SCRATCHING THE SURFACE

Risk factors and consequences of childhood eczema phenotypes

CHEN HU

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Copromotor	Dr. L. Duijts

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CONTENTS

MANUSCRIPTS THAT FORM THE BASIS OF THIS THESIS

Chapter 2.1

Hu C, Duijts L, Erler NS, Elbert NJ, Piketty C, Bourdes V, Blanchet-Réthoré S, de Jongste JC, Pasmans SGMA, Felix JF, Nijsten T. Most associations of early-life environmental exposures and genetic risk factors poorly differentiate between eczema phenotypes: the Generation R Study. *Br J Dermatol. 2019 Dec;181(6):1190-1197*.

Chapter 2.2

Hu C, Duijts L, van Meel ER, Looman KIM, Kiefte-de Jong JC, Pardo LM, Hijnen D, Pasmans SGMA, de Jongste JC, Moll HA, and Nijsten T. Association between nasal and nasopharyngeal bacterial colonization in early life and eczema phenotypes. *Clin Exp Allergy. 2021 May;51(5):716-725*.

Chapter 2.3

Hu C, van Meel ER, Medina-Gomez C, Kraaij R, Barroso M, Kiefte-de Jong JC, Radjabzadeh D, Pasmans SGMA, de Jong NW, de Jongste JC, Moll HA, Nijsten T, Rivadeneira F, Pardo LM, and Duijts L. A population-based study on associations of stool microbiota with atopic diseases in school-age children. *J Allergy Clin Immunol, 2021 Apr 15;S0091-6749(21)00563-7*.

Chapter 3.1

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Chapter 3.2

Hu C, Nijsten T, Pasmans SGMA, de Jongste JC, Jansen P, and Duijts L. Associations of eczema phenotypes with emotional and behavioural problems from birth until school age. The Generation R Study. *Br J Dermatol. 2020 Aug;183(2):311-320*.

1

General introduction and design

BACKGROUND

Eczema is one of the major common chronic health problems in children worldwide with a prevalence of up to 25%.[1] The prevalence of eczema in The Netherlands varies from 18.4% in children age 6 months to 5 years to 5.7% in children age 13 years.[2] It is important to understand the origins and consequences of childhood eczema in more depth in order to develop more effective prevention and management strategies to reduce the prevalence of eczema. Eczema is a heterogeneous and multifactorial chronic skin disease, and its underlying pathophysiology has not fully been unraveled. Due to the rise of personalized medicine, there has been a shift in eczema research from considering eczema as a single overlapping disease to a more differentiated entity.[3] Eczema could be differentiated based on external (phenotypes) factors, such as clinical symptoms, severity, and age of onset, and/or internal factors (endotypes), such as genetic risk factors and serum biomarkers.[3]

Eczema is a condition of recurrent red patchy scaly skin lesions with intense itchiness located over the entire body. Skin lesions in infants are often located in the face, and at the extensor site of the limbs, and sometimes the trunk. In older children, skin lesions are mostly located in the flexural folds. The clinical definition of eczema is based on the criteria of Hanifin and Rajka published in 1980, and consists of 4 major and 23 minor criteria.[4] However, the Hanifin and Rajka criteria have not been standardized and validated for use in epidemiological studies. For epidemiological studies, a questionnaire-based definition of eczema is more practical and efficient. Therefore, the United Kingdom Working Party's (UKWP) criteria were developed, consisting of 1 major and 5 minor criteria, and validated in pediatric patients with eczema in different countries.[5] Even more practical for large-scale population studies is a single parental-reported physician diagnosis question, which has demonstrated to be sufficiently valid for such studies.[6] This single question definition of eczema as a dichotomous trait is used in many studies. However, this definition does not take the age of onset and persistence of eczema into account, which varies considerably during childhood. Generally, the prevalence is highest in early childhood, and declines in later childhood. Therefore, defining eczema as a dichotomous trait might be an oversimplification. Few studies identified eczema phenotypes, taking into account age of onset and persistence of eczema, and were performed in children of mainly European ancestry, or until the age of 4 years.[7, 8] It is unclear whether similar eczema phenotypes can be found in multi-ethnic children until age 10 years. Additionally, using eczema phenotypes might enable better identification of specific genetic, and early life risk factors, and later life consequences. Genetic, early environmental and microbial factors could potentially be most influential risk factors of eczema phenotypes. Consequently, specific eczema phenotypes might predispose to

higher risk of asthma and allergies, and emotional and behavioural problems. Eventually, studying eczema in more depth, including identifying specific early life risk factors and consequences, could provide new insights for better prevention and treatment strategies focused on early life to reduce the prevalence and burden of childhood eczema.

GENETIC AND EARLY LIFE ENVIRONMENTAL FACTORS

Both genetic and early life environmental factors affect the risk of developing childhood eczema and the course of the disease.[9] The heritability of eczema in previous pediatric cohort studies in twins ranged from 39-90%.[10] The most important known genetic risk factors for eczema are loss-of-function mutations in the gene encoding filaggrin (*FLG*), an indispensable protein for epidermal differentiation and maintenance of an optimal skin barrier.[11] Multiple cohorts additionally identified approximately 40 *FLG* mutations, of which the four most common *FLG* mutations in Caucasians are 2282del4, R2447X, R501X, and S3247X.[12, 13] Furthermore, 31 single nucleotide polymorphisms (SNPs) have been identified in genome-wide association (GWA) studies of mostly population-based cohorts to be associated with childhood eczema.[7, 14, 15] However, the combination of these SNPs and four *FLG* mutations together explain up to approximately 14.91% of the variance in liability of eczema in children of European ethnicity.[14] A previous study that identified eczema phenotypes in children of European ethnicity showed that the genetic risk score based on 23 European SNPs was associated with increased risk of persistent eczema.[7] The eczema-liability of these genetic factors in children with non-European ethnicity has not been studied.

Previous population-based studies showed that a variety of main early life environmental risk factors for childhood eczema are higher maternal education, having older siblings, shorter duration or non-exclusiveness of breastfeeding, day care attendance and having no pets.[16-22] Also, children of non-European ethnicity, and more specifically those of Surinamese-Creole and Surinamese-Hindustani origin have an increased risk of eczema.[23] Only two previous studies have examined the association between early life environmental factors and eczema phenotypes.[7, 8] Furthermore, genetic and environmental risk factors were studied independently of each other in univariate analyses, and in children of predominantly European ethnicity. It remains unclear whether the genetic variants and early life exposures are associated with eczema phenotypes in children of multi-ethnicity, and if they are still risk factors when they are adjusted for each other in multivariate analyses.

MICROBIAL FACTORS

Recently, research on human microbiota have gained major interest in the development of eczema and other atopic diseases, since genetic susceptibility and established

environmental risk factors alone do not explain the increasing prevalence of eczema, and related other atopic diseases.[24, 25] According to the biodiversity hypothesis, which builds upon the hygiene hypothesis, urbanization and modern public health practices lead to less microbial exposure, and thereby a less stimulated immune system, and subsequently an increased risk of eczema, allergy and asthma.[25, 26] In addition, children with eczema are more prone to develop skin infections such as herpes simplex and impetigo.[27] A less diverse skin microbiome has been associated with increased risk of eczema and eczema severity.[28-30] However, the evidence of a causal relation between skin microbiome and eczema is lacking.[29, 30]

One of the most well-known microorganism associated with increased risk of eczema is *Staphylococcus aureus* (*S. aureus*), a commensal bacteria on humans, that often spreads from the nose to other body sites, and can occasionally cause severe infections.[31] Early life nasal carriage of *S. aureus* increases the risk of eczema and its severity in children at age 1-2 years, but persistent effects in later childhood are not clear.[32] Other commensal airway bacteria, such as *Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pneumonia,* are suspected to play a role in the development of childhood atopic respiratory diseases.[33, 34] Therefore, it might be that early life nasopharyngeal carriage with *H. influenzae, M. catarrhalis* or *S. pneumoniae* is also associated with risk of eczema. However, few studies examined these associations.[28, 35, 36] Also, it remains unclear whether there is a causal relation between bacterial nasal/nasopharyngeal carriage and eczema.[37]

Another relevant microbiota site related to eczema and other atopic diseases is the gut. Previous studies has shown that the gut microbiota affects the development and regulation of the immune system greatly, especially in early childhood.[38] Infants with a less diverse stool microbiota, and with greater abundance of *Bacteroidaceae*, *Clostridiaceae* and *Enterobacteriaceae*, and lower abundance of *Bifidobacteriaceae* and *Lactobacillaceae*, had a higher risk of eczema, allergy or asthma until age 3 years.[39] However, the majority of studies linking the gut microbiota to atopic diseases were performed in relative small number of children, and the role of gut microbiota in eczema, and other atopic diseases in later childhood is less clear.

ATOPIC DISEASE CONSEQUENCES

Eczema is strongly related to asthma and allergies.[40] According to the atopic march hypothesis, children with eczema and food allergies in early life are at risk to develop asthma and allergic rhinitis in later life.[41] Microbial and/or genetic factors combined with environmental factors may lead to dysfunction of the epithelial barrier, leading to transcutaneous sensitization, and type 2 inflammation, and thereby predisposing to asthma and allergic conditions.[9, 42-44] However, studies on early intervention of eczema in

order to prevent epithelial barrier dysfunction progressing into a proinflammatory state to for example prevent food allergy show contradictory results.[45-48] Early introduction of peanut consumption in infants decreases the risk of peanut allergy, but not the risk of other food and inhalant allergies, eczema or asthma.[49, 50] The underlying mechanism of the atopic march remains unclear. In addition, previous longitudinal population-based cohort studies only found a small proportion of children with eczema that follow this atopic march.[51] An explanation for this contradicting observation might be due to the use of a dichotomous definition of eczema. Few studies that identified eczema phenotypes suggest that children with early onset and persistent eczema have higher risks of asthma and allergy in later childhood.[7, 8] However, it is unclear whether eczema phenotypes in a multi-ethnic pediatric population are similarly related to asthma and allergies in later life, and whether eczema phenotypes are also associated with lung function, and more comprehensive allergy outcomes.

EMOTIONAL AND BEHAVIOURAL CONSEQUENCES

In children, previous studies showed that eczema was associated with more emotional problems, anxiety, depression, attention deficit hyperactivity disorders, and conduct disorders.[52-55] In addition, children with early onset and/or chronic eczema had increased risks of emotional and behavioural problems at school-age.[56, 57] A possible explanation is that eczema related symptoms such as chronic itchiness, red patchy skin appearance and disturbed sleep could negatively affect mental health via social isolation, low self-image, lack of concentration and more irritability. [55, 58, 59] On the other hand, children with emotional and behavioural problems had more severe eczema and eczema exacerbations.[54, 55, 60, 61] It has been suggested that stress, as proxy of psychopathological problems, could shift the balance towards type 2 T-helper cells via the hypothalamic-pituitary-adrenal axis and sympathetic adrenomedullary system leading to more susceptibility of atopic inflammation and diseases, and resulting in a vicious cycle.[62] Maternal psychiatric symptoms during pregnancy were associated with an increased risk of childhood eczema.[63] Therefore, it is unclear what the (causal) direction is between eczema and emotional and behavioural problems.[54] Additionally, using eczema phenotypes might clarify which children with eczema in early life are most at risk of emotional and behavioural problems later in life.

HYPOTHESIS

The hypothesis of this thesis is that genetic, early life environmental, and microbial factors modulate the immune system, and affect the age of onset and persistence of

eczema, and that specific eczema phenotypes might predispose to higher risk of other atopic diseases, and emotional and behavioural problems.

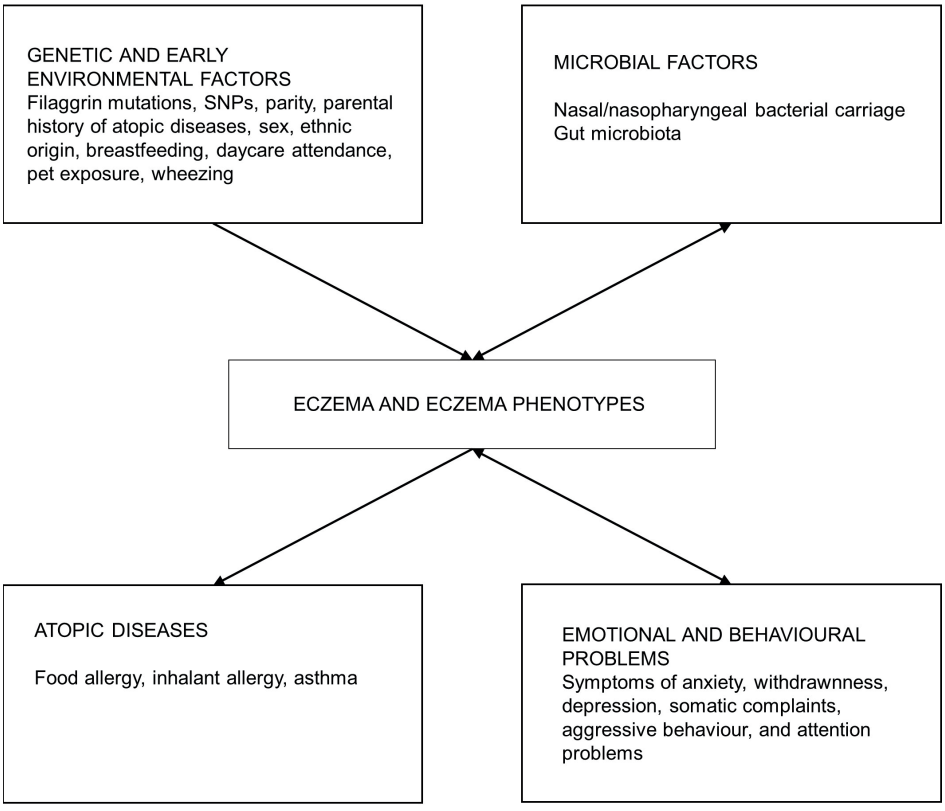

Figure 1. Overview of the specific risk factors, and consequences of eczema and eczema phenotypes studied in this thesis

OBJECTIVES

The major aims of these thesis are:

1. To identify eczema phenotypes
2. To assess the association of genetic, early life environmental and microbial factors with eczema, and eczema phenotypes
3. To assess the relation of eczema phenotypes with other other atopic diseases, and emotional and behavioural problems

GENERAL DESIGN

The studies included in this thesis were embedded in the Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands, following pregnant women and their children from fetal life until adulthood (www. generationr. nl).[64] The aim of the study was to identify genetic and early environmental factors, and causal pathways leading to normal and abnormal growth, and development of health and diseases during fetal life, childhood and adulthood. Women were enrolled primarily during the first trimester, and until birth of the child. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study, and the response rate at baseline was 61%.

Data collection for this thesis comprised parent-reported questionnaires for health and life style habits during pregnancy, and after birth, child's physical examination, and biological samples, including cord blood, nasal swabs and stool samples (Figure 2). Umbilical cord blood was collected in order to genotype the child's DNA by Illumina 670K platform, and modified Taqman allelic discrimination assays were used to identify the four most prevalent *FLG* mutations in Caucasians (2282del4, R2447X, R501X, and S3247X).[12, 13, 64] Between ages 6 months to 6 years, nasal swabs for *Staphylococcus aureus*, and nasopharyngeal swabs for *Streptococcus pneumoniae, Moraxella catarrhalis* and *Haemophilus influenzae* were collected and cultured. At age 10 years, stool samples were collected and analyzed using 16S rRNA gene sequencing.[65] Birth characteristics were obtained from midwife and hospital registries. Information on other covariates, including demographic, socioeconomic, and health-related and lifestyle factors, was mainly obtained by postal questionnaires in the first years of life. Between age 6 months and 10 years, physician-diagnosed eczema, food and inhalant allergy, and asthma was obtained by questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC).[18, 66] At age 10 years, we measured lung function via spirometry according to the American Thoracic Society / European Respiratory Society (ATS/ERS) criteria, and sensitization for food and inhalant allergens using skin prick testing.[67-70] Between the ages of 1.5 to 10 years, emotional and behavioural problems were measured repeatedly using the Child Behavior Checklist.[71-73]

OUTLINE OF THIS THESIS

Chapter 2 focuses on the association of risk factors with eczema and eczema phenotypes. In *Chapter 2.1*, the identification of eczema phenotypes, and its association with genetic and early life environmental factors are presented. In *Chapter 2.2*, the associations of

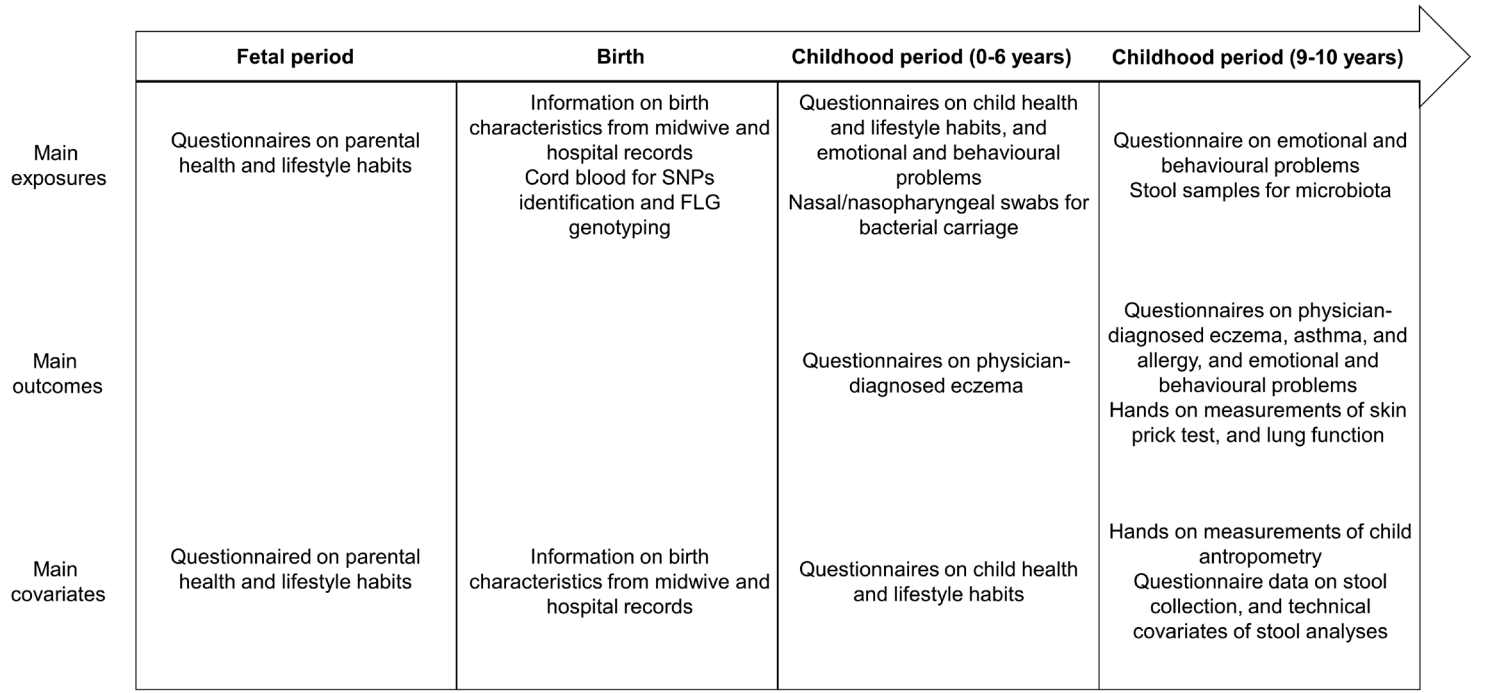

Figure 2. Data collection in the Generation R Study for this thesis

bacterial nasal and nasopharyngeal carriage with eczema phenotypes are described. The associations of child's stool microbiota with atopic diseases are explored in *Chapter 2.3*. **Chapter 3** focuses on the consequences of eczema phenotypes. In *Chapter 3.1,* the risk of eczema phenotypes with allergy and asthma are presented. Associations of eczema phenotypes with emotional and behavioural problems from birth until school age are described in *Chapter 3.2*. **Chapter 4** includes the general discussion with the main observations, and discusses the clinical implication of the studies described by this thesis. In **Chapter 5,** an English and a Dutch summary are presented.

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2

Risk factors of eczema phenotypes

2.1

Early life environmental exposures, genetic risk factors, and eczema phenotypes

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Chen Hu
Liesbeth Duijts
Nicole S. Erler
Niels J. Elbert
Christophe Piketty
Valérie Bourdès
Sandrine Blanchet-Réthoré
Johan C. de Jongste
Suzanne G.M.A. Pasmans
Janine F. Felix
Tamar Nijsten

ABSTRACT

Background Childhood eczema is variable in onset and persistence.

Objective To identify eczema phenotypes during childhood, and their associations with early life environmental and genetic factors.

Methods In this study among 5,297 children of a multi-ethnic population-based prospective cohort study, phenotypes based on parental-reported physician-diagnosed eczema from age 6 months until 10 years were identified using latent class growth analysis. Information on environmental factors was obtained by postal questionnaires. Four filaggrin mutations were genotyped and a risk score was calculated based on 30 genetic variants. Weighted adjusted multinomial models were used for association analyses.

Results We identified five eczema phenotypes: never (76%), early (8%) , mid- (6%) and late transient (8%), and persistent eczema (2%). Early transient and persistent eczema were most common in first born children, those with a parental history of eczema, allergy or asthma and those with persistent wheezing (odds ratio (95% confidence interval): range 1.37 (1.07,1.74) and 3.38 (1.95, 5.85)). Early transient eczema was most common in males only (1.49 (1.18,1.89)). Children with late transient or persistent eczema were more often of Asian ethnicity (2.04(1.14;3.65) and 3.08 (1.34;7.10), respectively). Children with early, late transient and persistent eczema more often had a filaggrin mutation or additional risk alleles (range 1.07 (1.02,1.12) and 2.21 (1.39, 3.50)). Eczema phenotypes were not associated with maternal education, breastfeeding, day care attendance and pet exposure.

Conclusion Five eczema phenotypes were identified in a multi-ethnic pediatric population with limited differences in risk profiles, except for sex and ethnicity.

INTRODUCTION

Childhood eczema is a major common chronic health problem with a prevalence of up to 25%.[1] The age of onset and the persistence of eczema during childhood vary. To better predict the natural course of eczema and to prevent the onset and worsening of eczema, there is a need for defining more detailed eczema phenotypes and for understanding their specific underlying risk factors. Defining eczema as a dichotomous trait is an oversimplification. Eczema phenotypes that take into account the age of onset and persistence over time may enable better identification of specific environmental exposures and genetic mechanisms that might play a role in the development of eczema.[2-4] Previous studies suggest that higher maternal education, non-European ethnicity, having older siblings, shorter duration or non-exclusiveness of breastfeeding, day care attendance and no pet exposure are associated with an increased risk of childhood eczema.[5-11] In addition, loss-of-function mutations in the gene encoding filaggrin (*FLG*), an indispensable protein for epidermal differentiation and maintenance of an optimal skin barrier, are well known to be associated with eczema.[12] Furthermore, genome-wide association (GWA) studies identified 31 variants to be associated with childhood eczema.[13-15] It is unclear whether these early life exposures and genetic variants are related to the various eczema phenotypes. Two previous longitudinal birth cohorts identified different eczema phenotypes with sex, parental history of eczema, asthma or allergies, breastfeeding, pet exposure, *FLG* mutations, genetic risk score, asthma and other allergenic comorbidities as determinants[15, 16] However, those cohorts consisted predominantly children of European ethnicity, did not take the correlation between repeated measurement of eczema into account in the cluster analysis, or only explored the risk of early life exposures and genetic variants in unadjusted analyses.

Therefore to better predict and prevent the natural course of eczema, we aimed to identify eczema phenotypes in a multi-ethnic population-based prospective cohort study among 5,297 children. We further examined the associations of socioeconomic and lifestyle exposures in early life and genetic risk factors with the identified eczema phenotypes.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards.[17] Written informed consent was obtained from both parents or legal guardians. Of 7,893 live born children participating after

birth, those without data on physician-diagnosed eczema available on at least 3 time points were excluded, leaving a total of 5,297 children for the current analyses.

Eczema definition

Information on eczema was obtained from parental questionnaires at age 6 months, and at ages 1, 2, 3, 4 and 10 years (response rates: 72 -76%). Physician-diagnosed eczema was defined as a positive response to the question 'Was your child diagnosed with eczema in the last 6 months/last year by a physician?' (no; yes).[18] This question was adapted from the core questionnaire of the International Study of Asthma and Allergies in Childhood (ISAAC).[19]

Early life environmental exposures

Information on parity (nulliparous; multiparous), maternal education (primary or secondary school; higher than secondary school), and parental history of eczema, allergy or asthma (no; yes) was available from parental questionnaires obtained at enrolment. Child sex was obtained from midwives and hospital records. Ethnic origin (European; non-European) of the child was based on the parents' country of birth according to Statistics Netherlands.[17] Postnatal questionnaires provided information on breastfeeding (never; ever) at 2, 6 or 12 months after birth, pet exposure at ages 2 and 6 months (no; yes, exposure to cat, dog, rodent or bird at home) and day care attendance at age 12 months (yes; no). Questionnaires adapted from the ISAAC study were used to determine wheezing at ages 1-6 years (no, yes).[19] Wheezing patterns were classified based on time of onset and persistence into 'never', 'early' (wheezing at age ≤3 years only), 'late' (wheezing at ages >3-6 years only), or 'persistent' wheezing (wheezing at age ≤3 years and at age >3-6 years) for children with data on wheezing available on at least 2 time points.[20]

Genetic risk factors

The most prevalent *FLG* mutations in Caucasians (2282del4, R2447X, R501X, and S3247X) were genotyped by modified Taqman allelic discrimination assays, using previously described primers.[21, 22] Children without any mutant alleles were classified as wild type. Because we only observed two cases of homozygous *FLG* mutations, we created a combined *FLG* genotype (no mutation; ≥1 mutations).[23] A recent and large GWA study identified and replicated 30 single nucleotide polymorphisms (SNPs) that were associated with childhood eczema.[13] Information on these SNPs was available from the GWA screen performed on DNA isolated from cord blood leukocytes or, in a small minority of children with missing cord blood samples, at age 6 years using the Illumina 670K platform.[17] Genotype data were imputed for all polymorphic SNPs to the 1000 Genomes panel. A genetic risk score for each individual was calculated by summation of the number of eczema-increasing risk alleles (between 0-2 for each SNP) across all SNPs.[13]

Statistical analysis

First, we compared characteristics of those included and not included in our study by using independent sample T-, Mann-Whitney U, and Pearson's Chi-square tests. Second, eczema phenotypes were identified using latent class growth analysis based on parental-reported-physician-diagnosed eczema data. This type of analysis assumes that a number of different latent classes exist in the study population that describe the variation of observed responses over time, and clusters subjects with similar patterns while taking into account correlations between measurements from the same subject.[24-26] Details on model selection are provided in the supplementary material. For comparison with previous studies on eczema subgroups, we performed longitudinal latent class analysis to identify eczema phenotypes.[15, 16] Third, we examined the associations of early environmental exposures and genetic risk factors with the identified eczema phenotypes using weighted mutually adjusted multinomial regression models. Multiple imputation using chained equations was used to impute missing values of environmental exposures (range 0 to 30% per variable). Twenty completed datasets were created and the results pooled using Rubin's rules.[27] Physician-diagnosed eczema, *FLG* genotype and the calculated genetic risk score were not imputed since they could not be appropriately predicted from the available data.[28] Last, in order to examine the associations between different ethnicities and eczema phenotypes in more detail, we divided ethnicity based on similarities in skin type and cultural background into European (European, American or Oceanian), Mediterranean (Turkish or Moroccan), Asian (Asian, Indonesian, Surinamese-Hindustani or Surinamese-mixed) and African (African, Dutch-Antillean or Surinamese-Creole) subgroups.[11, 29] All measures of association are presented as odds ratios (OR) with corresponding 95% confidence intervals (95% CI). Latent class analyses were performed using Mplus (version 7.11) for Windows (Muthén and Muthén, Los Angeles, CA, USA), imputation and weighted multinomial regression analyses were performed using the packages 'mice' (version 2.46.0)[30] and 'nnet' (version 7.3.12) in R version 3.4.3[31], respectively.

