. **Datagrunnlaget** 2507 study women were enrolled and 2201 person-years of follow-up observed among their infants. **Exposure:** This was a randomised, double-blind, placebo-controlled trial of anthelminthic in pregnancy, using albendazole versus placebo and praziquantel versus placebo in a 2 × 2 factorial design. **Primary outcome** Prospectively noted during routine or interim clinic-visits within first year; Doctor-diagnosed Infantile eczema defined as an recurrent itchy rash, with dry/scaly or wet/weeping skin with typical infant distribution. **Secondary outcome** - Reported allergic events at 1-year. Collected using the validated questionnaire from ISAAC. **Statistiske metoder** The effect of treatment on incidence of allergic conditions was assessed using Cox regression analysis, with robust standard errors to adjust for clustering within children. Logistic regression was used to assess the effect of anthelminthic treatment on reported outcomes. Wald tests were used to assess for interactions between treatments. IgE data (transformed to log(IgE + 1) to correct skewness) and SPT positivity were each related to clinical allergy incidence by Cox regression, with robust standard errors.	Table 1 44.5% had hookworm, 21.3%*M. perstans*, 18.3%*S. mansoni*, 12.3%*S. stercoralis*, 9.1%*Trichuris trichiura*, 2.3%*Ascaris lumbricoides* and 0.5% other worms. Table 2: Doctor-diagnosed infantile eczema incidence was 10.4/100 infant years. Treatment of pregnant women with albendazole (compared with placebo) was strongly associated with an increased risk of infantile eczema in their offspring Cox Hazard ratio1.82 (1.26–2.64) the strongest effect of albendazole was among infants whose mothers had no worm infections at all [HR (95% CI) 2.21 (1.23–3.96)], compared with one species [1.87 (1.04–3.35)], two species [1.42 (0.56–3.64)] and three or more worm species [0.97 (0.19–4.86)]. Treatment of pregnant women with praziquantel (compared with placebo) showed no overall effect on the risk of infantile eczema At 1.year visit Maternal treatment with albendazole (compared with placebo) was weakly associated with reported eczema [OR (95% CI), p-value: 1.29 (0.96–1.72), 0.09] and strongly associated with reported recurrent wheeze [1.58 (1.13–2.22), 0.008]	Enrolled participants were allocated to one of four treatment groups according to a random sequence generated in block of 100 by the trial statistician. Participants and staff were blinded to treatment allocation. Strengths; - Reported effects of treatment were equal for doctor-diagnosed outcomes and outcomes reported at the 1 year clinic visit suggesting good internal validity - Randomized, double-blinded, well-sized sample. Weakness - Single-centre, homogenous population - Lower power in the study because the eczema rate was lower than predicted. - Exposure drug was given in either second or third trimester. The comparison groups differ in treatment and worm status throughout the antenatal period.

Referanse: Riechelmann H, A E, T D, A R, B R, M W. Nasal carriage of Staphylococcus aureus in house dust mite allergic patients and healthy controls. Allergy Eur J Allergy Clin Immunol Journal Translated Name Allergy: European Journal of Allergy and Clinical Immunology. 2005;60(11):1418-23.			**Studiedesign: Kasus-kontroll** **Grade - kvalitet** 2C B
Formål	**Materiale og metode**	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
The frequency of *Staphylococcus aureus* (SA) nasal carriage and its possible influence on persistent allergic rhinitis was investigated. **Konklusjon** Nasal SA carriage is frequent in patients with persistent allergic rhinitis. The data of this study suggest that they are not only secondary bystanders, but actively modulate the disease by promoting local IgE production. **Land** Germany **År data innsamling** 2003- 2004	**Populasjon** Forty non-smoking participants were included in this study, aged 19-36 and all students from University of Ulm. Recruited from an outpatient allergy-unit Case; 22 patients with house dust mite allergy. **Control; 18 healthy controls defined as not having nasal allergy** **Excluded:** Not included were pregnant or lactating women, patients with chronic rhinosinusitis, acute rhinosinusitis within the last 6 weeks, clinically relevant nasal septal deviation, regular use of nasal decongestants, previous sinus surgery, bronchial asthma, lung emphysema, atopic dermatitis, previous immunotherapy, systemic therapy with corticosteroids or anti-inflammatory drugs, or nasal corticosteroid therapy within the last 6 weeks **Hovedeksponering:** *Staphylococcus aureus carriage.