RESULTS

Subject characteristics

Maternal and child characteristics are presented in Table 1. The prevalence of eczema declined from 16% (n=662) at age 6 months to 7% (n=347) at age 10 years. Subjects that were not included in the current analyses partly had less favourable socio-economic and environmental factors (Supplementary Table 1).

Table 1. Characteristics of children and their mothers after multiple imputation

	Subjects n=5,297
Parental characteristics	
Parity (nulliparous) % (n)	59 (3,096)
Maternal education (higher) % (n)	57 (3,009)
History of eczema, allergy and asthma (yes, at least one parent) % (n)	60 (3,189)
Child characteristics	
Sex (male) % (n)	50 (2,632)
Ethnicity (non-European) % (n)	26 (1,359)
Breastfeeding (ever) % (n)	92 (4,885)
Day care attendance (yes) % (n)	59 (3,145)
Pet exposure (yes) % (n)	39 (2,038)
Wheezing pattern % (n)	
Never	55 (2,887)
Early	28 (1,495)
Late	5 (261)
Persistent	12 (655)
FLG genotype (≥ 1 mutations) % (n)*	8 (247)
Genetic risk score (mean (SD))*	31 (3.45)
Physician-diagnosed-eczema % (n)*	
6 months	16 (662)
1 years	13 (637)
2 years	14 (719)
3 years	9 (442)
4 years	8 (378)
10 years	7 (347)

Values are based on 20 imputed datasets. *Data on *FLG* genotype, genetic risk score and physician-diagnosed eczema were not imputed. Data were missing for *FLG* genotype (41% (n=2,186)), genetic risk score (36%(n=1,880), and physician-diagnosed eczema at 6 months (30% (n=1,569)), 1 year (14% (n=730)), 2 years (9% (n=457)), 3 years (14% (n=726)), 4 years (15% (n=789)) and 10 years (27% (n=1,418)).

Eczema phenotypes

In children with data on physician-diagnosed eczema available from at least 3 time points (n=5,297), latent class growth analysis identified the model with five eczema phenotypes as the best fit (Figure 1, Supplementary Table 2 and 3). The five eczema phenotypes were described as never eczema (76%), early transient eczema (8%), mid-transient eczema (6%), late transient eczema (8%) and persistent eczema (2%). Similar results were observed in children with data on physician-diagnosed eczema available at all 6 time points (n=1,975) (Supplementary Table 2). To compare our results with previ-

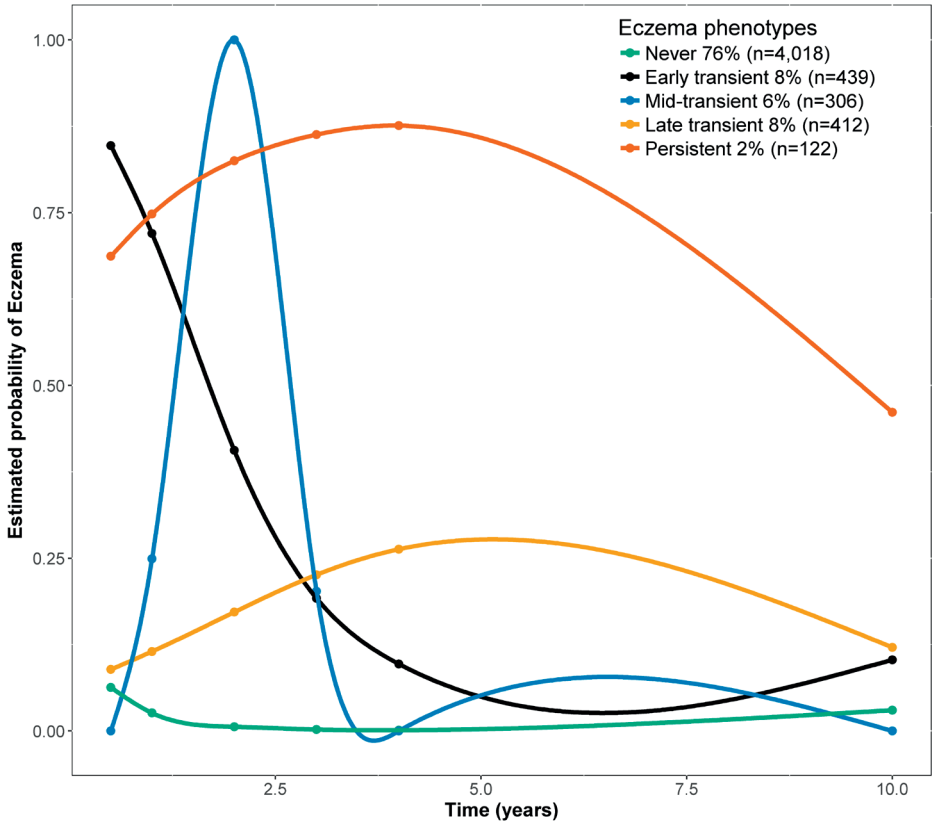

Figure 1. Eczema phenotypes trajectories from latent class growth analysis

ous studies, we used longitudinal latent class analysis and identified that the model with three, not five, eczema phenotypes best fitted our data (Supplementary Table 3, Supplementary Figure 1a and 1b). The three phenotypes were similar in pattern to the never, early transient and persistent eczema phenotypes identified using latent class growth analysis.

Early environmental exposures and eczema phenotypes

No major differences in the magnitude or the direction of the effect estimates were observed between analyses with imputed data and analyses with complete cases only. We only present the results based on imputed data. Nulliparity and parental history of eczema, allergy or asthma were positively associated with early transient and persistent eczema compared with never eczema and reference groups (OR range (95% CI): 1.37 (1.07,1.74) and 2.01 (1.20,3.36), respectively) (Table 2). Boys were significantly more likely to have early transient eczema (1.49 (1.18,1.89)), while non-European ethnicity was associated with late transient (1.35 (1.03,1.78)) and persistent eczema (1.76 (1.10, 2.82)). Chil-

Table 2. Associations of early environmental exposures with eczema phenotypes

	Early transient eczema	Mid-transient eczema	Late transient eczema	Persistent eczema
Environmental exposure model, n=5,297	n=439	n=306	n=412	n=122
Parity (nulliparous)	**1.37 (1.07, 1.74)**	1.38 (0.98, 1.95)	1.10 (0.88, 1.39)	**1.65 (1.06, 2.55)**
Maternal education (higher)	1.04 (0.80, 1.36)	1.11 (0.76, 1.61)	1.04 (0.80, 1.35)	0.70 (0.44, 1.12)
Parental history of eczema, allergy or asthma (yes)	**1.71 (1.29, 2.25)**	1.14 (0.79, 1.64)	1.20 (0.93, 1.55)	**2.01 (1.20, 3.36)**
Sex (male)	**1.49 (1.18, 1.89)**	0.98 (0.71, 1.35)	0.83 (0.66, 1.04)	1.21 (0.80, 1.83)
Ethnicity (non-European)	1.02 (0.76, 1.36)	1.04 (0.69, 1.58)	**1.35 (1.03, 1.78)**	**1.74 (1.09, 2.79)**
Breastfeeding (ever)	0.90 (0.58, 1.39)	0.83 (0.45, 1.53)	0.91 (0.59, 1.40)	0.69 (0.34, 1.39)
Child day care(yes)	1.06 (0.81, 1.39)	1.22 (0.82, 1.80)	1.21 (0.92, 1.58)	1.49 (0.88, 2.50)
Pet exposure (yes)	0.88 (0.68, 1.14)	0.82 (0.57, 1.19)	1.10 (0.85, 1.42)	0.67 (0.41, 1.11)
Wheezing pattern (early)	1.20 (0.88, 1.62)	1.05 (0.71, 1.55)	1.14 (0.85, 1.52)	0.87 (0.47, 1.58)
Wheezing pattern (late)	**2.65 (1.67, 4.20)**	1.23 (0.54, 2.80)	1.23 (0.68, 2.25)	**3.63 (1.71, 7.71)**
Wheezing pattern (persistent)	**2.67 (1.94, 3.69)**	1.27 (0.74, 2.17)	**1.89 (1.36, 2.65)**	**3.50 (2.03, 6.04)**

Values are pooled odds ratios with their 95% confidence intervals. All environmental exposure were entered simultaneously in the model. Reference groups are never eczema phenotype group (n=4,018), and multiparous, primary education, no parental history of eczema, allergy or asthma, female sex, never breastfeeding, no day care attendance, no pet exposure or never wheezing groups. Bold values indicate statistical significance at the α=0.05 level.

Table 3. The associations of genetic risk factors with eczema phenotypes and three separate sensitivity analysis investigating the associations between early environmental exposures, genetic risk factors and/or ethnicity and eczema phenotypes

	Early transient eczema	Mid-transient eczema	Late transient eczema	Persistent eczema
Genetic risk factor model, n=2,981	n=258	n=177	n=235	n=76
FLG genotype (≥ 1 mutations)	**2.21 (1.40, 3.49)**	1.52 (0.73, 3.15)	**2.09 (1.30, 3.34)**	1.68 (0.69, 4.09)
Genetic risk score (per additional allele)	**1.08 (1.03, 1.13)**	1.06 (0.99, 1.13)	1.02 (0.98, 1.06)	**1.09 (1.01, 1.18)**
Environmental exposure model and genetic risk factors, n=2,981	n=258	n=177	n=235	n=76
FLG genotype (≥ 1 mutations)	**2.21 (1.39, 3.50)**	1.56 (0.75, 3.24)	**2.02 (1.26, 3.24)**	1.80 (0.73, 4.47)
Genetic risk score (per additional allele)	**1.07 (1.02, 1.12)**	1.05 (0.99, 1.12)	1.01 (0.97, 1.06)	**1.09 (1.00, 1.18)**
Environmental exposure model in ethnic subgroups, n=5,297	n=439	n=306	n=412	n=122
Ethnicity (Mediterranean)	0.78 (0.48, 1.26)	0.44 (0.18, 1.05)	1.12 (0.72, 1.74)	1.10 (0.50, 2.40)
Ethnicity (Asian)	1.27 (0.78, 2.06)	1.66 (0.91, 3.01)	**1.83 (1.20, 2.80)**	**2.26 (1.11, 4.60)**
Ethnicity (African)	1.35 (0.89, 2.05)	1.11 (0.60, 2.06)	**1.49 (1.00, 2.23)**	**2.01 (1.06, 3.79)**
Environmental exposure and genetic model in ethnic subgroups, n=2,981	n=258	n=177	n=235	n=76
Ethnicity (Mediterranean)	0.99 (0.53, 1.86)	0.57 (0.19, 1.70)	1.41 (0.79, 2.49)	1.00 (0.35, 2.83)
Ethnicity (Asian)	1.43 (0.75, 2.73)	1.95 (0.88, 4.31)	**2.04 (1.14, 3.65)**	**3.08 (1.34, 7.10)**
Ethnicity (African)	1.35 (0.77, 2.37)	1.68 (0.82, 3.43)	1.43 (0.81, 2.50)	1.80 (0.77, 4.17)

Values are pooled odds ratios with their 95% confidence intervals. The genetic risk factor model was adjusted for ethnicity only. The environmental exposure model was mutually adjusted for all environmental exposures as presented in Table 1, and additionally adjusted for (1) genetic risk factors; (2) ethnic subgroups; and (3) for genetic risk factors and ethnicisubgroups. Reference groups are never eczema phenotype group, and European ethnicity, or no *FLG* mutation group. Effect estimates for the association of early environmental exposures with eczema phenotypes additionally adjusted for the genetic risk factors and ethnicities subgroups are shown in supplemental tables 4-6. Bold values indicate statistical significance at the α=0.05 level.

dren with late onset wheezing had higher risks for early transient and persistent eczema (2.65 (1.67-4.20) and 3.63 (7.71, 7.71), respectively) than children with never wheezing and never eczema. Children with persistent wheezing more often had early and late transient, and persistent eczema (2.67 (1.94,3.69), 1.89 (1.36,2.65), and 3.50 (2.03,6.04), respectively) than children with never wheezing and never eczema. No other significant associations of early environmental exposures with eczema phenotypes were observed.

Genetic risk factors and eczema phenotypes

Children with one or more *FLG* mutations had increased risks of early and late transient eczema (2.21 (1.40, 3.49) and 2.09 (1.30,3.34), respectively) compared with children without *FLG* mutations and never eczema (Table 3). Per additional risk allele in the genetic risk score, children had increased risks of early transient and persistent eczema (1.08 (1.03,1.13) and 1.09 (1.01, 1.18), respectively). The size and the direction of the effect estimates of the associations of genetic risk factors with eczema phenotypes remained similar when we additionally adjusted for all early environmental exposures. Also, the size and the direction of the effect estimates of the associations of early environmental exposures with eczema phenotypes remained similar, although some attenuated into non-significant, when we additionally adjusted for *FLG* genotype and the genetic risk score (Supplementary Table 4).

Ethnicity and eczema phenotypes

After dividing ethnicity into more detailed subgroups in our early environmental exposure model, we observed that Asian and African ethnicity were positively associated with late transient (1.83 (1.20,2.80) and 1.49 (1.00,2.23), respectively) and persistent eczema (2.26 (1.11,4.60) and 2.01 (1.06,3.79), respectively), compared with never eczema and European ethnicity (Table 3, Supplementary Table 5). When we additionally adjusted for genetic risk factors, only the associations of Asian ethnicity with an increased risk on late transient (2.04 (1.14,3.65)) and persistent eczema (3.08 (1.34,7.10)) remained (Table 3, Supplementary Table 6).

DISCUSSION

Five eczema phenotypes were identified in a multi-ethnic pediatric population followed from birth until age 10 years based on age of onset and persistence of eczema. Several known risk factors for eczema were associated with distinct phenotypes, but no clear patterns emerged suggesting that the previously known eczema risk factors have limited differentiating capacities for eczema phenotypes. Most of the associations were found in relation to early transient and persistent eczema. Early transient and persis-

tent eczema were most common in first born children, those with a parental history of eczema, allergy or asthma and those with persistent wheezing. Early transient eczema was most common in males only. Children with late transient or persistent eczema were more often of Asian and African ethnicity. Eczema phenotypes were not associated with maternal education, breastfeeding, day care attendance and pet exposure. Children with early and late transient and persistent eczema more often had a filaggrin mutation or additional risk alleles in the genetic risk score. Most effect estimates did not materially change when we mutually adjusted our analyses for both environmental and genetic factors. The explanation of why early transient and persistent eczema phenotypes share several determinants is not clear, but both patterns are dominant around the age of 1 year. This may be an important age in the exposure to environmental factors and the expression of genetic predisposition in the maturation of the skin and the immune system leading to the development of eczema.[32]

Comparison with previous literature

Never, early and late transient and persistent eczema showed a similar pattern as those identified in a cohort study among 1,038 children followed from birth until age 6 years.[16] Compared to a different study among 3,652 and 9,894 children followed from birth until age 11 and 16 years, higher early eczema probabilities were observed with more steeper resolving curves and no phenotype was identified with an onset after age 6 years.[15] The patterns were more similar when we used longitudinal latent class analysis. However, this analysis does not take repeated measurements into account, which we considered relevant in this study due to eczema measurements at different time intervals. The remaining discrepancy in number and pattern of phenotypes might be explained by the differences in follow-up time (10 versus 6 and 11-16 years), number of repeated eczema measurements (6 versus 7 and 10-12), eczema definition (physician-diagnosed-eczema versus itchy rash on specific locations) and population characteristics (multi-ethnic versus mostly European ethnicity).

The observations in this study supports that nulliparity, parental history of eczema, allergy and asthma, late onset and persistent wheezing, *FLG* genotype and the genetic risk score based on previously identified SNPs are risk factors for childhood eczema.[6, 12, 15, 16] The functions of many of these SNPs are not yet determined, but might be related to autoimmunity and skin barrier.[13] A previous study suggest that children of Surinamese-Creole and Surinamese-Hindustani origin have an increased risk of eczema.[23] In addition, this study showed that Asian and African children had an up to 3.6-fold increased risk of late transient and persistent eczema compared to European children. Possible underlying mechanisms include differences in skin barrier properties, parental psychological distress, microbiome development and other genetic factors.[11, 23, 33]

In contrast with literature, no associations were observed between breastfeeding and eczema phenotypes. However, most literature focused on the presence or absence of eczema and not distinct phenotypes or have used univariate analysis.[15] Even though favorable effects have been found between pet exposure and eczema, we did not found any effect between postnatal pet exposure eczema phenotypes.[8] This is in line with previous studies on eczema phenotypes.[15, 16] Differences with literature might be due to timing of the measurements, different distribution of risk factors or mild severity of included eczema cases as is illustrated by the low prevalence of *FLG* mutations in our multi-ethnic pediatric population.[12]

Interpretation of results

The number of eczema phenotypes is based on statistical fit and depends on clinical relevance. The three eczema phenotypes identified by latent class growth analysis might present a more useful model for clinical practice, because it makes a clearer distinction between transient and persistent eczema. All patients with eczema should receive optimal care, but it would be useful to identify children with a higher chance of developing persistent eczema, as they may well benefit from earlier more aggressive treatment.[34] For future studies, it would be clinically relevant to know whether specific eczema phenotypes are more prone to develop other atopic diseases such as asthma and/or food allergies in time. From an etiological point of view, it is important to better identify specific early life environmental exposures and genetic risk factors in the development and persistency of eczema. Sufficient number of cases of eczema and detailed information on endogenic factors are needed to compare immunological response, skin barrier defects and genetic predisposition in children with different eczema phenotypes.

Strengths and limitations

The strengths of this study include the prospective population-based design, multi-ethnic population with detailed information on eczema, early environmental exposures and genetic factors. Our eczema phenotypes model seems valid for a multi-ethnic population in an ever-globalizing world. Latent class growth analysis is an objective method to identify classes within a population. More precise and unbiased effect estimates are obtained by using multivariate multinomial models based on imputed data. However, some methodological limitations need to be considered in this study. We assumed that data was missing at random. Missing not at random is always a possibility, which might lead to biased estimates.[34] Including children with at least 50% of the data observed ensures a more reliable model. Selection bias might be present if the associations of the selected environmental and genetic factors with eczema phenotypes were different between children included and not included in the analyses. Furthermore, we cannot rule out underreporting or misreporting of eczema. There is, however, evidence that

parental-reported physician-diagnosed eczema is sufficient for epidemiological research and eczema prevalence in our study is similar to that of the Dutch population.[35, 36] No information was available to determine the severity of eczema or subtypes of eczema, which could have influenced the observed effect estimates and associations. Residual confounding might exist. Also a longer follow-up period could influence the number and pattern of eczema phenotypes. The uncertainty of class assignment of children is only partially accounted for by using weights in the multinomial analyses. Moreover, the results were not adjusted for multiple testing. We extracted the SNPs from the most recent GWA study including non-European populations, still most of the genetic risk factors, including *FLG* mutations, have been discovered in European populations.[13] Although early transient and persistent eczema appear to share several determinants, most of the selected environmental and genetic factors did not strongly differentiate between the various eczema phenotypes. This may partly be explained by minimizing the number of categories per factor in order to maximize the number of factors to maintain appropriate statistical models. Future large-scale studies examining early life environmental exposures in more detail could provide better differentiation between eczema phenotypes.

Conclusion

Five eczema phenotypes were identified in a multi-ethnic pediatric cohort followed from birth until the age of 10 years. Previously known eczema risk factors differentiated between the different phenotypes to a limited degree. Male sex and Asian and African ethnicity were differently associated with eczema phenotypes and therefore could be useful for prediction purposes. Further studies are needed to compare the trajectories of different eczema phenotypes and identify other potential predictive factors, ideally in a 'hypothesis free approach' because known risk factors are relatively poor discriminators, in order to improve eczema management and prevention strategies.

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Supplementary Table 1. Characteristics of children and their mothers of those included and not included in the analyses

	Included n=5,297	Not included n=2,596	P-value for difference
Parental characteristics			
Parity % (n)			
Nulliparous	59 (3,019)	48 (1,156)	<0.001
Multiiparous	41 (2,134)	52 (1,271)	
Maternal education % (n)			
Primary or secondary	43 (2,166)	79 (1,494)	<0.001
Higher	58 (2,925)	21 (406)	
History of eczema, allergy or asthma % (n)			
No	50 (2,528)	58 (1,120)	<0.001
Yes, at least one parent	50 (2,539)	42 (802)	
Child characteristics			
Sex % (n)			
Male	50 (2,632)	52 (1,350)	0.052
Female	50 (2,665)	48 (1,244)	
Ethnicity % (n)			
European	74 (3,920)	41 (891)	<0.001
Non-European	26 (1,347)	59 (1.291)	
Breastfeeding % (n)			
Never	8 (395)	10 (164)	0.003
Ever	92 (4,756)	90 (1,468)	
Day care attendance % (n)			
No	40 (1,683)	68 (274)	<0.001
Yes	60 (2,556)	32 (130)	
Pet exposure % (n)			
No	66 (3,119)	74 (748)	<0.001
Yes	34 (1,614)	26 (260)	
Wheezing pattern % (n)			
Never	50 (1,600)	4 (1)	<0.001
Early	29 (929)	15 (4)	
Late	5 (143)	0 (0)	
Persistent	16 (511)	82 (22)	
FLG genotype % (n)			
No mutation	92 (2,864)	96 (1,235)	<0.001
≥ 1 mutations	8 (247)	4 (54)	
Genetic risk score(mean (SD))	31 (3.5)	32 (3.3)	0.030

Values are percentages (absolute numbers) or means (SD) based on observed data. Observed characteristics of not included children were missing for parity (7% (n=169)), maternal education (27%(n=696)), parental history of eczema, allergy or asthma (26% (n=674)), ethnicity (16% (n=414)), breastfeeding (37% (n=964)), day care attendance (84% (n=2,192)), pet exposure (61% (n=1,588)) and wheezing pattern (99% (n=2,569)), *FLG* genotype (50% (n=1,307)), genetic risk score (47% (n=1,227)).

Supplementary Table 2. Model fit after latent class growth analysis

	Individuals with complete data (6/6 time points) (n=1.975)						Individuals with incomplete data (≥3/6 time points) (n=5.297)					
Class	BIC	AIC	BLRT p-value	VLMR LRT p-value	Entropy	Minimal n per class	BIC	AIC	BLRT p-value	VLMR LRT p-value	Entropy	Minimal n per class
2	7113.499	7074.381	<0.0001	<0.0001	0.746	354	16 429.93	16 383.91	<0.0001	<0.0001	0.734	747
3	7090.430	7028.959	<0.0001	0.0001	0.810	116	16 350.97	16 278.64	<0.0001	<0.0001	0.774	311
4	7101.697	7017.872	<0.0001	0.0040	0.852	40	16 331.61	16 232.99	<0.0001	<0.0001	0.573	226
5	**7112.813**	**7006.635**	**<0.0001**	**0.0483**	**0.628**	**47**	**16 310.54**	**16 185.61**	**<0.0001**	**<0.0001**	**0.678**	**122**
6	7137.288	7008.756	0.6667	0.0253	0.652	10	16 334.53	16 183.31	1.0000	0.0400	0.635	46
7	7160.502	7009.617	0.1429	0.2284	0.652	5	16 357.07	16 179.55	1.0000	0.0575	0.603	34

Growth factors used in shown model: intercept(I), slope (S), quadratic term (Q). Abbreviation used: BIC (Bayesian information criterion), AIC (Akaike information criterion), BLRT (bootstrap likelihood ratio test), VLMR LRT (Vuong-Lo-Mendel-Rubin likelihood ratio test). Bold values represent the best model fit.

Supplementary Table 3. Model fit after longitudinal latent class analysis

	Individuals with complete data (6/6 time points) (n=1,975)						Individuals with incomplete data (≥3/6 time points) (n=5,297)					
Class	BIC	AIC	BLRT p-value	VLMR LRT p-value	Entropy	Minimal n per class	BIC	AIC	BLRT p-value	VLMR LRT p-value	Entropy	Minimal n per class
2	7136.274	7063.626	<0.0001	<0.0001	0.752	303	16425.149	16339.676	<0.0001	<0.0001	0.739	758
3	**7124.276**	**7012.509**	**<0.0001**	**0.0059**	**0.795**	**91**	**16345.156**	**15877.547**	**<0.0001**	**<0.0001**	**0.776**	**349**
4	7157.705	7006.820	<0.0001	0.2098	0.685	63	16350.790	16173.267	<0.0001	0.0043	0.715	131
5	7199.320	7009.317	0.3750	0.6155	0.792	10	16399.507	16175.961	0.5000	0.0500	0.764	35
6	7244.461	7015.339	0.4286	0.2092	0.803	9	16448.139	16178.568	0.2083	0.1070	0.711	34
7	7291.981	7023.742	0.6000	0.0923	0.752	8	16502.115	16186.520	1.0000	1.0000	0.841	14

Abbreviation used: BIC (Bayesian information criterion), AIC (Akaike information criterion), BLRT (bootstrap likelihood ratio test), VLMR LRT (Vuong-Lo-Mendel-Rubin likelihood ratio test). Bold values represent the best model fit.

Supplementary Table 4. Associations of early environmental and genetic factors with eczema phenotypes

	Early transient eczema	Mid-transient eczema	Late transient eczema	Persistent eczema
	n=258	n=177	n=235	n=76
Parity (nulliparous)	1.33 (0.97, 1.83)	1.41 (0.90, 2.21)	1.05 (0.78, 1.42)	**2.10 (1.18, 3.75)**
Maternal education (higher)	1.10 (0.78, 1.56)	1.11 (0.68, 1.82)	1.04 (0.74, 1.47)	0.65 (0.36, 1.17)
Parental history of eczema, allergy or asthma (yes)	**1.79 (1.20, 2.66)**	1.21 (0.76, 1.92)	1.36 (0.95, 1.94)	1.78 (0.89, 3.55)
Sex (male)	**1.42 (1.04, 1.93)**	1.07 (0.70, 1.64)	0.83 (0.61, 1.12)	1.43 (0.84, 2.43)
Ethnicity (non-European)	1.16 (0.79, 1.71)	1.38 (0.82, 2.34)	**1.51 (1.05, 2.18)**	**1.84 (1.01, 3.36)**
Breastfeeding (ever)	0.81 (0.47, 1.39)	0.82 (0.37, 1.85)	0.77 (0.44, 1.35)	0.59 (0.25, 1.38)
Day care attendance (yes)	1.03 (0.72, 1.46)	1.18 (0.71, 1.95)	1.27 (0.89, 1.82)	1.10 (0.59, 2.08)
Pet exposure (yes)	0.95 (0.68, 1.32)	0.79 (0.49, 1.27)	1.00 (0.72, 1.39)	0.62 (0.33, 1.17)
Wheezing pattern (early)	1.26 (0.86, 1.84)	1.13 (0.69, 1.87)	0.90 (0.62, 1.31)	1.14 (0.53, 2.43)
Wheezing pattern (late)	**2.17 (1.15, 4.09)**	1.52 (0.59, 3.94)	1.43 (0.67, 3.06)	**4.47 (1.84, 10.88)**
Wheezing pattern (persistent)	**2.13 (1.36, 3.32)**	1.16 (0.57, 2.40)	1.39 (0.87, 2.23)	**3.64 (1.74,7.60)**
FLG genotype (≥ 1 mutations)	**2.21 (1.39, 3.50)**	1.56 (0.75, 3.24)	**2.02 (1.26, 3.24)**	1.80 (0.73, 4.47)
Genetic risk score (per additional allele)	**1.07 (1.02, 1.12)**	1.05 (0.99, 1.12)	1.01 (0.97, 1.06)	**1.09 (1.00, 1.18)**

Values are pooled odds ratios with their 95% confidence intervals. Model was mutually adjusted for all environmental exposures and genetic risk factors. *FLG* genotype and genetic risk score were not imputed. Reference groups are never eczema phenotype group (n=2,235), and multiparous, primary education, no parental history of eczema, allergy or asthma, female sex, European ethnicity, never breastfeeding, no day care attendance, no pet exposure, never wheezing or no *FLG* mutation. Bold values indicate statistical significance at the α=0.05 level.

Supplementary Table 5. Associations of early environmental and eczema phenotypes with ethnicity subdivision

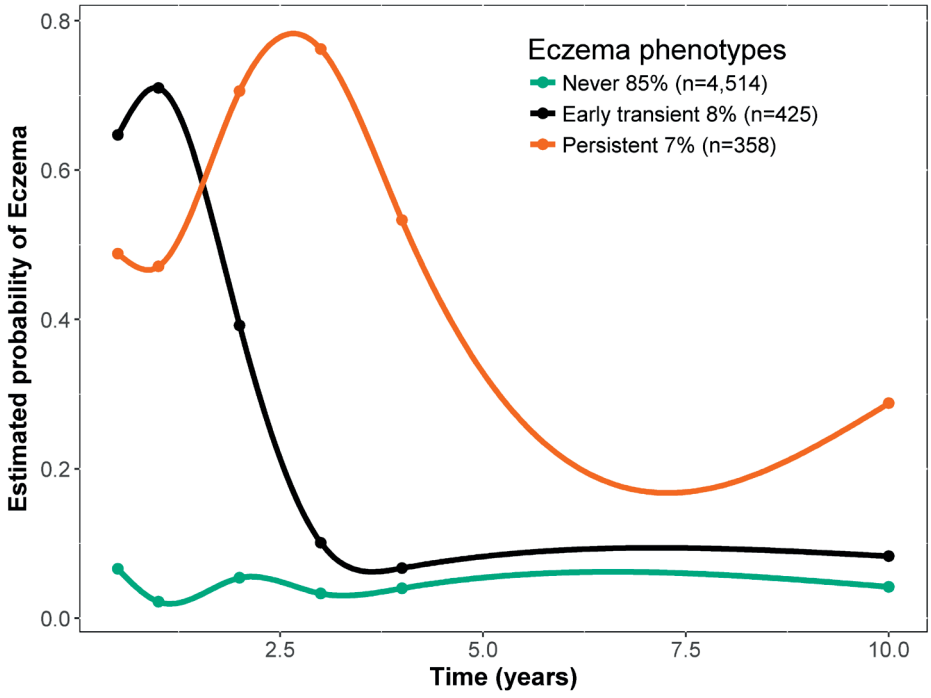
	Early transient eczema	Mid-transient eczema	Late transient eczema	Persistent eczema
	n=439	n=306	n=412	n=122
Parity (nulliparous)	**1.36 (1.07, 1.74)**	1.35 (0.96, 1.90)	1.11 (0.88, 1.40)	**1.61 (1.03, 2.49)**
Maternal education (higher)	1.05 (0.80, 1.36)	1.04 (0.72, 1.52)	1.05 (0.81, 1.36)	0.68 (0.42, 1.09)
Parental history of eczema, allergy or asthma(yes)	**1.71 (1.29, 2.26)**	1.12 (0.78, 1.61)	1.21 (0.94, 1.56)	**1.90 (1.10, 3.28)**
Sex (male)	**1.49 (1.18, 1.89)**	0.98 (0.70, 1.35)	0.83 (0.66, 1.04)	1.21 (0.80, 1.84)
Ethnicity (Mediterranean)	0.78 (0.48, 1.26)	0.44 (0.18, 1.05)	1.12 (0.72, 1.74)	1.10 (0.50, 2.40)
Ethnicity (Asian)	1.27 (0.78, 2.06)	1.66 (0.91, 3.01)	**1.83 (1.20, 2.80)**	**2.26 (1.11, 4.60)**
Ethnicity (African)	1.35 (0.89, 2.05)	1.11 (0.60, 2.06)	**1.49 (1.00, 2.23)**	**2.01 (1.06, 3.79)**
Breastfeeding (ever)	0.90 (0.58, 1.39)	0.87 (0.48, 1.60)	0.90 (0.59, 1.39)	0.70 (0.35, 1.43)
Day care attendance (yes)	1.04 (0.79, 1.39)	1.15 (0.78, 1.68)	1.18 (0.90, 1.55)	1.43 (0.86, 2.38)
Pet exposure (yes)	0.88 (0.68, 1.14)	0.80 (0.55, 1.15)	1.10 (0.86, 1.40)	0.65 (0.40, 1.06)
Wheezing pattern (early)	1.21 (0.89, 1.64)	1.04 (0.70, 1.54)	1.12 (0.85, 1.48)	0.90 (0.50, 1.61)
Wheezing pattern (late)	**2.63 (1.65, 4.19)**	1.25 (0.56, 2.78)	1.18 (0.63, 2.24)	**3.67 (1.71, 7.90)**
Wheezing pattern (persistent)	**2.68 (1.94, 3.71)**	1.29 (0.75, 2.20)	**1.91 (1.37, 2.66**)	**3.59 (2.11, 6.10)**

Values are pooled odds ratios with their 95% confidence intervals. Model was mutually adjusted for all environmental exposures. Reference groups are never eczema phenotype group (n=4,018), and multiparous, primary education, no parental history of eczema, allergy or asthma, female sex, European ethnicity, never breastfeeding, no day care attendance, no pet exposure or never wheezing. Bold values indicate statistical significance at the α=0.05 level.

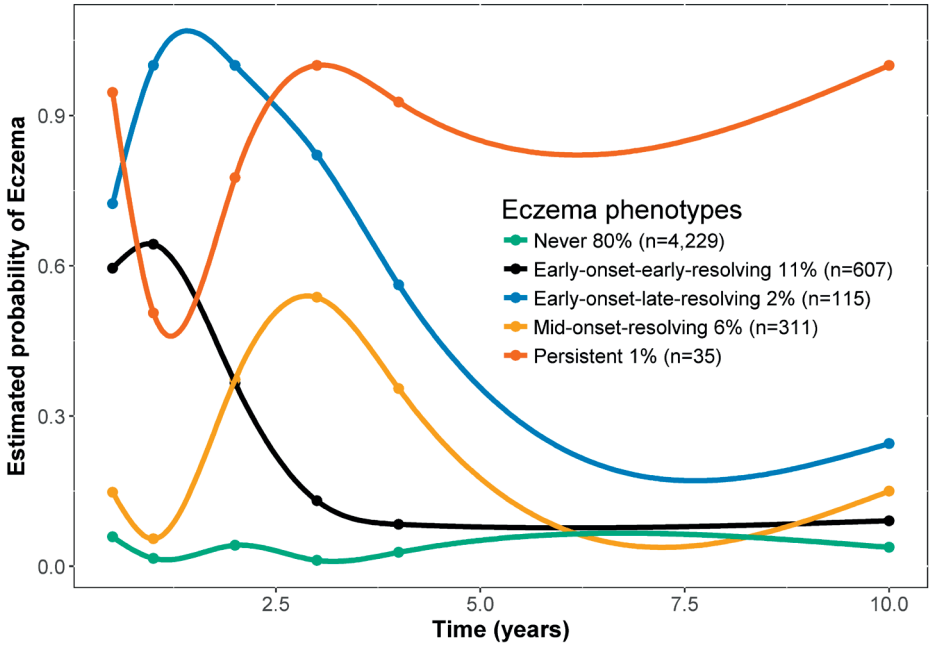
Supplementary Table 6. Associations of early environmental and genetic factors with eczema phenotypes with ethnicity subdivision

	Early transient eczema	Mid-transient eczema	Late transient eczema	Persistent eczema
	n=258	n=177	n=235	n=76
Parity (nulliparous)	1.32 (0.96, 1.81)	1.36 (0.86, 2.13)	1.04 (0.77, 1.41)	**1.99 (1.11, 3.56)**
Maternal education (higher)	1.08 (0.76, 1.53)	1.05 (0.64, 1.71)	1.04 (0.73, 1.47)	0.59 (0.33, 1.07)
Parental history of eczema, allergy or asthma (yes)	**1.76 (1.22, 2.56)**	1.21 (0.75, 1.95)	1.32 (0.95, 1.85)	1.68 (0.86, 3.28)
Sex (male)	**1.42 (1.04, 1.93)**	1.07 (0.69, 1.63)	0.83 (0.61, 1.12)	1.45 (0.85, 2.46)
Ethnicity (Mediterranean)	0.99 (0.53, 1.86)	0.57 (0.19, 1.70)	1.41 (0.79, 2.49)	1.00 (0.35, 2.83)
Ethnicity (Asian)	1.43 (0.75, 2.73)	1.95 (0.88, 4.31)	**2.04 (1.14, 3.65)**	**3.08 (1.34, 7.10)**
Ethnicity (African)	1.35 (0.77, 2.37)	1.68 (0.82, 3.43)	1.43 (0.81, 2.50)	1.80 (0.77, 4.17)
Breastfeeding (ever)	0.82 (0.47, 1.41)	0.87 (0.39, 1.95)	0.79 (0.45, 1.38)	0.64 (0.27, 1.49)
Day care attendance (yes)	1.02 (0.71, 1.47)	1.10 (0.67, 1.82)	1.28 (0.89, 1.84)	1.12 (0.60, 2.11)
Pet exposure (yes)	0.95 (0.68, 1.32)	0.76 (0.47, 1.22)	1.01 (0.73, 1.40)	0.59 (0.32, 1.10)
Wheezing pattern (early)	1.29 (0.89, 1.87)	1.13 (0.69, 1.86)	0.91 (0.63, 1.33)	1.14 (0.55, 2.38)
Wheezing pattern (late)	**2.18 (1.14, 4.16)**	1.56 (0.60, 4.03)	1.48 (0.75, 2.93)	**4.59 (1.89, 11.17)**
Wheezing pattern (persistent)	**2.10 (1.34, 3.32)**	1.20 (0.58, 2.49)	1.44 (0.91, 2.29)	**3.77 (1.88, 7.58)**
FLG genotype (≥1 mutations)	**2.22 (1.40, 3.52)**	1.54 (0.74, 3.20)	**2.04 (1.27, 3.28)**	1.78 (0.72, 4.43)
Genetic risk score (per additional allele)	**1.07 (1.02, 1.12)**	1.05 (0.99, 1.12)	1.02 (0.97, 1.06)	**1.09 (1.01, 1.18)**

Values are pooled odds ratios with their 95% confidence intervals. Model was mutually adjusted for all environmental exposures and genetic risk factors. *FLG* genotype and genetic risk score were not imputed. Reference groups are never eczema phenotype group (n=2,235), and multiparous, primary education, no parental history of eczema, allergy or asthma, female sex, European ethnicity, never breastfeeding, no day care attendance, no pet exposure, never wheezing or no *FLG* mutation. Bold values indicate statistical significance at the α=0.05 level.



Supplementary Figure 1a. Three eczema phenotypes after longitudinal latent class analysis



Supplementary Figure 1b. Five eczema phenotypes after longitudinal latent class analysis

Further detailed online resources can be found in the published article online:
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2.2

Bacterial nasal and nasopharyngeal carriage, and eczema phenotypes

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Chen Hu
Liesbeth Duijts
Evelien R. van Meel
Kirsten I.M. Looman
Jessica C. Kiefte-de Jong,
Luba M. Pardo
DirkJan Hijnen
Suzanne G.M.A. Pasmans
Johan C. de Jongste
Henriette A. Moll
Tamar Nijsten

ABSTRACT

Background An association has been reported between early life *Staphylococcus aureus* nasal carriage and higher risk of childhood eczema, but it is unclear whether this relationship is causal and associations with other bacterial species are unclear.

Objective To examine the associations of early life nasal and nasopharyngeal bacterial carriage with eczema phenotypes, and the direction of any associations identified.

Methods Among 996 subjects of a population-based prospective cohort study, nasal swabs for *Staphylococcus aureus*, and nasopharyngeal swabs for *Streptococcus pneumoniae, Moraxella catarrhalis* and *Haemophilus influenzae* were collected and cultured from age 6 weeks to 6 years. Never, early, mid-, late transient and persistent eczema phenotypes were identified from parental-reported physician-diagnosed eczema from age 6 months until 10 years. Multinomial regression models and cross-lagged models were applied.

Results *Staphylococcus aureus* nasal carriage at 6 months was associated with an increased risk of early transient and persistent eczema (OR (95% CI): 2.69 (1.34, 5.39) and 4.17 (1.12, 15.51)). The associations between *Staphylococcus aureus* nasal carriage and eczema were mostly cross-sectional, and not longitudinal. No associations of *Streptococcus pneumoniae, Moraxella catarrhalis* and *Haemophilus influenza* nasopharyngeal bacterial carriage with eczema and eczema phenotypes were observed (OR range (95% CI): 0.71 (0.35, 1.44) to 1.77 (0.84, 3.73)).

Conclusions Early life *Staphylococcus aureus* nasal carriage, but not *Streptococcus pneumoniae, Moraxella catarrhalis* and *Haemophilus influenza* nasopharyngeal carriage, was associated with early transient and persistent eczema. *Staphylococcus aureus* nasal carriage and eczema were mostly cross-sectionally associated, and not longitudinally, making a causal relationship in either direction unlikely.

INTRODUCTION

Childhood eczema is a common chronic skin disorder with variable age of onset and persistence.[1] We previously identified eczema phenotypes taking into account the variability of eczema onset and persistence within and between individuals over time. [2] The use of eczema phenotypes, instead of the simplified dichotomous outcome of eczema, might better reflect the natural course of eczema and help understand their specific underlying risk factors. Both genetic and environmental factors seem to influence the development and persistency of eczema.[3] Additionally, bacterial carriage of the main commensals *Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pneumoniae* in the nasal cavity and nasopharynx was suggested to be associated with eczema.[4, 5] The nasal and nasopharyngeal area may function as important reservoirs for bacteria to spread to different body sites. In addition, competitive and cooperative inter-bacterial, and host-bacterial interactions affect the microbial colonization dynamics and the priming of the host's immune responses, and thereby altering the susceptibility of developing atopic diseases.[6] A previous meta-analysis of mainly hospital-based cohorts showed that nasal carriage of *S. aureus* was associated with an increased risk of eczema in children and adults.[4] We previously showed in a population-based cohort that early life nasal carriage of *S. aureus* was associated with increased risk of eczema and eczema severity in children aged 1-2 years, but persistent effects at older ages were not clear.[7] Also *Haemophilus influenzae, Moraxella,* and *Streptococcus pneumoniae* in the nasopharynx are suggested to be associated with increased risk of eczema.[5, 8, 9] However, studies only used vaccinations against *Haemophilus influenzae* and *Streptococcus pneumoniae*, not bacterial carriage, and were performed in hospital-based or adult populations.[5, 8, 9] Furthermore, it remains unclear whether bacterial nasal and/or nasopharyngeal carriage leads to increased risk of the development of eczema, is a consequence of eczema, or occurs simultaneously with eczema due to other mechanisms.[10]

Therefore, we aimed to examine the associations of early life bacterial nasal and nasopharyngeal carriage with eczema phenotypes from birth until age 10 years among 996 subjects of a population-based prospective cohort study. Next, we aimed to disentangle whether the direction of associations was from bacterial nasal and nasopharyngeal carriage leading to an increased risk of eczema or reversely.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands.[11, 12] The study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre in Rotterdam (MEC 198.782/2001/31; MEC 217.595/2002/202; MEC-2007-413; and MEC-2012-165). Written informed consent was obtained from all participants. A total of 996 children were included for the current analysis (Figure 1).

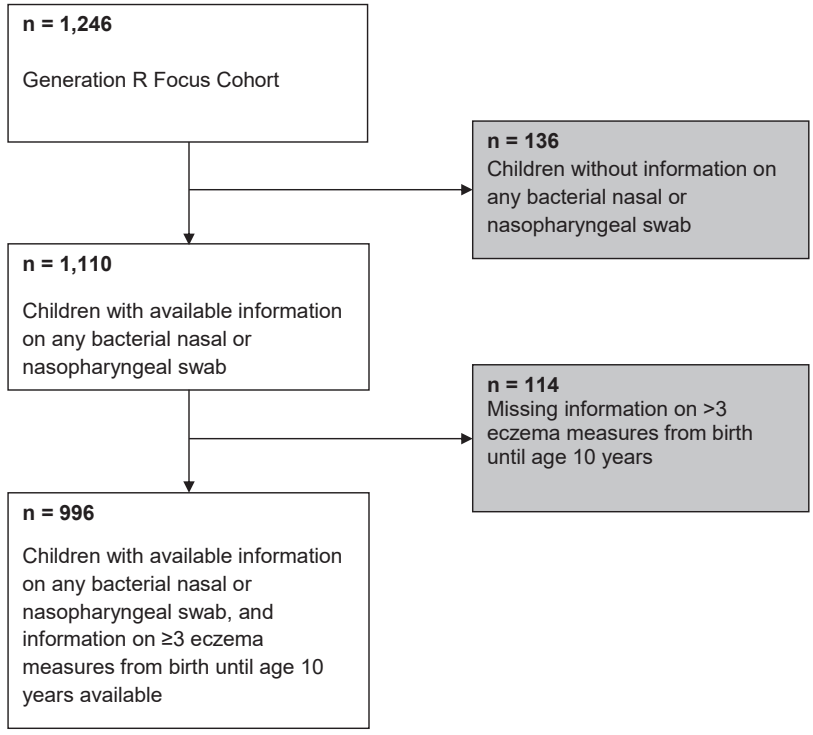

Figure 1. Flow chart of participants included for analysis

Bacterial nasal and nasopharyngeal carriage

Swabs of the nose and nasopharynx area were taken by trained research nurses at the research center at ages 6 weeks, 6 and 14 months, and 2, 3 and 6 years, as previously described.[13, 14] For this, sterile transport swabs with liquid Amies medium were used. Nasal swabs were put in phenol red mannitol broth at 35 ^{0}C for 5 days. Material from tubes that turned yellow were plated on a blood agar plate with 5% sheep blood at 35 ^{0}C for 1 day to isolate *Staphylococcus aureus*. Nasopharyngeal swabs were plated on a *Haemophilus*

selective agar plate, a blood agar plate with 5% sheep blood, and a chocolate agar plate for *Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae,* respectively. The plates were kept at 35 ^{0}C in a CO_2 rich environment for 2 days, and assessed daily for growth of bacteria. Swabs were classified as either negative or positive for *S. aureus, H. influenzae, M. catarrhalis* or *S. pneumoniae.* Nasopharyngeal carriage with any bacteria was classified as positive when one of the bacteria *H. influenzae, M. catarrhalis* or *S. pneumoniae* was positive, and negative if all three were negative. Additionally, to study the bacterial nasopharyngeal carriage in detail, sensitivity analysis was performed on each of the three bacteria separately.

Eczema phenotypes

Information on physician-diagnosed eczema was obtained from parental-reported questionnaires at the ages of 6 months, and 1, 2, 3, 4 and 10 years ('Was your child diagnosed with eczema in the last 6 months/last year by a general practitioner or physician in the hospital?') (no; yes). In children with available data on physician-diagnosed eczema on at least 3 time points, we previously identified five eczema phenotypes (never, early transient, mid-transient, late transient and persistent eczema) using latent class growth analysis, in which missing data is handled by maximum likelihood algorithm.[2] Subjects were assigned to the eczema phenotype for which they had the highest posterior probability. Children with early, mid- and late transient eczema had a high probability of developing eczema at approximately the age of 6 months, 2 years and 5 years, respectively, after which the eczema gradually declines. Children with persistent eczema had a high probability of eczema from birth until the age of 10 years. Ever eczema included those with early transient, mid-transient, late transient or persistent eczema. Due to relative low number of subjects in the persistent eczema phenotype in the five eczema phenotypes model, we performed sensitivity analyses with stronger statistical stability using three eczema phenotypes (never, early-mid transient, and late-persistent phenotype).

Covariates

Information on pet keeping and maternal psychiatric symptoms using the Global Severity Index (GSI) was obtained by questionnaires during pregnancy.[15] The mode of delivery was obtained from midwives and hospital records. Postnatal questionnaires provided information on daycare attendance, and antibiotic use in the first year after birth.

Statistical analysis

We compared characteristics of those included and not included in our study using Pearson's Chi-square and Mann-Whitney U tests. First, we examined the associations of bacterial nasal and nasopharyngeal carriage with ever eczema and with five eczema phenotypes from birth until age 10 years using logistic and weighted multinomial regres-

sion models, respectively. Weights were based on class probabilities. Next, cross-lagged models were used to examine bidirectional associations of bacterial nasal carriage with eczema from birth until 10 years. Cross-lagged models allow associations between two repeatedly measured variables to be examined in both directions simultaneously while accounting for continuity between the repeated measures over time. A conceptual model of the studied cross-lagged associations is presented in Supplementary Figure 1. For example, the effect estimates of the association of bacterial carriage at earlier age with eczema at later age will be adjusted for all earlier associations between and within bacterial carriages and eczema. With this method, we aimed to disentangle the predominant direction of the observed association between bacterial nasal carriage and eczema. We examined cross-lagged effects, cross-sectional effects, and stability effects in the period from birth until age 3 years, and only cross-lagged and stability effect in the period from 4 until 10 years due to the uneven distribution of repeated measures of bacterial nasal carriage and eczema at those ages. As a sensitivity analyses for increased statistical power, we applied generalized estimating equation (GEE) models with an unstructured and autoregressive correlation matrix to examine the associations between bacterial nasal and nasopharyngeal carriage at age 6 weeks with repeated measures of eczema from 6 months until 10 years. All analyses were adjusted for potential confounders, which were first selected from literature including known potential underlying biological mechanisms.[2, 16-18] Next, confounders were selected if they were associated with both the exposure (bacterial nasal/nasopharyngeal carriage) and the outcome (eczema phenotypes), and were not within the causal pathway based on epidemiological concept. Additionally, they were included if they changed the effect estimates or the unadjusted analyses with ≥10% in adjusted analyses. Family history of atopic diseases, maternal age, parity and education, and child's gestational age, birth weight, sex, and breastfeeding did not meet our defined statistical criteria of confounding, and therefore, were not included in the models. For better interpretation we adjusted all analyses models for the same confounders. We assumed that data were missing at random. Twenty datasets were created to handle missing data in covariates (≤12%) using multiple imputation by chained equations. Missing data in bacterial nasal and nasopharyngeal carriage and eczema was not imputed. The size and direction of the effect estimates were similar when we used complete-case-analyses, and therefore, we only present the results based on imputed data. We did not adjust for multiple testing in the main analyses (nasal carriage with *S. aureus* and nasopharyngeal carriage with any bacteria), because the bacterial carriages were examined under the same hypothesis. For the sensitivity analysis of the separate nasopharyngeal bacteria, we corrected for multiple testing using alpha 0.05 divided by the effective independent number of tests calculated based on the correlation structure between the bacteria.[19] All measures of association are presented as odds ratios (OR) together with their corresponding 95%

confidence intervals (95%CI). Imputation and regression analyses were performed using the packages 'mice' (version 3.6.0), 'stats' (version 3.6.1.) and 'nnet' (version 7.3.12), cross-lagged analyses were performed in Mplus (version 8.2), and using package 'MplusAutomation' (version 0.7-3), and GEE analyses were performed using the package 'geepack' (version 1.2-1) in R version 3.6.1.[20-25]

RESULTS

Subject characteristics

Characteristics of children and their mothers are shown in Table 1. Compared with children included in the analysis, those not included had mothers who had more psychiatric symptoms during pregnancy (Supplementary Table 1). The number of children eligible for inclusion during follow-up were 1,190 children at ages 6 weeks to 4 years, 1,166 children at age 5 years, and 1,109 at age 10 years. Physician-diagnosed eczema ranged from 13.4% at age 6 months to 6.0% at age 10 years (Supplementary Table 1).