* *Skin prick test;* various pollen, mites, molds and animal dander. Allergic rhinitis severity. Total IgE and Cytokine determination in nasal secretion **Statistiske metoder** Frequency tables were evaluated with Fisher's exact test. Symptom scores in SA carriers and noncarriers were compared with the Kruskal Wallis test. Biomarker concentrations in nasal secretions were log-transformed to the base 10 and means ± SD are provided for data description. The influence of SA colonization on nasal biomarkers in allergic patients was compared with *t*-tests. Due to the exploratory character of the study, no corrections for multiple tests were performed. A two-sided α-level of 0.05 was set as the significance level in all tests performed.	19/40 nasal lavages were positive for staphylococcus aureus. Staphylococcus aureus was significantly more frequent in patients with house dust mites allergy. 22 participants had house dust mite allergy. Nasal Staphylococcus Aureus colonization was not associated with polyvalent sensitization skin prick tests. S. Aureus colonization was associated with higher total IgE, high IL13 and low Interferon gamma levels. The results of this study are consistent with the concept that staphylococcal superantigens enhance the T_{H2}- and IgE-bias of the immune response in individuals with allergic rhinitis.	**Strengths;** - Combined symptoms-based questionnaire and diagnostic methods for determination of Allergic rhinitis. - Excluding potential confounders of nasal colonization of S. aureus. **Limitations** In general, poor external validity. - Small-sample. - Non-random biased selection of participants, young students from one university. - Limited statistical methods.

Referanse: **Worldwide time trends for symptoms of rhinitis and conjunctivitis: Phase III of the International Study of Asthma and Allergies in Childhood** Bengt Björkstén Tadd Clayton Philippa Ellwood Alistair Stewart David Strachan The ISAAC Phase III Study Group https://doi.org/10.1111/j.1399-3038.2007.00601.x			**Studiedesign: Kohortestudie** Grade - kvalitet: 2b - B
Formål	Materiale og metode	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
ISAAC is designed to allow comparison of the prevalence and time trends of asthma, rhinitis and eczema between populations in different countries. This article focuses on rhino-conjunctivitis. **Konklusjon** In conclusion, no consistent global time trends in the prevalence of childhood rhino conjunctivitis could be identified. **Land** From 36-56 depending on which cross-section and which age group. **År data innsamling** ISAAC phase II; 1991-1998 ISAAC phase III; 1999-2004	**Population:** The international Study of asthma and Allergies in Childhood study compared two age groups across different countries. In the first phase of the study a total of 468,801 children in the age group 13-14 spread across 155 centres and 56 countries and 257,601 in the age group of 6-7, spread across 91 centres in 38 countries. The same was done for the third phase. Briefly, two age-groups (13-14 years and 6-7 years)were chosen at random from a selected sample of schools. In total 194,404 children aged 6-7 years and 304,679 aged 14-14 years answered a cross-sectional questionnaire in 66 centres in 7 countries and 106 centres from 56 countries, respectively. **Main outcome** Symptom prevalence and annual change for rhino-conjunctivitis. **Confounding factors** Children lacking data on stem and branch questions were included in the surveys the age group 6-7 parents answered the questionnaire. Seasonal variation can give other findings. **Statistiske metoder** The data was presented in a tabular form with calculated prevalence and annual change for each question. The symptom prevalence was calculated by total amount of positive response of each question and dividing by the total of answered questionnaires. The annual change was calculated by taking the difference in phase one and two prevalence and diving it by the numbers of years between the surveys	**Hovedfunn** Slight increase of worldwide rhinoconjunctivitis, with increase in 62 centres and decrease in 44 among children aged 13-14. Mostly small changes across the two surveys, and no consistent pattern can be seen in any of the regions. **Bifunn** Allergic rhinoconjuncitviits increased more in the oldest age-group. Countries with the highest prevalence in phase I appear to have peaked according to phase III. In countries undergoing rapid socio-economic development, an increase is seen. There was a correlation between the changes in prevalence in the two age groups.(pearson correlation coeffcient R= 0.43 and P= 0.0005)	• **Strength** Randomly selected well-sized population with high attendance. Multicenter, multicultural population. Standardized questionnaire with study following the same rigorous procedure and manual in both cross-sectional study's. In the questionnaire they use symptoms instead of labels. • **Weakness :**In the age group 6-7, the parents reported symptoms and prevalence of a disease in their child resulting in unspecific questionnaire. Lack of clinical examination also provides to this weakness. Including only two cross-sectional surveys, complicates the interpretation of the mean yearly change to evaluate linearity. Most centres urban, thus, a centre might not be representative of its region or country.