Table 1. Characteristics of children and their mothers (n = 996)

	All (n=996)	**Never eczema** (n=768)	**Ever eczema** (n= 228)
Maternal characteristics			
Pet keeping, yes % (n)	43.1 (429)	42.4 (326)	44.3 (101)
Pyschiatric symptoms, median (IQR)	0.12 (0.06, 0.23)	0.10 (0.06, 0.21)	0.13 (0.04, 0.25)
Mode of delivery % (n)			
Vaginal	85.1 (848)	86.4(664)	80.9 (184)
Primary caesarian section	6.6 (66)	6.0 (46)	8.3 (19)
Secondary caesarian section	8.2 (82)	7.6 (58)	10.9 (25)
Childrens characteristics			
Daycare attendance, yes % (n)	68.5 (682)	68.8 (525)	67.1 (153)
Antibiotic use, yes % (n)	35.7 (356)	34.7 (266)	38.6 (88)
S. aureus carriage, yes % (n/total n)*			
Age 6 weeks	52.7 (302/573)	51.9 (232)	55.6 (70)
Age 6 months	20.9 (150/718)	18.2 (101)	30.2 (49)
Age 1 year	14.5 (98/676)	14.9 (78)	13.0 (20)
Age 2 years	13.4 (79/590)	12.1 (54)	17.4 (25)
Age 3 years	14.7 (89/604)	13.5 (62)	18.5 (27)
Age 6 years	28.0 (238/850)	26.3 (173)	34.0 (65)
Nasopharyngeal carriage with any bacteria, yes % (n/total n) *			
Age 6 weeks	22.9 (131/573)	21.9 (98)	26.2 (33)

Table 1. Characteristics of children and their mothers (n = 996) (continued)

	All (n=996)	**Never eczema** (n=768)	**Ever eczema** (n= 228)
Age 6 months	61.1 (438/717)	60.8 (338)	62.1 (100)
Age 1 year	67.0 (453/676)	67.4 (352)	65.6 (101)
Age 2 years	63.6 (375/590)	63.5 (283)	63.9 (92)
Age 3 years	50.0 (302/604)	50.0 (229)	50.0 (73)
Age 6 years	36.9 (314/850)	36.6 (241)	38.2 (73)
Eczema phenotypes, % (n)*			
Never eczema	77.1 (768)	100.0 (768)	0.0 (0)
Early transient eczema	7.5 (75)	0.0 (0)	32.9 (75)
Mid-transient eczema	6.7 (67)	0.0 (0)	29.4 (67)
Late transient eczema	7.1 (71)	0.0 (0)	31.1 (71)
Persistent eczema	1.5 (15)	0.0 (0)	6.6 (15)

Values are means (SD), valid percentages (absolute numbers) or medians (95% range) based on imputed data. *Nasal carriage of *S. aureus* and nasopharyngeal carriage with any bacteria (*H. influenzae, M. catarrhalis* or *S. pneumoniae*) were not imputed, and were missing (%) for the following ages: 43% at 6 weeks, 28% at 6 months, 32% at 1 year, 41% at 2 years, 39% at 3 years and 15% at 6 years. Eczema phenotypes had no missing values.

Early life bacterial nasal and nasopharyngeal carriage and eczema phenotypes

Compared with never eczema and no nasal carriage of *S. aureus*, nasal carriage of *S. aureus* at age 6 months was associated with an increased risk of ever eczema (OR (95% CI): 2.01 (1.33, 3.02)); Table 2). Nasal carriage of *S. aureus* at other ages, and nasopharyngeal carriage with any bacteria was not associated with ever eczema. When examining eczema phenotypes, nasal carriage of *S. aureus* at age 6 months was associated with an increased risk of early transient and persistent eczema, compared with no nasal carriage of *S. aureus* and never eczema phenotype (OR (95% CI): 2.69 (1.34, 5.39) and 4.17 (1.12, 15.51), respectively) (Table 2). Nasal carriage of *S. aureus* at ages 6 weeks, and 1,2 3, and 6 years were not associated with eczema phenotypes. Similar size and direction of estimates were observed when using the regrouped three eczema phenotypes, although the associations of nasal carriage of *S. aureus* at age 6 months with late-persistent eczema attenuated into non-significant (Supplementary Table 2). We found no associations of nasopharyngeal carriage with any bacteria between the ages of 6 weeks and 6 years with eczema phenotypes (Table 2). When we studied the nasopharyngeal carriage with *H. influenzae, M. catarrhalis* and *S. pneumoniae* separately in a sensitivity analyses, only nasopharyngeal carriage of *H. influenzae* at age 6 months was associated with an increased risk of early transient eczema phenotype (2.09 (1.03, 4.24)) (Supplementary Table 3). This association attenuated to non-significant after correcting for multiple testing. We observed no associations of nasal carriage of *S. aureus* or nasopharyngeal car-

Table 2. Associations of bacterial nasal and nasopharyngeal carriage with ever eczema and eczema phenotypes

	Ever eczema Odds ratio (95% Confidence Interval) (n=228)	**Never eczema Odds ratio (95% Confidence Interval)** (n=768)	**Early transient eczema Odds ratio (95% Confidence Interval)** (n=75)	**Mid-transient eczema Odds ratio (95% Confidence Interval)** (n=67)	**Late transient eczema Odds ratio (95% Confidence Interval)** (n=71)	**Persistent eczema Odds ratio (95% Confidence Interval)** (n=15)
***S. aureus* carriage**						
Age 6 weeks	1.21 (0.80 ,1.84)	Reference	1.42 (0.66, 3.09)	1.59 (0.57, 4.46)	0.93 (0.47, 1.85)	0.82 (0.16, 4.04)
Age 6 months	2.01 (1.33, 3.02)**	Reference	2.69 (1.34, 5.39)**	1.90 (0.74, 4.91)	1.48 (0.69, 3.21)	4.17 (1.12, 15.51)*
Age 1 year	0.88 (0.51, 1.52)	Reference	0.82 (0.30, 2.24)	0.97 (0.28, 3.42)	0.69 (0.23, 2.07)	1.14 (0.21, 6.38)
Age 2 years	1.64 (0.96, 2.77)	Reference	1.29 (0.50, 3.37)	2.02 (0.67, 6.06)	1.20 (0.43, 3.35)	2.08 (0.30, 14.15)
Age 3 years	1.57 (0.94, 2.61)	Reference	1.68 (0.66, 4.24)	1.64 (0.53, 5.07)	1.20 (0.48, 2.99)	2.25 (0.35, 14.60)
Age 6 years	1.40 (0.98, 2.00)	Reference	1.33 (0.68, 2.6)	1.22 (0.54, 2.78)	1.74 (0.94, 3.21)	1.82 (0.50, 6.63)
Nasopharyngeal carriage with any bacteria[†]						
Age 6 weeks	1.36 (0.85, 2.20)	Reference	1.01 (0.40, 2.60)	1.21 (0.38, 3.80)	1.77 (0.84, 3.73)	1.39 (0.23, 8.61)
Age 6 months	1.06 (0.72, 1.56)	Reference	1.11 (0.54, 2.27)	1.16 (0.45, 2.98)	0.80 (0.40, 1.61)	1.82 (0.45, 7.39)
Age 1 year	0.90 (0.59, 1.37)	Reference	1.12 (0.50, 2.48)	0.62 (0.24, 1.66)	0.98 (0.44, 2.17)	0.83 (0.19, 3.61)
Age 2 years	1.06 (0.70, 1.61)	Reference	0.71 (0.35, 1.44)	1.35 (0.50, 3.66)	1.05 (0.48, 2.29)	1.23 (0.22, 6.73)
Age 3 years	0.95 (0.64, 1.40)	Reference	1.53 (0.72, 3.22)	0.73 (0.29, 1.82)	1.02 (0.52, 1.97)	0.82 (0.17, 3.99)
Age 6 years	1.10 (0.79, 1.54)	Reference	1.06 (0.56, 2.00)	1.10 (0.51, 2.36)	1.23 (0.68, 2.23)	0.85 (0.23, 3.16)

Values are odds ratios (OR) with 95% confidence interval from logistic and multinomial regression models on imputed data. [†]Nasopharyngeal bacteria include *H. influenzae, M. catarrhalis* or *S. pneumoniae*. Models were adjusted for maternal psychiatric symptoms, pet keeping, mode of delivery, daycare attendance and antibiotic use. *p-value <0.05, **p-value <0.01.

riage with any bacteria at ages 6 weeks with overall eczema from birth until age 10 years in the sensitivity analyses with GEE models (data not shown).

Direction of associations between bacterial nasal and nasopharyngeal carriage and eczema

Figure 2 and Supplementary Table 4 show the bidirectional associations between bacterial nasal and nasopharyngeal carriage and eczema as dichotomous outcome from age 6 weeks to 10 years using cross-lagged models. Stability effect analysis showed that nasal carriage of *S. aureus* at earlier age was associated with increased risk of nasal carriage of *S. aureus* at later age between ages 6 weeks and 6 months, 2 to 3 years, and 3 to 6 years (OR (95% CI): 2.23 (1.36, 3.60), 2.39 (1.27, 4.48), and 1.73 (1.04, 2.89), respectively) (Supplementary Table 4). Stability effect analysis showed that eczema at earlier age was associated with eczema at later age between age 6 months and 10 years (OR (95% CI) range: 4.53 (1.99, 10.28) to 11.02 (6.36, 18.92)). Cross-sectional effect analysis showed that nasal carriage of *S. aureus* was associated with eczema at ages 6 months and 2 years (OR (95% CI): 2.39 (1.38, 4.14) and 2.20 (1.16, 4.18), respectively) (Figure 2A) (Supplementary Table 4). Cross-lagged effect analysis showed that children with eczema at age 2 years had an increased risk of nasal carriage of *S. aureus* at age 3 years (1.95 (1.02, 3.71)), but not at other ages. Reversely, no associations were observed of nasal carriage of *S. aureus* with eczema. For nasopharyngeal carriage with any bacteria, stability effect analysis showed that nasopharyngeal carriage with any bacteria at earlier age was largely associated with increased risk of nasopharyngeal carriage with any bacteria at later age between age 6 weeks and 6 years (OR (95% CI) range: 1.28 (0.86, 1.92) to 3.32 (2.29, 4.81)) (Supplementary Table 4). No cross-sectional or cross-lagged associations were observed between nasopharyngeal carriage with any bacteria and eczema (Figure 2B). Results from cross-lagged models were similar in effect size and direction when examining nasopharyngeal carriage with *H. influenzae, M. catarrhalis* and *S. pneumoniae* separately (Supplementary Table 5).

DISCUSSION

In this population-based prospective cohort study, we observed that only nasal carriage of *S. aureus* at age 6 months was associated with an increased risk of ever eczema, and specifically with an increased risk of early transient and persistent eczema phenotypes until age 10 years. The direction of effects between nasal carriage of *S. aureus* and eczema was largely cross-sectional, making causality either way unlikely. Nasopharyngeal bacterial carriage with *H. influenzae, M. catarrhalis* and/or *S. pneumoniae* from age 6

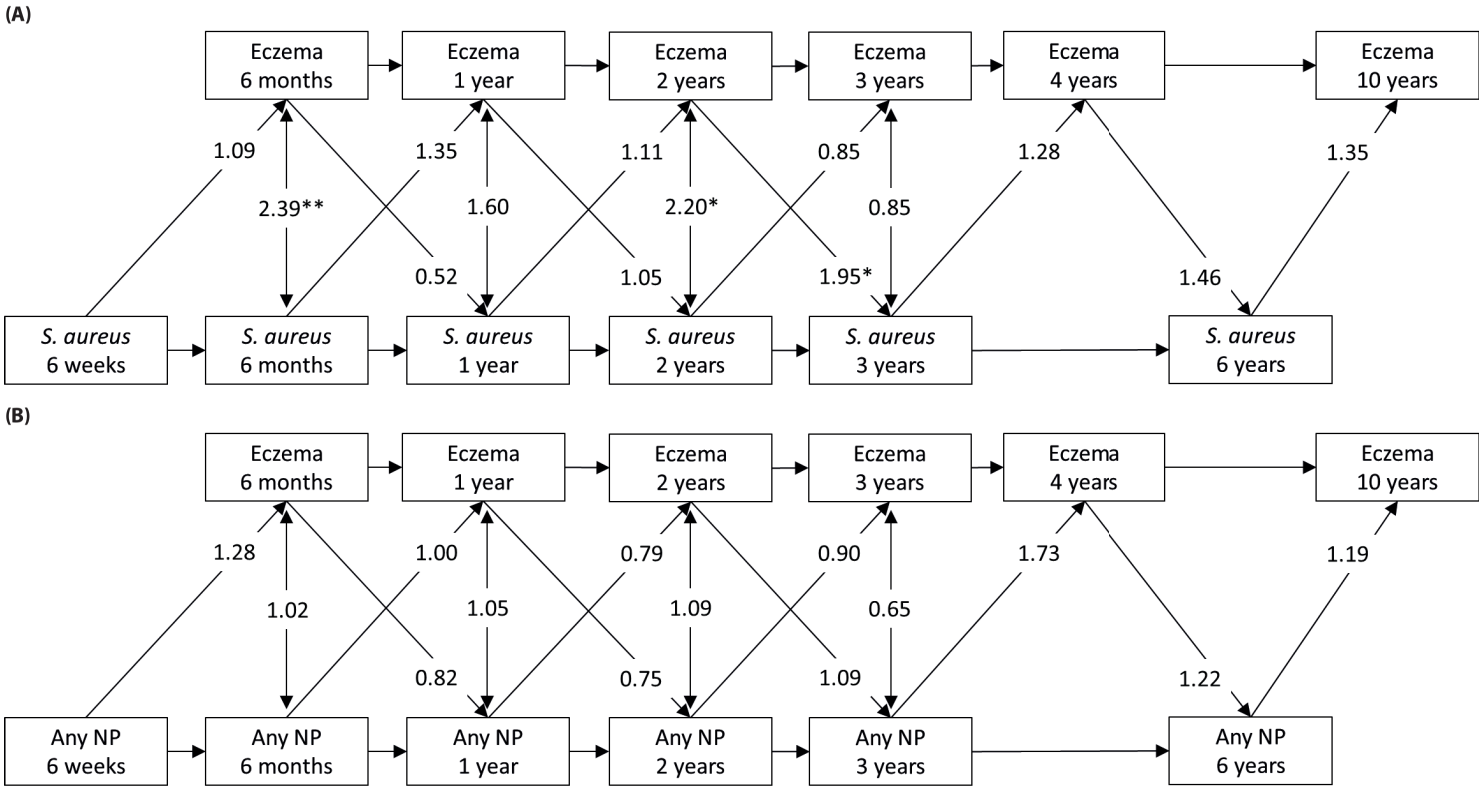

Figure 2. Bidirectional associations between nasal **(A)** *S. aureus* carriage and eczema, and **(B)** nasopharyngeal carriage with any bacteria (any NP), including *H. influenzae, M. catarrhalis* or *S. pneumoniae,* and eczema from birth until 10 years. Values are odds ratios (95% confidence interval) derived from logistic regression models, using cross-lagged modelling. Models were adjusted for maternal psychiatric symptoms, pet keeping, mode of delivery, daycare attendance and antibiotic use. *p-value <0.05, **p-value <0.01. The corresponding 95% confidence intervals of the cross-lagged and cross-sectional effects, and the effect estimates of the stability effects are shown in Supplementary Table 3.

weeks until age 6 years was not associated with ever eczema or eczema phenotypes from birth until age 10 years.

Comparison with previous studies

A previous meta-analysis, cohort and case-control studies showed that nasal carriage of *S. aureus* was associated with an up to 5-fold increased risk of eczema in children and adults.[4, 26] We observed in our current study that nasal carriage of *S. aureus* at age 6 months was associated with an increased risk of ever eczema at age 10 years, which is in line with results of our previous study in children until age 2 years.[7] We now additionally explored eczema phenotypes across childhood, taking the onset and persistence of eczema into account, and observed that nasal carriage of *S. aureus* at age 6 months was associated with an increased risk of early transient and persistent eczema. Nasal carriage of *S. aureus* at other ages until 6 years were not associated with ever eczema, and eczema phenotypes until age 10 years. Studies examining the direction of association between nasal carriage of *S. aureus* and eczema are scarce. Only two previous longitudinal studies, using one-directional statistical methods, examined the association between skin carriage of *S. aureus* and eczema in children, and showed conflicting results.[27, 28] Skin carriage of *S. aureus* 2 months prior to eczema onset was associated with an increased risk of eczema in a cohort of 149 children until age 2 years, while skin carriage of *S. aureus* at age 2 months was associated with a decreased risk of eczema in a nested case-control study of 20 children.[27, 28] The use of cross-lagged models allowed us to examine the effects between nasal carriage of *S. aureus* and eczema in both directions (bidirectional), and we observed that this association was largely cross-sectional, and not longitudinal.

Airway carriage of *H. influenzae, M. catarrhalis* and *S. pneumonia*e are suspected to play a role in the development of childhood atopic respiratory diseases by influencing the microbiota composition dynamics and/or the priming of the host's immune responses.[6, 16, 29] Therefore, we hypothesized that early life nasal and/or nasopharyngeal carriage with *H. influenzae, M. catarrhalis* or *S. pneumoniae* might also be associated with eczema, however few studies examined these associations. A cross-sectional cohort study in children with eczema showed that nasal carriage of different *Moraxella* species was associated with both increased, and decreased risk of eczema severity.[5] A previous case-control and cohort study showed that adults with eczema had higher risks of severe *S. pneumoniae* infections, and children with eczema had a delayed response to *Pneumococcal* vaccine, respectively.[9, 30] One cohort study showed that *H. influenzae* vaccination at age 6 months was associated with an increased risk of eczema at age 18 months.[8] We observed no associations of nasopharyngeal carriage of *H. influenzae, M. catarrhalis* and *S. pneumoniae* with ever eczema, and eczema phenotypes on a population-level. The difference between our results and the suggested positive associations of these

specific bacteria with eczema in previous studies might be explained by differences in study population (general versus hospital-based) and methods (microbial culture versus more sensitive rRNA sequencing, and nasal carriage versus vaccination responses), and potential publication bias towards positive findings.

Interpretation of results

Children with early transient and persistent eczema have a high probability of eczema at approximately 6 months of age.[2] Our observations of nasal carriage of *S. aureus* at age 6 months being associated with increased risks of early transient and persistent eczema, and not with mid-transient or late transient eczema, suggest that nasal carriage of *S. aureus* is associated with active eczema, and not with eczema developing in later life. This hypothesis is also supported by the results from our cross-lagged analyses that showed predominantly cross-sectional associations between nasal carriage of *S. aureus* and eczema rather than causal associations from nasal carriage of *S. aureus* to eczema or vice versa. Previous experimental and case-control studies showed that *S. aureus* might play a role in the development and worsening of eczema via the production of proteins, proteases and superantigens that can induce chronic inflammation of the skin.[31-33] However, a recent review found insufficient evidence for beneficial effects of *S. aureus* reducing interventions on eczema.[10] Therefore, *S. aureus* carriage or overgrowth might also be the consequence of a favorable skin environment in children with eczema. For instance, in eczema the skin has a higher pH compared to normal skin, and changes in skin microbiome composition may lead to more *S. aureus* growth.[31] Another possibility is that underlying immune dysregulation or skin characteristics, such as dry skin due to reduced levels of natural moisturizing factor and loss of function mutation in the filaggrin gene, prior to eczema development might benefit *S. aureus* growth.[31, 34, 35] Interestingly, nasal carriage of *S. aureus* at age 6 months, and not at older ages, was associated with eczema phenotypes. The prevalence of nasal carriage of *S. aureus* is highest at age 6 weeks, and declines at age 6 months in children with and without eczema. However, the prevalence of nasal carriage of *S. aureus* is higher in children with eczema than in those without eczema at age 6 months. A possible explanation is that the maturation of the skin and immune system is disrupted and/or delayed especially in children with early onset eczema, which increases the susceptibility of *S. aureus* (over)growth on the nose, and perhaps also on other body sites.[31] Future studies should focus on the microbiota on multiple body sites, such as nose, skin and gut in a longitudinal way, to further assess the relationship between bacteria and eczema.

Strengths and limitations

The strengths of this study include that it is embedded in a population-based prospective designs with detailed and repeated information on bacterial nasal carriage and

eczema, the use of eczema phenotypes and cross-lagged models to statistically examine longitudinal associations. However, methodological limitations of this study must also be taken into account when interpreting the results. Selection bias might occur when the associations between bacterial nasal carriage and eczema phenotypes were different in children included versus those not included in the analysis. Second, we used nasal swabs to examine *S. aureus* carriage. While the nose is the most frequent carriage site, other *S. aureus* prevalent body sites, such as the axilla and perineum, were not examined, thereby limiting the generalizability to nasal carriage of *S. aureus* only.[36, 37] Third, a relatively small number of children had nasal swabs, and not all of these had nasal swabs at every visit. Therefore, we were not able to cluster trajectories of bacterial nasal carriage in order to study the associations of bacterial nasal carriage phenotypes with eczema phenotypes. Fourth, non-differential misclassification of eczema remains possible due to use of self-reported questionnaires. Although these questionnaires have been validated for defining eczema in epidemiological research, cases with very mild eczema might be lacking since they are less likely to visit a physician.[38] In addition, most children in population-based settings have relatively mild disease, making it difficult to generalize findings to patients with moderate to severe eczema. Last, residual confounding might be present due to influencing factors not measured in our study. For example, the abundance of *S. aureus* and other nasopharyngeal bacteria, and horizontal transmission by surroundings could not be determined in our study.[36] It might be that children with early transient and persistent eczema have skin conditions (i.e. pH level, natural moisturizing factor, and filaggrin gene mutations) more suitable for *S. aureus* overgrowth, increased contact with parents due to frequent comforting, *S. aureus* contaminated topical ointments, and/or specific household conditions promoting *S. aureus* colonization (i.e. less frequent handwashing, shared towel use and shared bedrooms).[39-42] Also, since we only used one set of confounders mostly measured at early age, residual confounding could be greater for associations between exposures and outcomes at later ages.

Conclusion

We observed that early life nasal carriage with *S. aureus*, but not nasopharyngeal bacterial carriage with *H. influenzae, M. catarrhalis* or *S. pneumoniae,* was associated with increased risks of ever eczema, and early transient and persistent eczema phenotypes. The association between nasal carriage of *S. aureus* and eczema was more prominently cross-sectional, and not longitudinal. This suggest that nasal carriage of *S. aureus* is mainly associated with active eczema, and not with eczema development in later life. Future studies should focus on the longitudinal effects of the interaction of nasal and skin microbiome within individuals and their surroundings on eczema phenotypes.

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Supplementary Table 1. Characteristics of children included and not included

	Included (n=996)	**Not included** (n=250)	**p-value**
Maternal characteristics			
Pet keeping % (n)			0.140
No	57.6 (510)	51.9 (120)	
Yes	42.4 (375)	48.1 (111)	
Pyschiatric symptoms, median (IQR)	0.12 (0.06, 0.23)	0.13 (0.06, 0.29)	0.017
Mode of delivery % (n)			0.435
Vaginal	85.6 (786)	87.3 (186)	
Primary caesarian section	6.0 (55)	3.8 (8)	
Secondary caesarian section	8.4 (77)	8 .9 (19)	
Children's characteristics			
Day care attendance 1st year % (n)			0.194
No	30.8 (272)	37.8 (37)	
Yes	69.2 (612)	62.2 (61)	
Antibiotic use at age 1 year % (n)			0.395
No	64.3 (603)	59.6 (65)	
Yes	35.7 (335)	40.4 (44)	
Eczema % (n)			
Age 6 months, No	86.6 (587)	88.4 (76)	0.769
Yes	13.4 (91)	11.6 (10)	
Age 1 year, No	88.4 (823)	89.8 (97)	0.781
Yes	11.6 (108)	10.2 (11)	
Age 2 years, No	87.0 (815)	85.7 (78)	0.858
Yes	13.0 (122)	14.3 (13)	
Age 3 years, No	93.0 (837)	95.1 (58)	0.718
Yes	7.0 (63)	4.9 (3)	
Age 4 years, No	93.5 (829)	93.7 (59)	1.000
Yes	6.5 (58)	6.3 (4)	
Age 10 years, No	94 (778)	95.6 (43)	0.907
Yes	6.0 (50)	4.4 (2)	

Values are percentages (absolute values), mean (SD) or median (interquartile range) based on observed data.

Supplemental Table 2. Associations of bacterial nasal carriage with regrouped three eczema phenotypes

	Never eczema **Odds ratio (95% Confidence Interval)** (n=768)	**Early-mid transient eczema** **Odds ratio (95% Confidence Interval)** (n=142)	**Late-persistent eczema** **Odds ratio (95% Confidence Interval)** (n=86)
***S. aureus* carriage**			
Age 6 weeks	Reference	1.52 (0.82, 2.81)	0.90 (0.48, 1.69)
Age 6 months	Reference	2.39 (1.37, 4.15)**	1.88 (0.98, 3.61)
Age 1 year	Reference	0.98 (0.47, 2.06)	0.86 (0.35, 2.09)
Age 2 years	Reference	1.74 (0.87, 3.49)	1.36 (0.55, 3.33)
Age 3 years	Reference	1.68 (0.83, 3.40)	1.31 (0.58, 2.97)
Age 6 years	Reference	1.28 (0.77, 2.14)	1.73 (0.99, 3.00)
Nasopharyngeal carriage with any bacteria[†]			
Age 6 weeks	Reference	1.22 (0.60, 2.46)	1.76 (0.89, 3.50)
Age 6 months	Reference	1.13 (0.65, 1.97)	0.95 (0.51, 1.76)
Age 1 year	Reference	0.94 (0.52, 1.72)	0.92 (0.46, 1.84)
Age 2 years	Reference	0.89 (0.51, 1.57)	1.12 (0.55, 2.26)
Age 3 years	Reference	1.03 (0.59, 1.80)	0.95 (0.52, 1.74)
Age 6 years	Reference	1.06 (0.66, 1.72)	1.14 (0.67, 1.94)

Values are odds ratios (OR) with 95% confidence interval from multinomial regression models on imputed data. [†]Nasopharyngeal bacteria include *H. influenzae, M. catarrhalis* or *S. pneumoniae.* Models were adjusted for maternal psychiatric symptoms, pet keeping, mode of delivery, daycare attendance and antibiotic use. *p-value <0.05, **p-value <0.01.

Supplemental Table 3. Associations of nasopharyngeal carriage of *H. influenzae, M. catarrhalis* or *S. pneumoniae* with ever eczema and eczema phenotypes

	Ever eczema Odds ratio (95% Confidence Interval) (n=228)	**Never eczema Odds ratio (95% Confidence Interval)** (n=768)	**Early transient eczema Odds ratio (95% Confidence Interval)** (n=75)	**Mid-transient eczema Odds ratio (95% Confidence Interval)** (n=67)	**Late transient eczema Odds ratio (95% Confidence Interval)** (n=71)	**Persistent eczema Odds ratio (95% Confidence Interval)** (n=15)
***H. influenzae* carriage**						
Age 6 weeks	1.07 (0.46, 2.45)	Reference	0.43 (0.05, 3.60)	2.61 (0.56, 12.22)	1.20 (0.33, 4.39)	0.00 (0.00, >100)
Age 6 months	1.27 (0.84, 1.93)	Reference	2.09 (1.03, 4.24)	1.21 (0.46, 3.20)	0.53 (0.21, 1.38)	1.96 (0.50, 7.65)
Age 1 year	0.78 (0.51, 1.19)	Reference	1.32 (0.65, 2.67)	0.30 (0.08, 1.09)	0.86 (0.40, 1.87)	0.51 (0.09, 3.00)
Age 2 years	1.02 (0.64, 1.62)	Reference	0.88 (0.40, 1.95)	1.09 (0.38, 3.11)	1.15 (0.49, 2.67)	0.61 (0.06, 6.04)
Age 3 years	0.99 (0.62, 1.60)	Reference	1.03 (0.42, 2.53)	0.93 (0.30, 2.87)	1.12 (0.51, 2.50)	1.41 (0.22, 8.88)
Age 6 years	0.89 (0.52, 1.51)	Reference	0.89 (0.32, 2.48)	0.85 (0.25, 2.92)	0.93 (0.36, 2.42)	1.62 (0.29, 8.89)
***M. catarrhalis* carriage**						
Age 6 weeks	1.28 (0.68, 2.43)	Reference	1.23 (0.36, 4.21)	0.53 (0.07, 4.04)	1.96 (0.78, 4.92)	1.54 (0.15, 15.84)
Age 6 months	0.99 (1.67, 1.72)	Reference	0.89 (0.43, 1.84)	1.33 (0.54, 3.29)	0.99 (0.48, 2.06)	0.66 (0.14, 3.06)
Age 1 year	0.92 (0.61, 1.40)	Reference	1.08 (0.52, 2.24)	0.91 (0.33, 2.48)	0.75 (0.34, 1.67)	1.05 (0.25, 4.43)
Age 2 years	0.88 (0.17, 0.46)	Reference	0.79 (0.37, 1.69)	1.16 (0.43, 3.11)	0.90 (0.40, 2.03)	0.17 (0.01, 2.35)
Age 3 years	0.94 (0.59, 1.50)	Reference	1.82 (0.82, 4.03)	0.82 (0.27, 2.54)	1.04 (0.47, 2.27)	0.00 (0.00, >100)
Age 6 years	0.81 (0.46, 1.41)	Reference	0.74 (0.25, 2.19)	0.87 (0.24, 3.18)	0.82 (0.30, 2.23)	0.62 (0.06, 5.97)

Supplemental Table 3. Associations of nasopharyngeal carriage of *H. influenzae, M. catarrhalis* or *S. pneumoniae* with ever eczema and eczema phenotypes (continued)

	Ever eczema Odds ratio (95% Confidence Interval) (n=228)	**Never eczema Odds ratio (95% Confidence Interval)** (n=768)	**Early transient eczema Odds ratio (95% Confidence Interval)** (n=75)	**Mid-transient eczema Odds ratio (95% Confidence Interval)** (n=67)	**Late transient eczema Odds ratio (95% Confidence Interval)** (n=71)	**Persistent eczema Odds ratio (95% Confidence Interval)** (n=15)
***S. pneumoniae* carriage**						
Age 6 weeks	1.13 (0.55, 2.31)	Reference	0.78 (0.16, 3.67)	1.03 (0.19, 5.65)	1.14 (0.35, 3.73)	1.58 (0.13, 19.21)
Age 6 months	0.92 (0.62, 1.38)	Reference	0.92 (0.44, 1.91)	1.12 (0.45, 2.81)	0.76 (0.35, 1.64)	0.73 (0.15, 3.64)
Age 1 year	0.97 (0.65, 1.43)	Reference	1.05 (0.52, 2.09)	0.84 (0.33, 2.17)	0.98 (0.47, 2.02)	0.75 (0.18, 3.19)
Age 2 years	0.78 (0.51, 1.19)	Reference	0.51 (0.24, 1.09)	1.13 (0.44, 2.91)	0.61 (0.27, 1.38)	1.30 (0.27, 6.26)
Age 3 years	0.79 (0.51, 1.23)	Reference	1.11 (0.50, 2.46)	0.41 (0.12, 1.46)	1.02 (0.49, 2.09)	1.18 (0.22, 6.28)
Age 6 years	1.07 (0.73, 1.57)	Reference	0.92 (0.43, 1.94)	1.29 (0.57, 2.95)	1.02 (0.52, 2.03)	1.17 (0.28, 4.82)

Values are odds ratios (OR) with 95% confidence interval from logistic and multinomial regression models on imputed data. Models were adjusted for maternal psychiatric symptoms, pet keeping, mode of delivery, daycare attendance and antibiotic use. *adjusted p-value < 0.05

Supplementary Table 4. Direction of associations between bacterial nasal and nasopharyngeal carriage[†] and eczema from birth until age 10 years

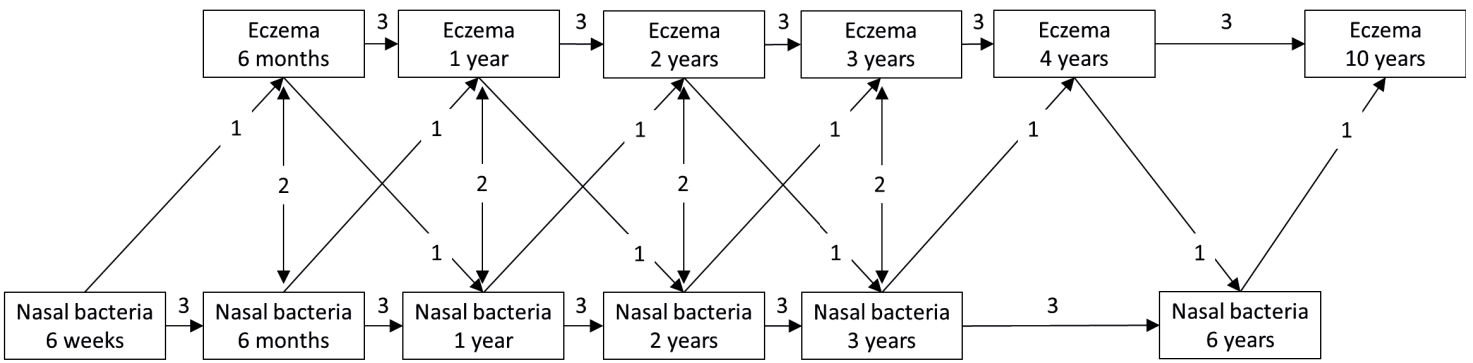
	***S. Aureus* carriage**	**Nasopharyngeal carriage with any bacteria**
Cross-lagged effects		
Bacterial nasal carriage 6w → Eczema 6m	1.09 (0.64, 1.86)	1.28 (0.68, 2.46)
Bacterial nasal carriage 6m → Eczema 1y	1.35 (0.76, 2.44)	1.00 (0.58, 1.72)
Bacterial nasal carriage 1y → Eczema 2y	1.11 (0.61, 2.01)	0.79 (0.47, 1.32)
Bacterial nasal carriage 2y → Eczema 3y	0.85 (0.35, 2.12)	0.90 (0.47, 1.75)
Bacterial nasal carriage 3y → Eczema 4y	1.28 (0.48, 3.42)	1.73 (0.85, 3.53)
Bacterial nasal carriage 6y → Eczema 10y	1.35 (0.70, 2.61)	1.19 (0.64, 2.20)
Eczema 6m → Bacterial nasal carriage 1y	0.52 (0.18, 1.46)	0.82 (0.43, 1.57)
Eczema 1y → Bacterial nasal carriage 2y	1.05 (0.52, 2.10)	0.75 (0.44, 1.28)
Eczema 2y → Bacterial nasal carriage 3y	1.95 (1.02, 3.71)*	1.09 (0.64, 1.86)
Eczema 4y → Bacterial nasal carriage 6y	1.46 (0.79, 2.72)	1.22 (0.68, 2.20)
Cross-sectional effects		
Bacterial nasal carriage ↔ Eczema 6m	2.39 (1.38, 4.14)**	1.02 (0.61, 1.73)
Bacterial nasal carriage ↔ Eczema 1y	1.60 (0.76, 3.39)	1.05 (0.57, 1.93)
Bacterial nasal carriage ↔ Eczema 2y	2.20 (1.16, 4.18)*	1.09 (0.63, 1.90)
Bacterial nasal carriage ↔ Eczema 3y	0.85(0.36, 2.01)	0.65 (0.34, 1.25)
Stability effects		
Bacterial nasal carriage 6w → 6m	2.23 (1.36, 3.60)**	1.72 (1.05, 2.83)*
Bacterial nasal carriage 6m → 1y	1.42 (0.82, 2.44)	3.32 (2.29, 4.81)**
Bacterial nasal carriage 1y → 2y	0.92 (0.41, 2.08)	2.29 (1.54, 3.39)**
Bacterial nasal carriage 2y → 3y	2.39 (1.27, 4.48)**	1.28 (0.86, 1.92)
Bacterial nasal carriage 3y → 6y	1.73 (1.04, 2.89)*	1.63 (1.15, 2.34)**
Eczema 6m → 1y	10.07 (5.75, 17.64)**	11.02 (6.36, 18.92)**
Eczema 1y → 2y	8.50 (5.31, 13.46)**	8.67 (5.42, 13.74)**
Eczema 2y → 3y	6.49 (3.71, 11.36)**	6.42 (3.71, 11.13)**
Eczema 3y → 4y	7.54 (3.82, 14.88)**	8.17 (4.06, 16.28)**
Eczema 4y → 10y	4.53 (1.99, 10.28)**	4.66 (2.12, 10.38)**

Values are odds ratios (95% confidence interval) derived from logistic regression models, using cross-lagged modelling. [†]Nasopharyngeal bacteria include *H. influenzae, M. catarrhalis* or *S. pneumoniae.* Models were adjusted for maternal psychiatric symptoms, pet keeping, mode of delivery, daycare attendance and antibiotic use. *p-value <0.05, **p-value <0.01. Abbreviation used: weeks (w), months (m), years (y).

Supplementary Table 5. Direction of associations between bacterial nasopharyngeal carriage and eczema from birth until age 10 years

	***H. influenzae* carriage**	***M. catarrhalis* carriage**	***S. pneumonia* carriage**
Cross-lagged effects			
Bacterial nasal carriage 6w → Eczema 6m	0.84 (0.28, 2.51)	1.35 (0.55, 3.32)	1.23 (0.47, 3.22)
Bacterial nasal carriage 6m → Eczema 1y	1.80 (1.03, 3.13)	1.19 (0.68, 2.10)	0.59 (0.33, 1.05)
Bacterial nasal carriage 1y → Eczema 2y	0.53 (0.28, 0.99)	0.70 (0.39, 1.25)	1.01 (0.61, 1.68)
Bacterial nasal carriage 2y → Eczema 3y	1.17 (0.57, 2.44)	0.68 (0.31, 1.46)	0.45 (0.21, 0.96)
Bacterial nasal carriage 3y → Eczema 4y	1.79 (0.84, 3.82)	1.49 (0.69, 3.19)	1.46 (0.72, 2.94)
Bacterial nasal carriage 6y → Eczema 10y	1.75 (0.77, 3.97)	1.02 (0.38, 2.75)	0.90 (0.44, 1.88)
Eczema 6m → Bacterial nasal carriage 1y	0.83 (0.44, 1.54)	0.99 (0.53, 1.86)	0.79 (0.43, 1.46)
Eczema 1y → Bacterial nasal carriage 2y	0.97 (0.52, 1.80)	0.66 (0.37, 1.20)	0.70 (0.39, 1.22)
Eczema 2y → Bacterial nasal carriage 3y	0.99 (0.50, 1.99)	0.99 (0.51, 1.93)	0.76 (0.41, 1.42)
Eczema 4y → Bacterial nasal carriage 6y	1.40 (0.61, 3.29)	1.21 (0.5, 2.92)	0.86 (0.43, 1.73)
Cross-sectional effects			
Bacterial nasal carriage ↔ Eczema 6m	1.14 (0.64, 2.03)	0.95 (0.53, 1.70)	1.17 (0.69, 1.99)
Bacterial nasal carriage ↔ Eczema 1y	0.85 (0.51, 1.45)	1.14 (0.65, 1.99)	1.35 (0.79, 2.32)
Bacterial nasal carriage ↔ Eczema 2y	0.75 (0.38, 1.49)	0.99 (0.55, 1.79)	1.13 (0.65, 1.95)
Bacterial nasal carriage ↔ Eczema 3y	0.85 (0.37, 1.95)	0.61 (0.26, 1.48)	1.00 (0.49, 2.05)
Stability effects			
Bacterial nasal carriage 6w → 6m	2.80 (1.23, 6.42)	1.08 (0.55, 2.12)	1.45 (0.74, 2.83)
Bacterial nasal carriage 6m → 1y	2.01 (1.32, 3.06)*	1.62 (1.11, 2.39)	2.97 (2.05, 4.35)*
Bacterial nasal carriage 1y → 2y	1.82 (1.17, 2.80)*	1.20 (0.78, 1.84)	1.65 (1.13, 2.41)*
Bacterial nasal carriage 2y → 3y	1.45 (0.86, 2.46)	0.85 (0.51, 1.43)	1.92 (1.25, 2.97)*
Bacterial nasal carriage 3y → 6y	0.87 (0.42, 1.79)	1.60 (0.88, 2.92)	1.62 (1.06, 2.46)
Eczema 6m → 1y	11.13 (6.42, 19.3)*	10.91 (6.36, 18.92)*	11.36 (6.55, 19.69)*
Eczema 1y → 2y	8.58 (5.37, 13.74)*	8.76 (5.47, 13.87)*	8.58 (5.42, 13.60)*
Eczema 2y → 3y	6.55 (3.78, 11.36)*	6.42 (3.71, 11.13)*	6.62 (3.78, 11.59)*
Eczema 3y → 4y	7.69 (3.94, 15.18)*	8.00 (4.06, 15.80)*	7.85 (3.97, 15.49)*
Eczema 4y → 10y	4.71 (2.12, 10.49)*	4.62 (2.08, 10.28)*	4.76 (2.16, 10.49)*

Values are odds ratios (95% confidence interval) derived from logistic regression models, using cross-lagged modelling. Models were adjusted for maternal psychiatric symptoms, pet keeping, mode of delivery, daycare attendance and antibiotic use. *adjusted p-value <0.05. Abbreviation used: weeks (w), months (m), years (y).



(1) Cross-lagged effects
(2) Cross-sectional effects
(3) Stability effects

Supplementary Figure 1. Overview of cross-lagged model design

2.3

Stool microbiota and atopic diseases in school-age children

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Chen Hu
Evelien R. van Meel
Carolina Medina-Gomez
Robert Kraaij
Monica Barroso
Jessica Kiefte-de Jong
Djawad Radjabzadeh
Suzanne G.M.A. Pasmans
Nicolette W. de Jong
Johan C. de Jongste
Henriette A. Moll
Tamar Nijsten
Fernando
Luba M. Pardo
Liesbeth Duijts

ABSTRACT

Background Infants with less diverse gut microbiota seem to have higher risks of atopic diseases in early life, but any associations at school age are unclear.

Objective To examine the associations of diversity, relative abundance and functional pathways of stool microbiota with atopic diseases in school-aged children.

Methods We performed a cross-sectional study within an existing population-based prospective cohort among 1,440 children aged 10 years. On stool samples, 16S rRNA gene sequencing was performed, and taxonomic and functional tables were produced. Physician-diagnosed eczema, allergy and asthma were measured by questionnaires, allergic sensitization by skin prick tests, and lung function by spirometry.

Results Alpha diversity of stool microbiota was associated with a decreased risk of eczema (OR (95%CI): 0.98 (0.97, 1.00)), and beta diversity was associated with physician-diagnosed inhalant allergy (R2 (p-value): 0.001 (0.047)). *Lachnospiraceae*, *Ruminococcaceae_UCG-005* and *Christensenellaceae_R-7_group* species were associated with decreased risks of eczema, inhalant allergic sensitization, and physician-diagnosed inhalant allergy (OR range (95%CI): 0.88 (0.79, 0.96) – 0.94 (0.88, 0.98)), while *Agathobacter* species was associated with an increased risk of physician-diagnosed inhalant allergy (1.23 (1.08, 1.42)). Functional pathways related to heme and terpenoid biosynthesis were associated with decreased risks of physician-diagnosed inhalant allergy and asthma (OR range (95%CI): 0.89 (0.80, 0.99) - 0.86 (0.73, 1.02)). No associations of stool microbiota with lung function were observed.

Conclusions The diversity, relative abundance and functional pathways of stool microbiota were most consistently associated with physician-diagnosed inhalant allergy in school-aged children, and less consistent with other atopic diseases.

INTRODUCTION

Atopic diseases such as eczema, allergy and asthma are a major public health concern. At school age, up to 30% of children is affected by at least one of these atopic diseases globally.[1, 2] Genetic susceptibility alone does not explain the high prevalence of atopic diseases as environmental exposures, partly through changes in the developing immune system, are likely to have major influence on the development of atopic diseases.[3, 4] The hygiene hypothesis suggests that the urbanization and modern public health practices lead to less microbial exposure, and thereby a less stimulated immune system, and subsequently an increased risk of eczema, allergy and asthma.[4] The gut microbiota has a prominent role in the development and regulation of the immune system.[5] Previous cohort and case-control studies have demonstrated that the diversity and relative abundance of stool microbiota, as proxy for gut microbiota, in early life are associated with the risk of atopic diseases.[6] Specifically, children with a less diverse stool microbiota before the age of 1 year, and with greater relative abundance of *Bacteroidaceae*, *Clostridiaceae* and *Enterobacteriaceae*, and lower relative abundance of *Bifidobacteriaceae* and *Lactobacillaceae*, have a higher risk of eczema, allergy or asthma until age 3 years.[6] Whether stool microbiota is also associated with atopic diseases in later childhood is less clear. Only few studies have been performed with limited power to detect the effects of diversity and differential relative abundance of stool microbiota on the risks of eczema, allergy and asthma on a population-based level. Lastly, information on the association of stool microbiota with lower lung function, one of the underlying mechanism in asthma, is lacking.

Therefore, we aimed to examine among 1,440 children participating in a prospective population-based cohort study the associations of diversity, relative abundance, and functional pathways of stool microbiota with eczema, allergic sensitization, allergy, lung function and asthma at school age.

METHODS

Design

This cross-sectional study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands[7]. The study has been approved by the Medical Ethical Committee of the Erasmus MC University Medical Centre Rotterdam, in Rotterdam, The Netherlands. Written informed consent was obtained from both parents or legal guardians. A total of 1,440 children with relevant data for stool microbiota and atopic diseases were available at a mean age of 9.8 years (SD: 0.27) for the current analysis (Supplementary Figure 1 and Methods).

Stool microbiota

Stool samples of children participating in the Generation R Study were collected at home using a Commode Specimen Collection System (Covidien, Mansfield, MA) and a feces collection tube (Minigrip Nederlands, Lelystad, The Netherlands) without preserving agent. Stool samples were stored at 4°C until mailing, and thereafter sent by post to the appropriate laboratory of the Erasmus MC, and locally stored at -20°C. Methods for DNA isolation, RNA sequencing, and microbiome quality criteria of stool samples have been described in detail previously, and are provided in the Supplementary Methods.[8] For current analyses, phylogenetic profiling and denoising was performed using DADA2 to produce amplicon- or exact sequence variants (ASVs).[9] High-throughput sequencing produced compositional data, therefore, we used the term 'relative abundance' to refer to the counts of ASVs reads generated by 16S rRNA gene sequencing.[10] The sequence data was then analysed for alpha diversity metrics (Chao richness index, Shannon diversity Index, and Inverse Simpson Index). Pair-wise beta diversities were calculated using Bray-Curtis dissimilarity metrics and Aitchison distance in centred log-ratio (CLR) transformed ASV relative abundances.[10] As a sensitivity analyses among the same subjects, we compared the observations of the current data processing approach using the DADA2 pipeline with the results obtained by our previously in-house developed wrapper pipeline wrapper pipeline (microRapTor) based on QIIME (version 1.9.0), TAGCleaner (version 0.16), PEAR (version 0.9.6), and UPARSE (version 8.1) software package.[11-14] More details are provided in the Supplementary Materials.

Atopic diseases

At age 10 years, information on current physician-diagnosed eczema, inhalant allergy (for pollen (hay fever), house dust mite, cat or dog), food allergy (for cashew nut or peanut) and asthma was obtained from a parental-reported questionnaire with questions adapted from the ISAAC core questionnaires.[15, 16] Current asthma was defined as physician diagnosis of ever asthma, with asthma medication use and/or wheezing in the past 12 months. Sensitization to the most relevant inhalant and food allergens on a population level was examined by skin prick tests. Inhalant allergens included house dust mite, 5-grass mixture, birch, cat, and dog (ALK-Abelló B.V., Almere, The Netherlands), and food allergens included hazelnut, cashew nut, peanut, and peach.[16, 17] Lung function was measured by spirometry at our research center according to ATS/ERS criteria, and values of forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were converted into sex-, height-, age-, and ethnicity-adjusted Z-scores according to GLI-reference values.[18-20] The ratio FEV_1 and FVC was dichotomized into obstructive and non-obstructive lung function (Z-score cut-off of ≤-1.64 and >1.64, respectively).[20]

We assumed that children with two or more atopic diseases tend to have a more severe variant of their atopic diseases.[21, 22] Therefore, we regrouped children into those with only one atopic disease (with any eczema, asthma or physician-diagnosed food or inhalant allergies), or two or more atopic diseases for sensitivity analyses. Detailed information on atopic diseases are provided in the Supplementary Methods.

Covariates

Information on lifestyle and socioeconomic confounders, and technical covariates were obtained from parental questionnaires, medical records, or measurements at our research center or laboratory, and are provided in detail in the Supplementary Methods.

Statistical analysis

We compared characteristics of those included and not included in our study using Pearson's Chi-square and independent sample *t*-tests. Analyzing high-throughput sequencing data is challenging, and rather than a golden standard, the use of multiple independent tools are recommended.[23] Therefore, we used both count-based and compositional approaches to examine the associations of stool microbiota with atopic diseases. All models were adjusted for lifestyle and socioeconomic confounders, and technical covariates. Unadjusted analyses are presented in Supplementary Tables 2, 4, and 6. We considered the adjusted model as our main model for interpretation of results. Functional pathways were predicted with PICRUSt2.[24, 25] The statistical analyses were performed in R version 4.0.0[26] using the packages microbiome[27], zCompositions[28], vegan[29], and phyloseq[30], and analysis of composition of microbiomes (ANCOM) with a detection cut-off of 0.60 (Supplementary Figure 2).[31] Measures of association are presented as odds ratios (OR) per unit increase of ASV relative abundance with their corresponding 95% confidence intervals (95%CI). Detailed information on statistical analyses, including which functions and parameters were used, are provided in the Supplementary Methods.

RESULTS

Subject characteristics

Table 1 shows the characteristics of the subjects. Compared with children included in the analysis, those not included had mothers who were lower educated, were more often of non-European ethnicity, and had a higher BMI (Table 1). Characteristics of the major phyla, families and genera of stool microbiota of the children included in the analyses are presented in Figure 1. Supplementary Figure 3 shows that the major phyla for children with any atopic disease and without any atopic diseases are similar.

Table 1. Characteristics of children and their mothers

	Included subjects n=1,440	**Not included subjects** n=3,187
Maternal characteristics		
Maternal education, higher % (n)	53.8 (719)	49.8 (1,436)*
Child characteristics		
Sex, female % (n)	50.3 (725)	50.4 (1,605)
Ethnicity, non-European % (n)	30.1 (430)	33.7 (1,039)*
Body mass index (BMI) at age 10 years, mean (SD)	17.4 (2.6)	17.7 (2.8)*
Antibiotic use in the past 3 months, yes % (n)	3.9 (56)	0.0 (0)
Stool sample		
Collection time in days, median (IQR)	2.00 (1.00-3.00)	1.50 (1.00-2.00)
DNA isolation batch, number 2 %(n)	89.0 (1,281)	100.0 (3)
Alpha diversity metrics, mean (SD)		
Chao index	150.6 (37.7)	150.2 (31.7)
Shannon Index	4.0 (0.41)	4.0 (0.4)
Inverse Simpson Index	31.9 (14.3)	30.1 (15.9)
Eczema, yes % (n)	7.0 (89)	7.0 (182)
Sensitization for food allergens, yes % (n)	6.4 (80)	7.2 (188)
Sensitization for inhalant allergens, yes % (n)	30.8 (385)	32.3 (842)
Physician-diagnosed food allergy, yes % (n)	2.3 (29)	2.1 (49)
Physician-diagnosed inhalant allergy, yes % (n)	11.8 (150)	12.4 (296)
Obstructive lung function, yes % (n)	5.1 (67)	4.5 (123)
Asthma, yes % (n)	5.0 (64)	6.0 (145)

Values are percentages (absolute values), mean (standard deviation (SD)) or median (interquartile range (IQR)) based on observed data. For the included subjects, data were missing for maternal education (n=103), ethnicity (n=13), BMI (n=5), eczema (n=147), sensitization for food (n=195) and inhalant allergens (n=189), physician-diagnosed food (n= 201) and inhalant allergy (n=170), lung function (n=118), and asthma (n= 172). *p-value for difference <0.05.

Diversity of stool microbiota and atopic diseases

In the adjusted analyses, all alpha diversity indices and Aitchison beta diversity showed that the diversity, and overall compositional variation of stool microbiota was not different between those with and without atopic diseases, except for eczema, namely inverse Simpson index was associated with a decreased risk of eczema (OR (95%CI): 0.98 (0.97, 1.00)) (Table 2). The Bray-Curtis beta diversity showed that the overall compositional variation of stool microbiota was different between children with and without physician-diagnosed inhalant allergies only (Bray-Curtis beta-diversity R^2 (adjusted p-value): 0.001 (0.047)) (Table 2). We observed no associations of alpha and beta diversity of stool microbiota with two or more atopic diseases, compared to those with only one

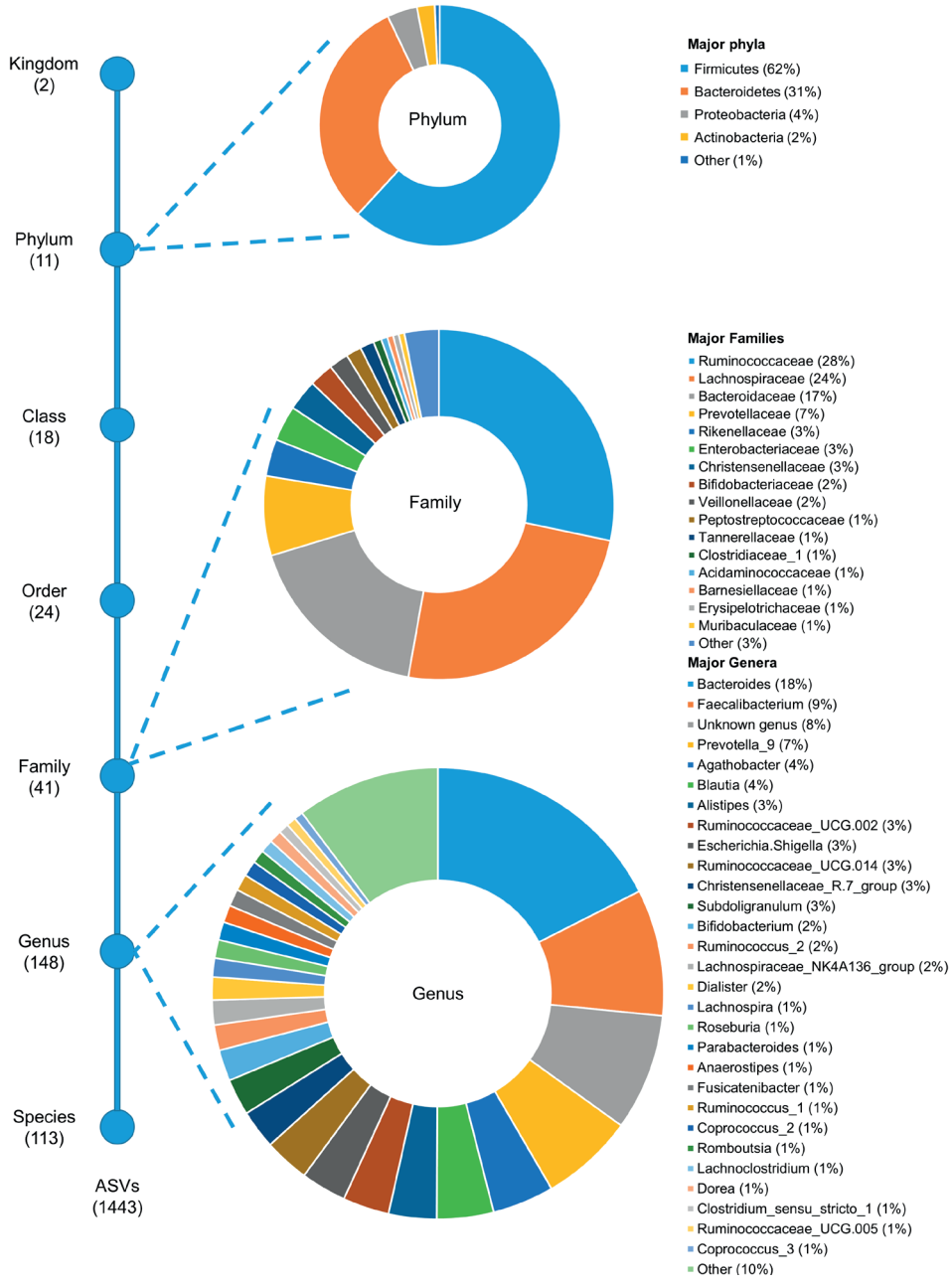

Figure 1. Characteristics of stool microbiota of the study population (n=1,440)
Left: Number of observed taxa at each taxonomy level. Within brackets represents the number of unique amplicon sequence variants (ASVs) identified in each taxonomic class.
Right: Donut plots of average relative abundance of the top major phyla, families and genera and within brackets the percentage coverage of the total relative abundance.

Table 2. Associations of alpha and beta diversity with atopic diseases at age 10 years

	Eczema (n=1,217)	**Sensitization for food allergens** (n=1,156)	**Sensitization for inhalant allergens** (n=1,161)	**Physician-diagnosed food allergy** (n=1,169)	**Physician-diagnosed inhalant allergy** (n=1,197)	**Obstructive lung function** (n=1,229)	**Asthma** (n=1,192)
Alpha diversity	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***
Chao index	1.00 (0.99, 1.01)	1.00 (1.00,1.01)	1.00 (1.00, 1.00)	1.00 (0.99, 1.01)	1.00 (0.99, 1.00)	0.99 (0.99,1.00)	1.00 (0.99,1.01)
Shannon index	0.75 (0.45, 1.30)	1.46 (0.78,2.88)	1.03 (0.75, 1.42)	0.81 (0.35, 2.14)	0.78 (0.51, 1.20)	0.76 (0.44,1.40)	0.85 (0.46,1.68)
Inverse Simpson index	0.98 (0.97, 1.00)*	1.01 (0.99,1.03)	1.00 (0.99, 1.01)	0.99 (0.96, 1.02)	0.99 (0.98, 1.00)	0.99 (0.97,1.00)	0.99 (0.97,1.01)
Beta diversity	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***
Bray-Curtis	0.001 (0.552)	0.001 (0.964)	0.001 (0.683)	0.001 (0.230)	0.001 (0.047)*	0.001 (0.548)	0.001 (0.559)
Aitchison	0.001 (0.787)	0.001 (0.703)	0.001 (0.494)	0.001 (0.368)	0.001 (0.119)	0.001 (0.216)	0.001 (0.850)

Values are odds ratios (95% confidence intervals) from adjusted logistic regression models for alpha diversity, and R-squared (Benjamin Hochberg -adjusted p-values) from adjusted PERMANOVA analysis for 999 iterations for beta diversity. Models were adjusted for maternal education, child's sex, ethnicity, body mass index at age 10 years, antibiotic use in the past 3 months, DNA isolation batch and general collection time. *p-value <0.05, **p-value<0.01.

atopic disease (data not shown). Sensitivity analyses with previous in-house developed pipeline showed that alpha diversity indices and Bray-Curtis beta diversity were not associated with any atopic diseases. Aitchison beta diversity showed that overall compositional variation of stool microbiota was different between children with and without physician-diagnosed inhalant allergy (Aitchison beta-diversity R^2 (adjusted p-value): 0.001 (0.014))

Differential relative abundance of stool microbiota and atopic diseases

Supplementary Table 3 shows all ASVs of stool microbiota included in the analyses, which ASVs were associated with atopic diseases from unadjusted and adjusted ANCOM analyses, if they belonged to the top 10% or 25% of the most abundant ASVs, and the percentage of samples that contained the ASV. The associated ASVs with atopic diseases are depicted in Supplemental Figure 4. In the adjusted ANCOM and logistic regression analyses, *Lachnospiraceae_unknowngenus_5* was associated with a decreased risk of eczema (OR (95%CI): 0.88 (0.79, 0.96)), and with a decreased risk of sensitization for inhalant allergens (0.94 (0.89, 0.98)) (Table 3). *Ruminococcaceae_UCG-005_unknownspecies_2* and *Christensenellaceae_R-7_group_unknownspecies_2* were associated with a decreased risk of physician-diagnosed inhalant allergy (0.89 (0.82, 0.96) and 0.94 (0.88, 0.99), respectively), while *Agathobacter_unknownspecies* was associated with an increased risk of physician-diagnosed inhalant allergy (1.23 (1.08, 1.42)). No associations were observed for specific ASVs of stool microbiota with sensitization for food allergens, physician-diagnosed food allergy, lung function, and asthma in the adjusted ANCOM analyses. No specific ASVs were associated with two or more atopic diseases compared to only one atopic disease in the adjusted ANCOM analyses (data not shown). Sensitiv-

Table 3. Differential relative abundance of stool microbiota and atopic diseases at age 10 years

	Amplicon sequence variants (ASV)	Odds ratio (95%CI)
Eczema	Lachnospiraceae unknowngenus_5	0.88 (0.79, 0.96)**
Sensitization for inhalant allergens	Lachnospiraceae unknowngenus_5	0.94 (0.89, 0.98)**
Physician-diagnosed inhalant allergy	Ruminococcaceae_UCG-005 unknownspecies_2	0.89 (0.82, 0.96)**
	Christensenellaceae_R-7_group unknownspecies_2	0.94 (0.88, 0.99)*
	Agathobacter unknownspecies	1.23 (1.08, 1.42)**

The number of subjects included for the adjusted ANCOM and adjusted logistic regression analyses for eczema (n=1,217), sensitization for food allergens (n=1,156), sensitization for inhalant allergens (n=1,161), physician-diagnosed food allergy (n=1,169), physician-diagnosed inhalant allergy (n=1,197), lung function (n=1,229), and asthma (n=1,192). Models were adjusted for maternal education, child's sex, ethnicity, body mass index at age 10 years, antibiotic use in the past 3 months, DNA isolation batch and general collection time, and other associated Amplicon sequence variants (ASVs) with the outcome of interest. No associations were found for stool microbial ASVs with sensitization for food allergens, physician-diagnosed food allergy, lung function, and asthma at age 10 years. *p-value <0.05, **p-value<0.01.

ity analyses with the previous in-house developed pipeline showed similar results for *Lachnospiraceae* genus with sensitization for inhalant allergens (0.99 (0.99, 1.00)), and *Ruminococcaceae_UCG-005* species and *Christensenellaceae_R-7_group* species with physician-diagnosed inhalant allergy (0.99 (0.98, 1.00) and 1.00 (1.00, 1.00)).

Functional pathways of stool microbiota and atopic diseases

Supplementary Table 5 shows all identified functional pathways based on the full ASV table using PICRUSt2, and which were associated with atopic diseases. In the adjusted ANCOM and logistic regression analyses, N-acetylneuraminate catabolism was associated with a decreased risk of sensitization for food allergens (OR (95%CI): 0.71 (0.54, 0.92)) (Table 4). Ethylmalonyl-CoA, L-leucine degradation I, Adenosylcobalamin biosynthesis I (anaerobic) and Aerobic respiration I (cytochrome c) pathways were associated with a decreased risk of sensitization for inhalant allergens in the adjusted ANCOM analyses, but the associations attenuated to non-significant in the adjusted logistic regression

Table 4. Associations of functional pathways of stool microbiota with atopic diseases at age 10 years

	Functional pathway	Odds ratio (95%CI)
Sensitization for food allergens	N-acetylneuraminate catabolism; sialic acid degradation	0.71 (0.54, 0.92)**
Sensitization for inhalant allergens	Ethylmalonyl-CoA pathway	0.80 (0.60, 1.08)
	L-leucine degradation I pathway	0.91 (0.75, 1.09)
	Adenosylcobalamin biosynthesis I (anaerobic) pathway	1.16 (0.98, 1.41)
	Aerobic respiration I (cytochrome c) pathway	1.01 (0.94, 1.07)
Physician-diagnosed food allergy	L-lysine biosynthesis II pathway	0.67 (0.52, 0.89)**
Physician-diagnosed inhalant allergy	Taxadiene biosynthesis	0.89 (0.78, 1.01)
	Geranylgeranyldiphosphate biosynthesis I (via mevalonate)	0.90 (0.83, 0.98)*
	Superpathway of heme b biosynthesis from glycine	0.92 (0.85, 0.99)*
	Methylphosphonate degradation I pathways	0.94 (0.85, 1.06)
Asthma	Superpathway of heme b biosynthesis from glycine	0.89 (0.80, 0.99)*
	L-glutamate degradation V (via hydroxyglutarate)	0.88 (0.79, 0.99)*
	Taxadiene biosynthesis	0.86 (0.73, 1.02)

The number of subjects included for the adjusted ANCOM and adjusted logistic regression analyses for eczema (n=1,217), sensitization for food allergens (n=1,156), sensitization for inhalant allergens (n=1,161), physician-diagnosed food allergy (n=1,169), physician-diagnosed inhalant allergy (n=1,197), lung function (n=1,229), and asthma (n=1,192). Models were adjusted for maternal education, child's sex, ethnicity, body mass index at age 10 years, antibiotic use in the past 3 months, DNA isolation batch and general collection time, and other associated pathways with the outcome of interest. No associations were found for stool microbial functional pathways with eczema, and lung function at age 10 years. *p-value <0.05, **p-value<0.01.

analyses (OR range (95%CI: 0.80 (0.60, 1.08) - 1.16 (0.98, 1.41)). L-lysine biosynthesis II pathway was associated with a decreased risk of physician-diagnosed food allergy (0.67 (0.52, 0.89)) in the adjusted ANCOM and logistic regression analyses. Geranylgeranyl-diphosphate biosynthesis I (via mevalonate) and superpathway of heme b biosynthesis from glycine were associated with a decreased risk of physician-diagnosed inhalant allergy (0.90 (0.83, 0.98) and 0.92 (0.85, 0.99), respectively), while the identified pathways of Taxadiene biosynthesis and Methylphosphonate degradation I from adjusted ANCOM analyses attenuated to non-significant in the adjusted logistic regression model (0.89 (0.78, 1.01) and 0.94 (0.85, 1.06), respectively). The superpathway of heme b biosynthesis from glycine, and L-glutamate degradation V (via hydroxyglutarate) were associated with a decreased risk of asthma (0.89 (0.80, 0.99) and 0.88 (0.79, 0.99), respectively), while the association of Taxadiene biosynthesis with a decreased risk of asthma in adjusted ANCOM analyses attenuated to non-significant in the adjusted logistic regression (0.86 (0.73, 1.02)). No functional pathways of stool microbiota were associated with eczema or lung function. No specific functional pathways were associated with two or more atopic diseases compared to only one atopic disease (data not shown).

DISCUSSION

In this population-based prospective cohort study among children aged 10 years, we observed that the diversity, relative abundance, and functional pathways of stool microbiota were associated with physician-diagnosed inhalant allergy. Associations of diversity, relative abundance, and functional pathways of stool microbiota with other atopic outcomes were less consistent.

Comparison with previous studies

Compared to our previous study on diversity, compositional, and functional differences of stool microbiota of children and adults, the top four major phyla and the top 10 major families remained the same, and of the top most abundant genera 24 were also in the top 30 genera of the in the current study population.[8] In addition, we gained more unique ASV numbers on each taxonomic level using the DADA2 pipeline with the final dataset, including 1443 different ASVs in the current study compared to 661 operational taxonomic units in our previous study. We observed that inverse Simpson index (alpha diversity) of stool microbiota was associated with a decreased risk of eczema only, and not with other current atopic diseases at age 10 years. We observed that the overall composition of stool microbiota, based only on Bray-Curtis beta diversity, is different in children with and without physician-diagnosed inhalant allergy at the age of 10 years. A recent systematic review of previous cohort and case-control studies performed in

children mostly before the age of 6 months showed associations of alpha diversity of stool microbiota with eczema, inhalant and food allergies, and asthma, while studies performed in older children using modern sequencing methods showed conflicting results.[6] Therefore, the relative abundance of stool microbiota might be more important in atopic diseases than the diversity at school age. The ASVs that we identified as being associated with atopic diseases at 10 years were in line with results of previous studies among children younger than 5 years These demonstrated that genera from *Lachnospiraceae, Ruminococcaceae*, and *Prevotellaceae* families, and higher taxonomic order of *Clostridiaceae* measured at ages 0-2 years were associated with atopic diseases at ages 0-8 years.[6] However, the direction of associations between specific stool microbiota and atopic diseases was not consistent. For example, the family of *Lachnospiraceae* and the order of *Clostridiaceae* were associated with both increased and decreased risks of atopic diseases.[6] The differences in results of diversity and relative abundance of stool microbiota with atopic diseases in our and previous studies might be due to differences in characteristics of children included in the analysis, such as age and geographic location, and the prospective or cross-sectional design. It might be more challenging to find associations of stool microbiota with atopic diseases in older children, since the complexity of gut microbiota increases with age, despite its stabilization in later life.[8] Also, the heterogeneity of the bioinformatics and statistical approaches of the studies due to the compositional nature of stool microbiota data, and limitation of sequencing technique could explain the differences in results.[10] Homogenization of microbiota tables is needed to adequately compare results of gut microbiota with atopic diseases between different studies. Future studies should analyse gut microbiota at lower taxonomic levels, because specific microbial species within a genus may influence atopic diseases differently.

No specific functional pathways of stool microbiota were consistently associated with all atopic diseases, which might be explained by differences in the etiology of atopic diseases, and only a small proportion of children that follows the atopic march in our population-based cohort.[32-34] The most consistent observations included functional pathways related to heme and terpenoid biosynthesis, which were associated with decreased risks of physician-diagnosed inhalant allergy and asthma. Taxadiene biosynthesis, Geranylgeranyldiphosphate biosynthesis I (via mevalonate) and Mevalonate pathway I (eukaryotes and bacteria) are part of the pathways class of biosynthesis of terpenoids, which are a class of naturally occurring organic compounds, derived from five carbon isoprene units, and often used in cosmetics, pharmaceuticals, or biofuels.[35] Murine studies showed that mevalonate biosynthesis plays a role in T-helper 2 cell differentiation, and that inhibiting mevalonate pathway can lower the allergic inflammation and airway hyperreactivity, making it a possible novel therapeutic target for

atopic diseases.[36, 37] This is in line with findings of a recent review suggesting that terpenoids have anti-inflammatory effects, and might be effective in treating respiratory inflammation and atopic dermatitis.[38] Although the specific superpathway of heme b biosynthesis from glycine has not been previously found to be associated with atopic diseases, hemeoxygenase-1 (HO-1) protein, which catabolizes heme to biliverdin, free iron and carbonoxide, is increased in murine lung tissue in allergic airway inflammation and in skin lesions of patients with eczema.[39, 40] In addition, iron-deficiency has also been related to increased risk of atopic diseases, possibly through changes in the gut microbiome.[41] Interestingly, we observed that N-acetylneuraminate catabolism (also known as sialic acid degradation) pathway was associated with a decreased risk of sensitization of food allergens. Further, this finding might explain the increased sialic acid content that a recent study found on total IgE from individuals with a peanut-allergy as compared to those without any allergies.[42] We did not observe an association of N-acetylneuraminate catabolism with physician-diagnosed food allergy, which might be due to the used definition of allergy. Allergic sensitization measured by skin prick tests reflects children who are sensitized but partly do not experience symptoms of food allergy. Another interesting observation included the association of L-glutamate degradation V (via hydroxyglutarate) with a decreased risk of current asthma. Via this pathway, bacteria are able to ferment amino acids into short chain fatty acids (SCFA) among other products. This observation supports the hypothesis of SCFA having anti-inflammatory properties, and its association with decreased risks of atopic diseases.[43, 44]

Interpretation of results

To the best of our knowledge, our study is one of the few studies to examine the associations of stool microbiota with atopic diseases in school-age children from the general population. Our results contribute to the body of knowledge on the role of gut microbiota in the development of atopic diseases in later childhood. We observed no consistent associations of gut microbiota with eczema, allergy and asthma using DADA2 pipeline and our previously in-house developed pipeline, which suggests that the role of gut microbiota on atopic diseases might be limited in later childhood. The relation between gut microbiota and atopic diseases might be influenced by the severity of the condition, steroid use, and persistence of atopic diseases. Therefore, observations might be different in hospital-population or at an individual based level, when children have multiple and persistent atopic diseases, and greater disease severity. Furthermore, due to the cross-sectional design of our study, we could not examine the influence of early life gut microbiota on later life gut microbiota, and subsequently atopic diseases in later life. Our observations that diversity and relative abundance of stool microbiota were not prominently associated with atopic diseases at school age support the hypothesis of an 'early window of opportunity' in which the gut microbiota plays an more important role

in the maturation of the immune system in early childhood, and to a lesser extent in later childhood.[45] This has also been suggested by murine studies that showed that changes in gut microbiota in neonatal mice, but not in adult mice are associated with the of development of atopic diseases.[46, 47] Gut microbiota protects the host from potential pathogenic colonization, contributes to the intestinal barrier function, and helps develop and regulate the immune system.[5] Especially in early childhood, when maturation of the gut and immune system is still ongoing, disruption of systems contributing to the maturation of the gut and immune system might increase the risk of developing atopic diseases. T-regulatory cells play an important role in the immune responses to allergens, the regulation of type 2 T-helper cells, and the production of immunoglobulin type E.[48] Gut microbiota can induce the differentiation of T-regulatory cells, and reduce pro-inflammatory cytokines through different pathways, such as via the production of short-chain fatty acids leading to activation of G protein–coupled receptors signaling pathways and epigenetic changes.[45] Also, gut microbiota might affect atopic diseases through interaction with other microbiota, such as on the lung or skin.[49, 50] Gut bacteria and their metabolites can migrate to the blood, the lymphatic system, skin, and lung via an impaired intestinal barrier, and can produce short chain fatty-acids that can exhibit antimicrobial effects against for example Staphylococcus aureus, which on the skin and nose has been associated with increased risk of eczema and eczema severity.[49, 50] Our study was designed cross-sectionally and prospective cohort studies should examine the role of early life microbiome and its longitudinal development on later life atopic diseases.

Strengths and limitations

The strengths of this study include its cross-sectional design within a well-characterized population-based prospective study, a large number of subjects, and examining stool microbiota with atopic diseases at school age using a combination of compositional and non-compositional approaches. Also, we used novel and open source microbiome analyses tools such as DADA2 and PICRUSt2, which enables better comparison with other (future) studies, since the used DADA2 pipeline produced sequences reads with counts that can be compared directly across studies.[9, 24] However, limitations of this study should also be considered in the interpretation of the results. Children not included in the analyses had less favourable socio-economic factors, were more often of non-European ethnicity, and had a higher BMI, which could have resulted in selection bias if the associations of gut microbiota with atopic diseases would have been different in children included and not included in the analysis. Non-differential misclassification of eczema, asthma and physician-diagnosed allergies remains possible due to collection via self-reported questionnaires and might have led to dilution of results. We used validated questionnaires to minimize this bias.[15, 51] In addition, residual confounding

might still be present as in any observational study. Besides inhaled asthma medication, no information was available on steroid use. However, on a population level most atopic diseases are of mild severity, and therefore the effect of steroid use on the associations of stool microbiota with atopic disease will most likely be constrained. Further, although the 16S rRNA gene amplification and shotgun metagonomics approaches provide different information, the phylogeny and biomolecular function have been shown to be strongly correlated.[25] Shotgun metagenomic sequencing would allow greater precision in the functional predictions obtained from PICRUSt2.[28] Unfortunately, metagenomic profiling was not available considering the high costs of performing shotgun sequencing in our large population-based cohort. Also, high inter-individual variability of the gut microbiota and high-dimensionality of the microbiota data can hinder the power of analysis. Uniform and harmonized methodological approaches for better identification and comparison of results between large-scale studies are urgently needed.[52] Lastly, although we consider the applied methods as the most appropriate following the current standards set in the field, analytical tools in the field of microbiota are still developing and as such results may depend on the method applied. While microbiome profiling methods continue to evolve further, our study already provides some leads that await replication to confirm the involvement of the gut microbiota in the etiology of atopic diseases.

Conclusion

We observed that the diversity, relative abundance and pathways derived from stool microbiota were consistently associated with physician-diagnosed inhalant allergy, and less consistent with current eczema, food allergy outcomes and asthma at school age. The role of stool microbiota on atopic diseases therefore seems limited in later childhood. Despite the relatively large sample of our study, we cannot exclude that weak associations with other atopic diseases were not detected given the large dimensionality and heterogeneity of the stool microbiome data, and the low prevalence of some of the atopic diseases. Additionally, the effect of the stool microbiota on atopic diseases with a different etiology might differ. Future large-scale studies should repeatedly examine the longitudinal associations of stool microbiota and atopic diseases from infancy to school-age. This might clarify any age specific influences of gut microbiota on the development of atopic diseases throughout childhood.

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Supplementary Table 1. Descriptives of concurrent atopic diseases

	Eczema	Sensitization for food allergens	Sensitization for inhalant allergens	Physician-diagnosed food allergy	Physician-diagnosed inhalant allergy	Obstructive lung function	Asthma
Eczema	100.0% (89/89)	13.5% (12/89)	37.1% (33/89)	6.7% (6/89)	31.5% (28/89)	1.1% (1/89)	14.6% (13/89)
Sensitization for food allergens	15.0% (12/80)	100% (80/80)	91.3% (73/80)	18.8% (15/80)	40.0% (32/80)	6.3% (5/80)	15.0% (12/80)
Sensitization for inhalant allergens	8.6% (33/385)	18.9% (73/385)	100.0% (385/385)	3.9% (15/385)	24.7% (95/385)	4.4% (17/385)	7.5% (29/385)
Physician-diagnosed food allergy	20.7% (6/29)	51.7% (15/29)	51.7% (15/29)	100% (29/29)	79.3% (23/29)	6.9% (2/29)	34.5% (10/29)
Physician-diagnosed inhalant allergy	18.7% (28/150)	21. 3% (32/150)	63. 3% (95/150)	15. 3% (23/150)	100.0% (150/150)	6.0% (9/150)	21. 3% (32/150)
Obstructive lung function	1.5% (1/67)	7.5% (5/67)	25.4% (17/67)	3.0% (2/67)	13.5% (9/67)	100.0% (67/67)	13.4% (9/67)
Asthma	19.4% (13/67)	17.9% (12/67)	43.3% (29/67)	14.9% (10/67)	47.8% (32/67)	13.4% (9/67)	100.0% (67/67)

Supplementary Table 2. Unadjusted associations of alpha and beta diversity with atopic diseases at age 10 years

	Eczema (n=1,293)	**Sensitization for food allergens** (n=1,245)	**Sensitization for inhalant allergens** (n=1,251)	**Physician-diagnosed food allergy** (n=1,239)	**Physician-diagnosed inhalant allergy** (n=1,270)	**Obstructive lung function** (n=1,322)	**Asthma** (n=1,268)
Alpha diversity	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***	***Odds Ratio (95%CI)***
Chao index	1.00 (0.99, 1.00)	1.00 (1.00,1.01)	1.00 (1.00, 1.00)	1.00 (0.99, 1.01)	1.00 (0.99, 1.00)	0.99 (0.99,1.00)	1.00 (0.99,1.00)
Shannon index	0.69 (0.44, 1.14)	1.12 (0.65,2.04)	0.98 (0.73, 1.32)	0.95 (0.42, 2.47)	0.79 (0.54, 1.19)	0.90 (0.52,1.64)	0.63 (0.38,1.11)
Inverse Simpson index	0.98 (0.96, 1.00)*	1.01 (1.00,1.01)	1.00 (0.99, 1.01)	0.99 (0.97, 1.02)	0.99 (0.98, 1.00)	0.99 (0.97,1.01)	0.98 (0.96,1.00)*
Beta diversity	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***	***R^2 (p-value)***
Bray-Curtis	0.001 (0.453)	0.001 (0.988)	0.001 (0.434)	0.001 (0.283)	0.001 (0.014)*	0.001 (0.843)	0.001 (0.473)
Aitchison	0.001 (0.375)	0.001 (0.756)	0.001 (0.400)	0.001 (0.401)	0.001 (0.057)	0.001 (0.645)	0.001 (0.381)

Values are odds ratios (95% confidence intervals) from unadjusted logistic regression models for alpha diversity, and R-squared (Benjamin Hochberg -adjusted p-values) from unadjusted PERMANOVA analysis for 999 iterations for beta diversity. *p-value <0.05, **p-value<0.01. n=number of children included in the analysis.

Supplementary Table 4. Unadjusted differential relative abundance of stool microbiota and atopic diseases at age 10 years

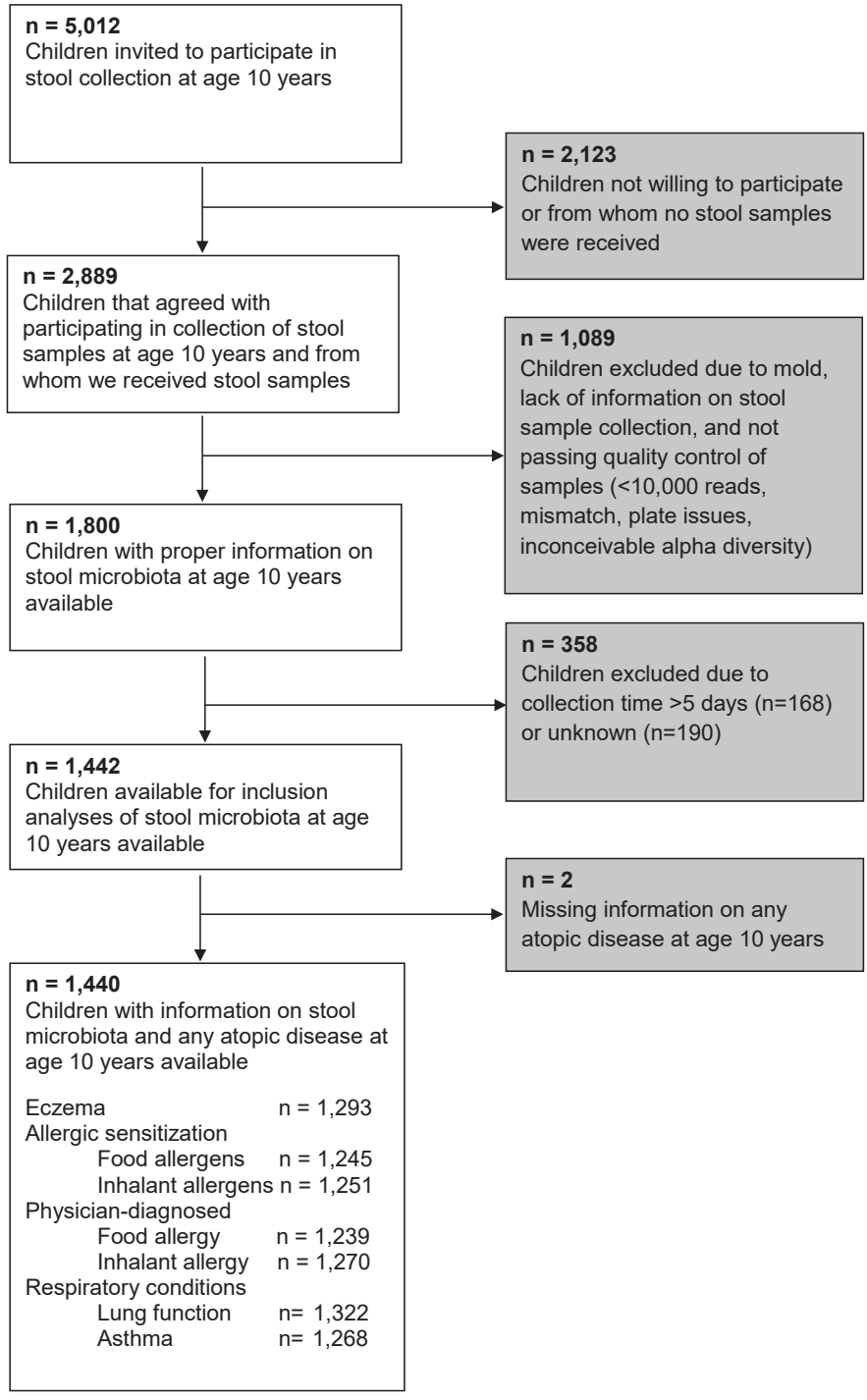
	Amplicon sequence variants (ASVs)	Odds ratio (95%CI)
Eczema	Lachnospiraceae unknowngenus_5	0.86 (0.79, 0.94)**
Sensitization for inhalant allergens	Lachnospiraceae unknowngenus_5	0.93 (0.89, 0.97)**
Physician-diagnosed food allergy	Ruminococcus_2 bromii	1.16 (1.03, 1.30)*
Physician-diagnosed inhalant allergy	Ruminococcaceae_UCG-005 unknownspecies_2	0.87 (0.81, 0.93)**
	Christensenellaceae_R-7_group unknownspecies_2	0.92 (0.87, 0.97)**
	Agathobacter unknownspecies	1.26 (1.11, 1.45)**
	Prevotella_9 unknownspecies_4	0.88 (0.80, 0.95)**

The number of subjects included for the unadjusted ANCOM and unadjusted logistic regression analyses were for eczema (n=1,293), sensitization for food allergens (n=1,245), sensitization for inhalant allergens (n=1,251), physician-diagnosed food allergy (n=1,239), physician-diagnosed inhalant allergy (n=1,270), lung function (n=1,322), and asthma (n=1,268). No associations were found for stool microbial Amplicon sequence variants (ASVs) with sensitization for food allergens, lung function, and asthma at age 10 years. *p-value <0.05, **p-value<0.01.

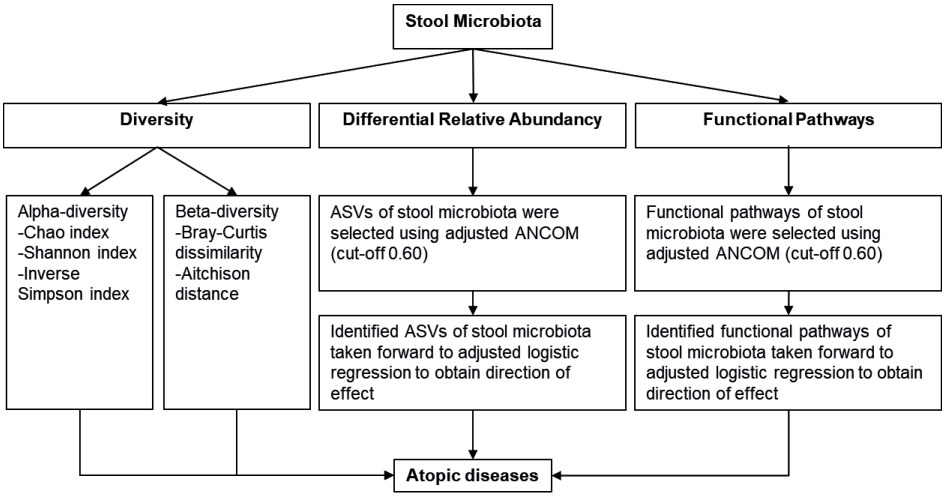
Supplementary Table 6. Unadjusted analysis of functional pathways of stool microbiota with atopic diseases at age 10 years

	Functional pathway	Odds ratio (95%CI)
Sensitization for inhalant allergens	Ethylmalonyl-CoA pathway	0.91 (0.85, 0.96)**
	L-leucine degradation I pathway	0.91 (0.86, 0.97)*
	Adenosylcobalamin biosynthesis I (anaerobic) pathway	0.94 (0.90, 0.98)**
	Aerobic respiration I (cytochrome c) pathway	0.95 (0.91, 0.99)*
	Glycerol degradation to butanol pathway	1.07 (1.01, 1.14)*
Physician-diagnosed food allergy	L-lysine biosynthesis II pathway	0.67 (0.52, 0.89)**
Physician-diagnosed inhalant allergy	Taxadiene biosynthesis	0.82 (0.73, 0.92)**
	Geranylgeranyldiphosphate biosynthesis I (via mevalonate)	0.92 (0.86, 0.99)*
	Mevalonate pathway I (eukaryotes and bacteria)	0.92 (0.85, 0.99)*
	Peptidoglycan biosynthesis V (β-lactam resistance)	0.95 (0.90, 1.00)
	(S)-propane-1,2-diol degradation	1.09 (1.01, 1.19)*
Asthma	Superpathway of heme b biosynthesis from glycine	0.87 (0.79, 0.96)**
	L-glutamate degradation V (via hydroxyglutarate)	0.87 (0.79, 0.97)*
	4-aminobutanoate degradation V	0.76 (0.61, 0.97)*

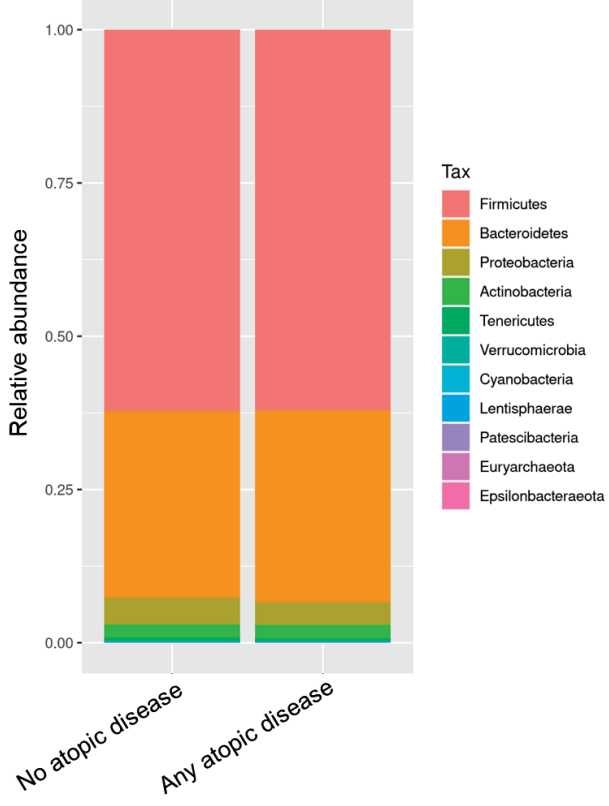
The number of subjects included for the unadjusted ANCOM and unadjusted logistic regression analyses were for eczema (n=1,293), sensitization for food allergens (n=1,245), sensitization for inhalant allergens (n=1,251), physician-diagnosed food allergy (n=1,239), physician-diagnosed inhalant allergy (n=1,270), lung function (n=1,322), and asthma (n=1,268). No associations were found for stool microbial functional pathways with eczema, sensitization for food allergens, and lung function at age 10 years. *p-value <0.05, **p-value<0.01.



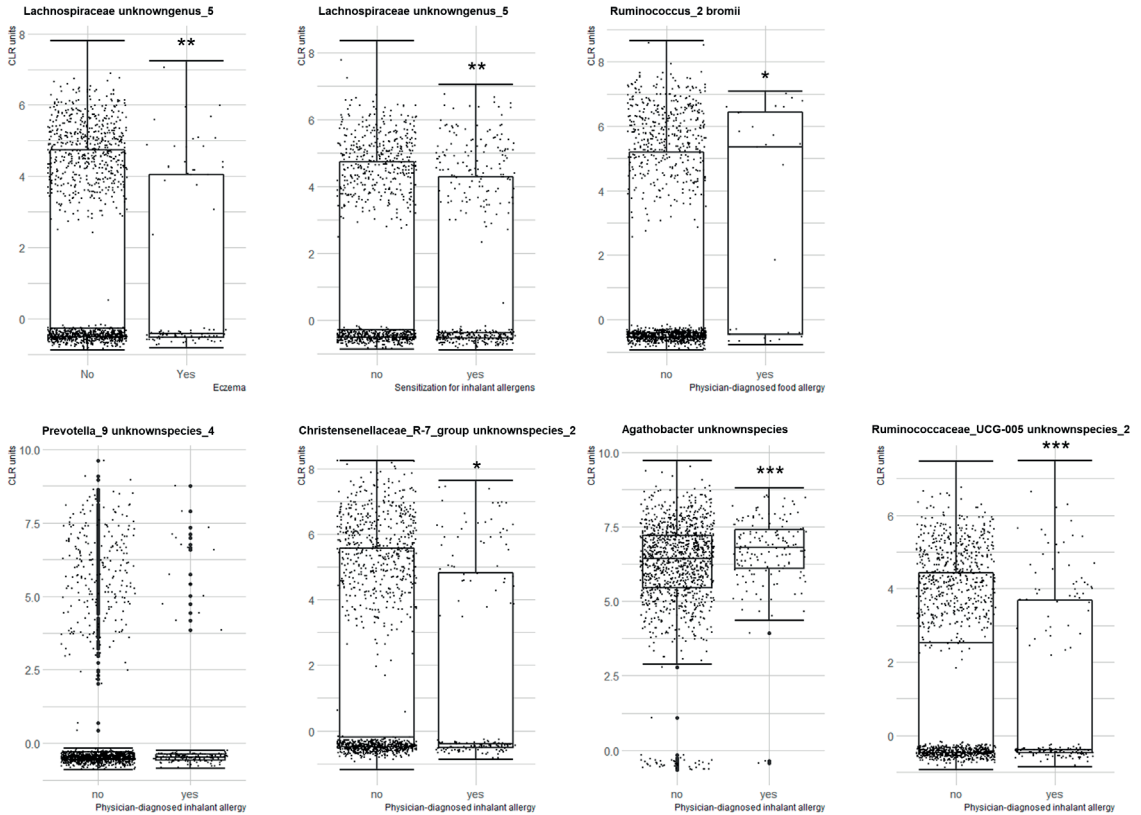
Supplementary Figure 1. Flow chart of participants included for analysis.



Supplementary Figure 2. Study design



Supplementary Figure 3. Relative abundance of phyla for children with any atopic disease outcome compared to those without any atopic diseases.



Supplementary Figure 4. Associated ASVs from adjusted ANCOM analyses for each atopic disease

Boxplots showing the centered-log ratio (CLR) transformed relative abundance of associated ASVs with atopic diseases from adjusted ANCOM analyses. *p-value<0.05, **p-value<0.01, ***p-value <0.001

Further detailed online resources can be found in the published article online:
https://www.sciencedirect.com/science/article/pii/S0091674921005637?via%3Dihub

3

Consequences of eczema phenotypes

3.1

Eczema phenotypes and risk of allergic and respiratory conditions in school age children

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Chen Hu
Tamar Nijsten
Evelien R. van Meel
Nicole S. Erler
Christophe Piketty
Nicolette W. de Jong
Suzanne G.M.A. Pasmans
Johan C. de Jongste
Liesbeth Duijts

ABSTRACT

Background Eczema phenotypes based on eczema onset and persistence might better identify groups prone to allergic and respiratory conditions than a binary definition of eczema. We examined the associations of childhood eczema phenotypes with allergic sensitization, allergy, asthma and lung function at school age.

Methods This study among 4,277 children was embedded in a multi-ethnic population-based prospective cohort study. Five eczema phenotypes (never, early transient, mid-transient, late transient, persistent) based on parental-reported physician-diagnosed eczema from age 6 months until 10 years were identified. At age 10 years, allergic sensitization was measured by skin prick tests, physician-diagnosed allergy and asthma by parent-reported questionnaires, and lung function by spirometry. Adjusted linear, logistic and multinomial regression models were applied.

Results Compared with never eczema, all eczema phenotypes were associated with increased risks of asthma (odds ratios (OR) range (95% confidence interval): 2.68 (1.58,4.57) to 11.53 (6.65,20.01)), food and inhalant allergic sensitization (1.72 (1.25, 2.36) to 12.64 (7.20, 22.18)), and physician-diagnosed inhalant allergy (1.92 (1.34, 2.74) to 11.91 (7.52, 18.86)). Strongest effect estimates were observed of early and persistent eczema with the risk of physician-diagnosed food allergy (OR (95% CI) 6.95 (3.76,12.84) and 35.05 (18.33,70.00), respectively) and combined asthma and physician-diagnosed allergy (7.11 (4.33, 11.67) and 29.03 (15.27, 55.22), respectively). Eczema phenotypes were not associated with lung function measures.

Conclusion Eczema phenotypes were differentially associated with risks of respiratory and allergic conditions in school-aged children. Children with early transient and persistent eczema might benefit from more intense follow-up for early identification and treatment of asthma and allergies.

BACKGROUND

Childhood eczema is a chronic disease with variable onset and persistence over time. The prevalence of eczema is up to 25% in infancy and diminishes over time.[1] Eczema is strongly associated with asthma and allergic sensitization.[2] It has been suggested that children with eczema and food allergies in early life develop asthma and allergic rhinitis in later life, which has been referred to as the atopic march.[3] However, previous results of longitudinal cohorts only found a small proportion of children with eczema that follow this atopic march.[4] This might partly be explained by the definition of eczema used in these studies. In recent years, eczema phenotypes have been introduced in epidemiologic research to replace the binary definition of eczema, as they incorporate the variability in age of onset and persistence of eczema, and therefore allow identification of specific underlying risk factors which can be used to optimize personalized preventative strategies and improve public health.[5] Also, eczema phenotypes could better identify children that may be at risk for developing asthma and allergy. Results of previous studies using longitudinal birth cohorts showed that all identified eczema phenotypes in early life were associated with up to 7-fold increased risks of asthma and allergy in later life, compared to the never eczema phenotype.[6, 7] The strongest association was observed for the persistent eczema phenotype in relation to asthma and allergy. However, the eczema phenotypes are not yet determined in non-Caucasian children, related to lung function or comprehensive allergy outcomes in older childhood.

Therefore, we examined in a multi-ethnic population-based prospective cohort of 4,277 children the associations of eczema phenotypes from birth until 10 years with lung function, asthma, allergic sensitization, and allergy at school-age.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands[8]. The study has been approved by the Medical Ethical Committee of the Erasmus MC University Medical Centre in Rotterdam. Written informed consent was obtained from parents or legal guardians. Children were excluded from the current analyses if information was missing on physician-diagnosed eczema for more than 3 time points and if information on lung function, asthma and allergic sensitization were missing. A total of 4,277 children were included for the current analyses (Supplementary Figure 1).

Eczema phenotypes

Information on eczema was obtained from parental-reported questionnaires at the age of 6 months, and 1, 2, 3, 4 and 10 years ('Was your child diagnosed with eczema in the last 6 months/last year by a general practitioner or physician in the hospital?')[9]. As previously described, in children with available data on at least 3 time points between age 6 months to 10 years, latent class growth analysis was used to assign children to their latent classes based on their respective posterior probabilities.[10] Five eczema phenotypes were identified based on the various eczema trajectories: never, early transient, mid-transient, late transient and persistent eczema (Supplementary Figure 2). Data on ever eczema was collected by parental-reported questionnaires at 10 years of age ('Has your child ever had eczema diagnosed by a doctor?').

Lung function, asthma and allergy

Children visited the research center at a median age of 9.7 years (2.5-97.5th percentile range 9.3-10.3 years). Information on lung function was measured by spirometry and included forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and forced expiratory flow after exhaling 75% of FVC (FEF_{75}). Lung function measures were converted into sex-, height-, age-, and ethnicity-adjusted z-scores[11, 12]. Information on current asthma, and physician-diagnosed inhalant and food allergy were adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)[13]. Current asthma (no; yes) was defined as ever diagnosis of asthma with wheezing or medication use in the past 12 months at 10 years of age. Parental reported questionnaires were used to define physician-diagnosed inhalant allergy ("Was your child ever diagnosed by a physician with an allergy to pollen (hay fever)/house dust mite/cat/dog?") (no; yes) and food allergy ("Was your child ever diagnosed by a physician with an allergy to cashew nut/peanut?") (no; yes) at age 10 years. Additionally, information on allergic rhinitis, a more detailed question on inhalant allergy, was obtained by a parental reported questionnaire ("Did your child had any sneezing, running nose or stuffed nose in the last 12 months, even though he or she did not have a cold or flu?" (no; yes). Information on allergic sensitization was collected by skin prick tests using the scanned area method.[14, 15] We examined the most prevalent food allergens for children at age 10 years at a population-based level, and therefore allergens for milk and egg were excluded.[16, 17] Inhalant allergens included house dust mite, 5-grass mixture, birch, cat, and dog. Food allergens included hazelnut, cashew nut, peanut and peach. Details on the collection of lung function, asthma and allergy measures are provided in the supplementary material.

Covariates

Information on parity, maternal education, and parental history of eczema, allergy or asthma was available from parental questionnaires obtained at enrolment. Child's

sex was obtained from midwives and hospital records, and ethnic origin based on the parents' country of birth according to Statistics Netherlands.[18] Postnatal questionnaires provided information on breastfeeding at 2, 6 or 12 months after birth.

Statistical analysis

Linear, logistic and multinomial regression models were used to examine the association of eczema phenotypes with lung function measures, risk of asthma, allergic sensitization or physician-diagnosed allergy, and combined allergic outcomes, respectively, using the packages 'mice' (version 3.3.0) , 'stats' (version 3.5.2) and 'nnet' (version 7.3-12) in R version 3.5.2.[19-21] The analyses were adjusted for potential confounders, selected from literature if they were related with both eczema phenotypes and the outcome and were not in the causal pathway. In order to examine inhalant allergies in detail, we also examined the correlation between physician-diagnosed inhalant allergy and allergic rhinitis, and the associations of eczema phenotypes with allergic rhinitis. To study the role of ethnicity in more detail, we performed a sensitivity analysis by stratifying for ethnicity (European or non-European). We only present the results based on imputed data, because the size and direction of effects were similar in complete-case-analysis. We did not adjust for multiple testing, because the respiratory and allergic measures were related to each other, and examined under the same hypothesis. More information on the statistical analyses is provided in the supplementary material. All measures of association are presented as pooled z-score change or odds ratios (OR) with their corresponding 95% confidence intervals (95%CI).

RESULTS

Subject characteristics

Characteristics of children and their mothers are summarized in Table 1. For each eczema phenotype, the prevalence of current asthma, physician-diagnosed food allergy and inhalant allergy are presented in Figure 1. Co-occurrence of these comorbidities was most prevalent in the persistent eczema group (range 1% to 19%). Main results of loss-to-follow-up analysis showed that children not included in the analyses more often had mothers of younger age, multiparity, lower education and no history of eczema, allergy or asthma, and more often had lower birth weight, a male sex and a non-European ethnicity mostly of Moroccan, Turkish and Cape Verdean ethnicity (Supplementary Table 1).

Table 1. Characteristics of children and their mothers

	Subjects n=4,277
Maternal characteristics	
Age at enrollment, years mean (SD)	31.7 (4.5)
Parity, nulliparous % (n)	59 (2,526)
Maternal education, higher % (n)	59 (2,510)
History of eczema, allergy and asthma, yes % (n)	61 (2,597)
Child characteristics	
Sex, female % (n)	51 (2,181)
Gestational age at birth, weeks median (2.5-97.5th percentile)*	40.1 (35.5-42.3)
Birth weight, grams mean (SD)*	3443.1 (566.9)
Ethnicity, non-European % (n)	24 (1,006)
Breastfeeding, ever % (n)	93 (3,961)
Eczema, ever % (n)*	23 (859)
Eczema phenotypes % (n)	
Never	76 (3,229)
Early transient	9 (363)
Mid-transient	6 (259)
Late transient	8 (333)
Persistent	2 (93)
Current asthma, yes % (n)†	5 (203)
Inhalant sensitization, yes % (n)†	32 (985)
Food sensitization, yes % (n)†	7 (209)
Physician diagnosed inhalant allergy, yes % (n)†	12 (447)
Allergic rhinitis, yes % (n)*	20.6 (734)
Physician diagnosed food allergy, yes % (n)†	2 (79)
Lung function, Z-scores mean (SD)*	
FVC	0.18 (0.91)
FEV_1	0.13 (0.96)
FEV_1/FVC	-0.12 (0.95)
FEF_{75}	-0.00 (0.91)

Values are percentages (absolute values), mean (SD) or median (2.5-97.5th percentile) after imputation. *Data was missing and not imputed for gestational age at birth (0.2%), birth weight (0.1%) ever eczema (11.6%) allergic rhinitis (26.9 %), and lung function (11.5%). †Data on the following outcomes were not imputed for the individual analysis and were missing for: current asthma (9.7%), inhalant (26.9%) and food sensitization (27.1%), physician diagnosed inhalant (10.9%) and food allergy (12.7%). They were imputed for the combined outcome analysis and values are for current asthma (yes) 6% (n=237), inhalant sensitization (yes) 33% (n=1,394), food sensitization (yes) 8% (n=336), physician-diagnosed inhalant allergy (yes) 12% (n=521) and physician-diagnosed food allergy (yes) 3% (n=105)

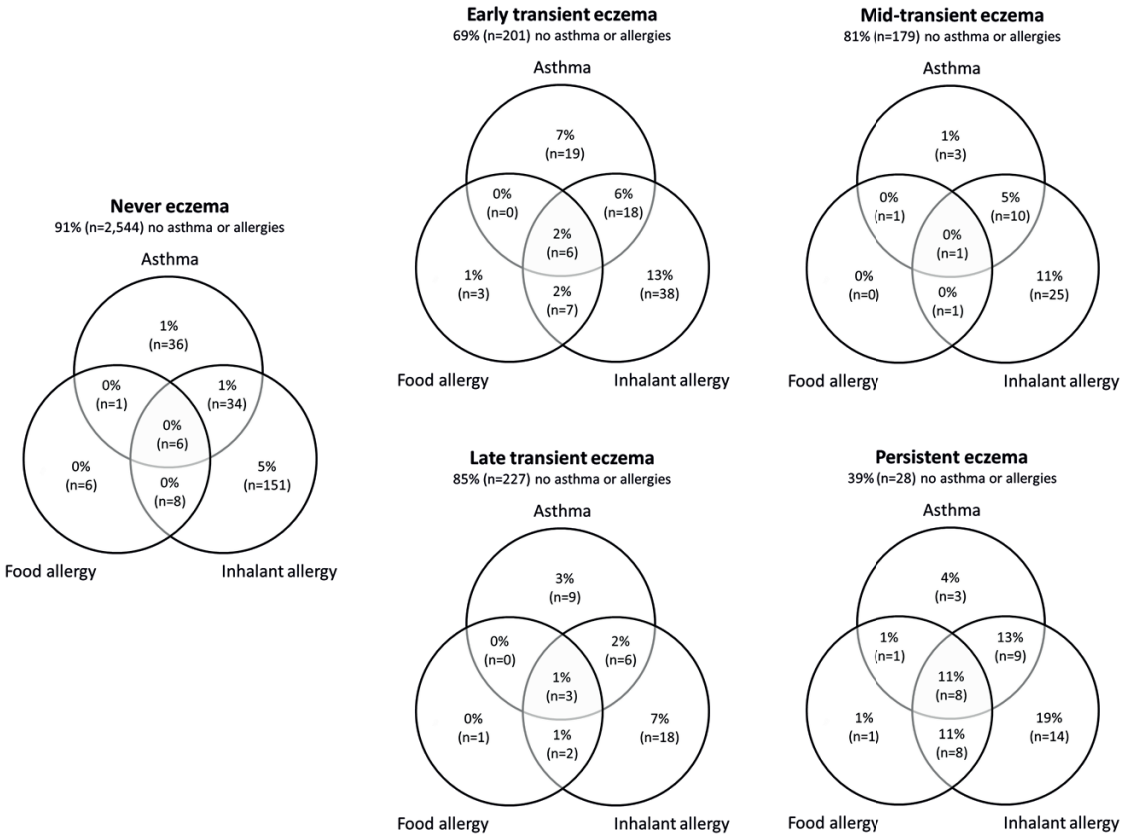

Figure 1. Prevalence of current asthma, physician diagnosed food and inhalant allergy in eczema phenotype

Values are percentages (absolute values) and based on observed data. n = number of participants with information on current asthma or physician diagnosed allergies, and at least 3 eczema measurements.

Table 2. Associations of eczema phenotypes with lung function and current asthma in children at age 10 years

	FVC Z-score (95%CI)	FEV_1 Z-score (95%CI)	FEV_1/FVC Z-score (95%CI)	FEF_{75} Z-score (95%CI)	Current asthma at 10 years Odds Ratio (95%CI)
Never eczema	Reference	Reference	Reference	Reference	Reference
Ever eczema	**0.08 (0.01,0.16)**	**0.08 (0.00,0.16)**	0.00 (-0.07,0.08)	0.02(-0.05,0.09)	**6.38 (4.61,8.83)**
Never	Reference	Reference	Reference	Reference	Reference
Early transient	0.04 (-0.07,0.14)	0.00 (-0.10,0.11)	-0.02 (-0.23,0.19)	-0.05 (-0.15,0.05)	**4.82 (3.29,7.08)**
Mid-transient	-0.07 (-0.20,0.05)	-0.08 (-0.21,0.05)	-0.04 (-0.15,0.07)	-0.03 (-0.15,0.09)	**2.68 (1.58,4.57)**
Late transient	0.11 (0.00,0.21)	0.05 (-0.06,0.16)	-0.11 (-0.22,0.00)	-0.03 (-0.13,0.08)	**3.07 (1.94,4.87)**
Persistent	0.04 (-0.16,0.24)	0.00 (-0.21,0.21)	-0.02 (-0.23,0.19)	-0.00 (-0.20,0.19)	**11.53 (6.65,20.01)**

Values are Z-score mean differences for lung function measurements and odds ratios (95% confidence intervals) for current asthma from linear and logistic regression models for never/ever eczema. Values are average Z-score mean differences for lung function measurements and average odds ratios (95% confidence intervals) for current asthma from linear and logistic regression models, respectively, after multiple sampling based on 150 imputed datasets for eczema phenotypes. Lung function outcomes are force expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), force expiratory flow at 75% of the exhaled FVC (FEF_{75}). Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex, ethnicity and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.

Eczema phenotypes, lung function and current asthma

Compared with never eczema, ever eczema was associated with a higher FVC and FEV_1 (Z score change (95%CI): 0.08 (0.01,0.16) to 0.08 (0.00,0.16), respectively), but not with FEV_1/FVC and FEF_{75}. Ever eczema was associated with an increased risk of current asthma (OR (95%CI): 6.38 (4.61,8.83)) (Table 2). When examining eczema phenotypes, we observed that compared with the never eczema phenotype, only late transient eczema was associated with a higher FVC (Z score change (95%CI): 0.11 (0.00,0.21)) (Table 2). All eczema phenotypes were associated with an increased risk of current asthma at the age of 10 years with the strongest effect estimates for early transient and persistent eczema (OR (95% CI): 4.82 (3.29,7.08) and 11.53 (6.65,20.01)). Similar size and direction of effect estimates were observed among children of European and non-European ethnicity (Supplementary Tables 2 and 3).

Eczema phenotypes, allergic sensitization and physician-diagnosed allergies

Compared with never eczema, ever eczema was associated with increased risks of allergic sensitization and physician-diagnosed allergies for both inhalant and food allergens. The strongest association was observed for ever eczema with physician diagnosed food allergy (OR (95%CI): 11.89 (6.85, 20.61)) (Table 3). Of the eczema phenotypes, the early transient and persistent phenotypes were most strongly associated with increased risks of inhalant allergic sensitization (OR (95%CI): 2.62 (2.01, 3.42) and 4.53 (2.65, 7.51)), food allergic sensitization (OR (95%CI): 5.73 (3.94, 8.31) and 12.64 (7.20, 22.18)), physician-diagnosed inhalant allergy (OR (95%CI): 3.72 (2.78, 4.97) and 11.91 (7.52, 18.86)) and

Table 3. Associations of eczema phenotypes with allergic sensitization and physician-diagnosed allergies in children at age 10 years

	Inhalant sensitization Odds ratio (95% CI)	**Food sensitization Odds ratio (95% CI)**	**Physician-diagnosed inhalant allergy Odds ratio (95% CI)**	**Physician-diagnosed food allergy Odds ratio (95% CI)**
Never eczema	Reference	Reference	Reference	Reference
Ever eczema	**2.91(2.41, 3.52)**	**4.90 (3.60, 6.67)**	**4.54 (3.65, 5.63)**	**11.89 (6.85, 20.61)**
Never	Reference	Reference	Reference	Reference
Early transient	**2.62 (2.01, 3.42)**	**5.73 (3.94, 8.31)**	**3.72 (2.78, 4.97)**	**6.95 (3.76,12.84)**
Mid-transient	**1.72 (1.25, 2.36)**	**2.13 (1.21, 3.76)**	**2.66 (1.86, 3.80)**	1.44 (0.43, 4.80)
Late transient	**1.77 (1.33, 2.35)**	**2.52 (1.56, 4.07)**	**1.92 (1.34, 2.74)**	**4.50 (2.19, 9.28)**
Persistent	**4.53 (2.65, 7.51)**	**12.64 (7.20, 22.18)**	**11.91 (7.52, 18.86)**	**35.05 (18.33,70.00)**

Values are odds ratios (95% confidence intervals) from logistic regression models for never/ever eczema and average odds ratios (95% confidence intervals) from logistic regression models after multiple sampling based on 150 imputed datasets for eczema phenotypes. Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex, ethnicity and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.

physician-diagnosed food allergy (OR (95%CI): 6.95 (3.76,12.84) and 35.05 (18.33,70.00)) (Table 3). Physician-diagnosed inhalant allergy and allergic rhinitis were correlated (Cramer's V (Chi-square p-value) 0.50 (<0.001)). The observed effect estimates of the associations of eczema phenotypes with allergic rhinitis were in the same direction, but less greater, versus those of eczema phenotypes with physician-diagnosed inhalant allergy (OR range (95%CI): 1.43 (1.02, 2.00) and 4.91 (3.14, 7.66) versus 1.92 (1.34, 2.74) and 11.91 (7.52, 18.86), respectively) (Supplementary Tables 4). Similar size and direction of effect estimates were observed among children of European and non-European ethnicity (Supplementary Tables 2 and 3). Effect estimates were in the same direction and stronger if a child had both allergic sensitization and physician-diagnosed allergy (Supplementary Table 5).

Table 4. Association of eczema phenotypes with combined asthma and physician-diagnosed allergy groups in children at age 10 years.

	Asthma, but no allergy n=97	**Allergy, but no asthma** n=413	**Asthma and allergy** n=140
Never eczema	Reference	Reference	Reference
Ever eczema	**5.83 (3.49, 9.74)**	**4.03 (3.17, 5.11)**	**8.98 (5.89, 13.69)**
Never	Reference	Reference	Reference
Early transient	**5.36 (3.07, 9.36)**	**3.68 (2.67, 5.08)**	**7.11 (4.33, 11.67)**
Mid-transient	1.37 (0.45, 4.19)	**2.21 (1.47, 3.32)**	**4.31 (2.33, 7.99)**
Late transient	**2.94 (1.47, 5.89)**	**1.76 (1.18, 2.64)**	**3.48 (1.88, 6.44)**
Persistent	**5.23 (1.55, 17.63)**	**10.02 (5.92, 16.96)**	**29.03 (15.27, 55.22)**

Values are odds ratios (95% confidence intervals) from logistic regression models for never/ever eczema and average odds ratios (95% confidence intervals) from multinomial regression models after multiple sampling based on 150 imputed datasets for eczema phenotypes. Reference group is no asthma and no allergy (n=3,627). n = number of participants with information on at least 3 eczema measurements. Missing data on asthma and physician-diagnosed allergy was imputed. Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex, ethnicity and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.

Eczema phenotypes, asthma and physician-diagnosed allergy combined

Compared with never eczema, ever eczema was associated with increased risks of both asthma only and physician-diagnosed allergy only (OR (95%CI): 5.83 (3.49, 9.74) and 4.03 (3.17, 5.11)), and most strongly with asthma and physician-diagnosed allergy combined (8.98 (5.89, 13.69)) (Table 4). Compared with never eczema phenotypes, early transient and persistent eczema were most strongly associated with asthma only (OR (95%CI): 5.36 (3.07, 9.36) and 5.23 (1.55, 17.63)), physician-diagnosed allergy only (3.68 (2.67, 5.08) and 10.02 (5.92, 16.96)), and asthma and physician-diagnosed allergy combined (7.11 (4.33, 11.67) and 29.03 (15.27, 55.22)). Effect estimates for eczema phenotypes were in the same direction and higher odds were observed when physician-diagnosed food

and inhalant allergy were combined and when physician-diagnosed food and inhalant allergies were combined with asthma (Supplementary Table 6).

DISCUSSION

In this multi-ethnic population-based prospective cohort study, eczema phenotypes were differentially associated with the risk of allergic and respiratory conditions in school-aged children. The early transient and persistent eczema phenotypes were most consistently associated with asthma, allergic sensitization, and physician-diagnosed allergies, including allergic rhinitis. Results were similar for children of European and non-European ethnicity. Stronger effect estimates were observed for early transient and persistent eczema phenotypes with food allergy related measures and combined asthma and physician-diagnosed allergies. Compared with never eczema, ever eczema was associated with higher FVC and FEV_1, but not with FEV_1/FVC. Eczema phenotypes were not associated with any lung function measurement.

Comparison with previous studies

When comparing results with previous studies, the difference in eczema phenotype definition and follow-up duration need to be taken into account. Previous cohort studies showed that children with early-onset and persistent eczema phenotypes have increased risks of asthma at ages 6 to 13 year.[6, 7] Results for mid- and late transient eczema phenotypes and the risk of asthma are inconsistent. Our observations in a multi-ethnic population are in line with previous findings and support that children with any eczema phenotype, but especially those with early onset and persistent eczema have increased risks of asthma at school-age.[22, 23] While eczema is strongly related to asthma and therefore hypothetically also with altered lung function, the relationship between eczema and lung function has not been studied. We observed that children with ever eczema had slightly higher FEV_1 and FVC, but no changes in FEV_1/FVC. These findings might be incidental, since there were no associations of eczema phenotypes with lung function measures. Other mechanisms might underlie the observed associations of ever eczema and eczema phenotypes with asthma, such as inhalant allergies and possible modulating effects of early allergic sensitization and allergic rhinitis.[24] Also all children included in our analysis had higher FEV_1 and FVC z-scores, which might be explained by a relatively healthy study population or well-controlled asthma.

Previous studies showed that persistent eczema was associated with elevated total Immunoglobulin E levels at ages 7-8 years, and with an increased risk of sensitization to inhalant allergens, but not to food allergens at age 6 years.[6, 7] We showed that children

with early transient and persistent eczema phenotypes had both allergic sensitization and physician-diagnosed allergies, with the strongest effect estimates for food allergy at age 10 years. These observed differences might be due to differences in number of children included for analysis, food allergy prevalence, eczema phenotypes definition and because our population has a longer follow-up which allowed the identification of more diverse phenotypes. A cohort study in children until age 6 years showed that children with early transient and persistent eczema had increased risks of food allergy and allergic rhinitis.[7] We observed similar results among children until age 10 years with allergic sensitization and physician-diagnosed food and inhalant allergies. Many children among the early transient and persistent eczema phenotype group had both asthma and multiple allergic conditions, and a large percentage of these (31% - 61%) had at least one diagnosis of asthma, food or inhalant allergy. Therefore, our results do not support the atopic march hypothesis in all children with eczema, but does show that in particular children with early transient and persistent eczema are likely to develop asthma and/or allergies later in childhood.

Possible mechanisms

Early transient and especially persistent eczema consistently showed the strongest associations with asthma and allergic conditions. A common trait of both phenotypes is the early onset of eczema, suggesting that the period before the age of 2 years of age was important for the development of asthma and allergic conditions. Maturation rates of the skin, lungs and immune system from birth until 2 years are high and any change or disruption of these maturation processes might have long term consequences.[25] Proposed mechanisms include dysfunction of the epithelial barrier due to microbial and/or genetic factors and transcutaneous sensitization, leading to type 2 inflammation, and thereby predisposing to asthma and allergic conditions.[25-27] Our recent study showed an association of the four most common filaggrin mutations in Europeans with early and late transient eczema, but not with persistent eczema.[10] Unfortunately, we were not able to study filaggrin mutations as mediators for the association of eczema phenotypes with asthma and allergic conditions due to lack of power. Also sensitivity analysis in more detailed non-European ethnic subgroups was not possible due to small sample size. Therefore, future studies with larger sample sizes are needed to examine the potential mediating role of filaggrin mutations on the associations of eczema phenotypes with asthma and allergic conditions, and the role of different ethnicities.

Strengths and limitations

The strengths of this study include the eczema phenotypes among a multi-ethnic population with detailed information on asthma, lung function, and multiple allergic conditions. By using multivariate regression models with multiple imputation and

sampling we achieved more precise and unbiased effect estimates. However, some methodological considerations need to be taken into account. Children not included in the analyses partly had less favourable socio-economic factors and more often parents with no history or eczema, allergy or asthma. Selection bias due to lost to follow-up might have been present if the associations of eczema phenotypes with respiratory and allergic conditions were different in children that were not included in the analyses compared to the children that were included in the analyses. We aimed to minimize bias by imputation methods.[20] Despite validated questions, misclassification of eczema, asthma and physician diagnosed allergies remains possible due to self-response.[13, 28] We included the most relevant allergens for children of age 10 years at population level, and excluded allergens with low sensitization rates at this age, such as milk and egg.[16, 17] Residual confounding might be present since there might be factors not measured or not included in our analysis. For example, there was no information available to determine the severity of eczema. Furthermore, we were unable to perform our analyses in more detailed ethnic groups due to lack of power.[29]

Conclusion

Eczema phenotypes were differentially associated with risks of asthma and allergic conditions among school-aged children, and were similar in children from European and non-European ethnicity. The strongest and most consistent associations were found in children with early transient and persistent eczema. This suggests that children with early transient and persistent eczema might benefit from more intense follow-up for early identification and treatment of asthma and allergies.

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Supplementary Table 1. Characteristics of children and their mothers of those included and not included in the analyses

	Included n=4,277	**Not included** n=3,116	**p-value for difference**
Maternal characteristics			
Age at enrollment, years mean (SD)	31.7 (4.5)	29.0 (5.5)	<0.001
Parity % (n)			
Nulliparous	59 (2,468)	51 (1,536)	<0.001
Multiparous	41 (1,696)	49 (1,460)	
Maternal education % (n)			
Primary or secondary	41 (1,677)	71 (1,847)	<0.001
Higher	59 (2,446)	29 (745)	
History of eczema, allergy or asthma % (n)			
No	49 (2,010)	55 (1,435)	<0.001
Yes, at least one parent	51 (2,092)	45 (1,168)	
Child characteristics			
Sex % (n)			
Male	49 (2,096)	52 (1,610)	0.024
Female	51 (2,181)	48 (1,505)	
Gestational age at birth, weeks median (2.5-97.5th percentile)	40.1 (35.5-42.3)	40 (35.3-42.3)	<0.001
Birth weight, grams mean (SD)	3443.1 (566.9)	3363.2 (577.9)	<0.001
Ethnicity % (n)			
European	76 (3,252)	51 (1,467)	<0.001
Non-European	24 (1,011)	49 (1,416)	
Breastfeeding % (n)			
Never	7 (303)	11 (161)	<0.001
Ever	93 (3,858)	90 (1,377)	
Eczema phenotypes % (n)			0.586
Never	76 (3,229)	77 (470)	
Early transient	9 (363)	8 (48)	
Mid-transient	6 (259)	5 (29)	
Late transient	8 (333)	8 (47)	
Persistent	2 (93)	3 (17)	
Eczema % (n)			1.000
Never	77 (2,923)	77 (714)	
Ever	23 (859)	23 (210)	
Current asthma % (n)*			0.045
No	95 (3,658)	93 (406)	

Supplementary Table 1. Characteristics of children and their mothers of those included and not included in the analyses (continued)

	Included n=4,277	**Not included** n=3,116	**p-value for difference**
Yes	5 (203)	7 (97)	
Inhalant sensitization % (n)*			0.088
No	69 (2,142)	65 (681)	
Yes	32 (985)	35 (356)	
Food sensitization % (n)*			0.177
No	93 (2,908)	92 (951)	
Yes	7 (209)	8 (86)	
Physician diagnosed inhalant allergy % (n)*			0.100
No	88(3,362)	87 (804)	
Yes	12 (447)	14 (125)	
Allergic rhinitis % (n)*			<0.001
No	79.4 (2,837)	73.2 (698)	
Yes	20.6 (734)	26.8 (255)	
Physician diagnosed food allergy % (n)*			0.088
No	98 (3655)	98 (886)	
Yes	2 (79)	2 (19)	
Lung function, Z-scores mean (SD)*			
FVC	0.18 (0.91)	0.22 (1.01)	0.018
FEV_1	0.13 (0.96)	0.20 (1.04)	0.125
FEV_1/FVC	-0.12(0.95)	-0.06 (1.01)	0.037
FEF_{75}	-0.00 (0.91)	0.10 (0.98)	<0.001

Values are percentages (absolute values), mean (SD) or median (2.5-97.5th percentile) based on observed data.

Supplementary Table 2. Associations of eczema phenotypes with asthma, allergic sensitization and physician-diagnosed allergies in children of European ethnicity at age 10 years

	Current asthma **Odds Ratio (95%CI)** n=3,021	**Inhalant sensitization** **Odds ratio (95% CI)** n=2,355	**Food sensitization** **Odds ratio (95% CI)** n=2,349	**Physician-diagnosed inhalant allergy** **Odds ratio (95% CI)** n=2,996	**Physician-diagnosed food allergy** **Odds ratio (95% CI)** n=2,941
Never eczema	Reference	Reference	Reference	Reference	Reference
n= 2,319	n= 2,318/2,319	n= 1,708/2,319	n= 1,707/2,319	n= 2,292/2,319	n= 2,260/2,319
Ever eczema	**5.38 (3.69,7.85)**	**3.05 (2.49, 3.73)**	**4.84 (3.48,6.73)**	**4.86 (3.77,6.25)**	**10.06 (5.33, 18.99)**
n= 663	n= 615/663	n= 471/663	n= 466/663	n= 645/663	n= 626/663
Never	Reference	Reference	Reference	Reference	Reference
n=2,490	n=2,320/2,490	n=1,809/2,490	n=1,807/2,490	n=2,277/2,490	n= 2,244/2,490
Early transient	**5.12 (3.27, 8.03)**	**2.97 (2.22, 3.96)**	**6.00 (3.93, 9.15)**	**4.36 (3.14, 6.05)**	**6.47 (3.14,13.30)**
n=274	n=247/274	n=190/274	n=186/274	n=250/274	n=240/274
Mid-transient	**3.15 (1.75, 5.67)**	**1.82 (1.28, 2.61)**	**2.60 (1.40, 4.85)**	**3.18 (2.12, 4.75)**	1.90 (0.56, 6.46)
n=204	n=184/204	n=140/204	n=140/204	n=189/204	n=183/204
Late transient	**3.16 (1.81, 5.49)**	**1.70 (1.22, 2.35)**	**2.63 (1.52, 4.54)**	**3.18 (1.29, 3.07)**	**3.94 (1.66, 9.36)**
n=244	n=219/244	n=175/244	n=175/244	n=222/244	n=218/244
Persistent	**11.71 (6.05,22.70)**	**5.31 (2.88, 9.79)**	**15.03 (7.98, 28.31)**	**15.83 (9.08, 27.59)**	**33.45 (15.39, 72.69)**
n=59	n=51/59	n=41/59	n=41/59	n=58/59	n=56/59

Values are odds ratios (95% confidence intervals) from logistic regression models for never/ever eczema and average odds ratios (95% confidence intervals) from logistic regression models after multiple sampling based on 150 imputed datasets for eczema phenotypes. n = number of participants with information on current asthma, allergic sensitization or physician diagnosed allergies and at least 3 eczema measurements. Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.

Supplementary Table 3. Associations of eczema phenotypes with current asthma, allergic sensitization and physician-diagnosed allergies in children of non-European ethnicity at age 10 years

	Current asthma Odds Ratio (95%CI) n=840	**Inhalant sensitization Odds ratio (95% CI)** n=772	**Food sensitization Odds ratio (95% CI)** n=768	**Physician-diagnosed inhalant allergy Odds ratio (95% CI)** n=813	**Physician-diagnosed food allergy Odds ratio (95% CI)** n=793
Never eczema	Reference	Reference	Reference	Reference	Reference
n=604	n= 604/604	n= 482/604	n= 480/604	n= 591/604	n= 581/604
Ever eczema	**9.44 (4.89,18.22)**	**1.98 (1.37,2.86)**	**3.43 (2.03, 5.79)**	**3.58 (2.37, 5.41)**	**13.21 (4.43,39.34)**
n=196	n= 177/196	n= 139/196	n= 137/196	n= 185/196	n= 181/196
Never	Reference	Reference	Reference	Reference	Reference
n=739	n=628/739	n=582/739	n=579/739	n=599/739	n=588/739
Early transient	**4.88 (2.43, 9.78)**	**2.08 (1.26, 3.45)**	**4.17 (2.22, 7.83)**	**2.56 (1.45, 4.51)**	**6.22 (2.12, 18.26)**
n=89	n=67/89	n=66/89	n=66/89	n=66/89	n=65/89
Mid-transient	1.26 (0.36, 4.39)	1.40 (0.75, 2.61)	0.96 (0.25, 3.71)	1.51 (0.70, 3.27)	0.00 (0.00, >100)
n=55	n=49/55	n=42/55	n=42/55	n=50/55	n=48/55
Late transient	**2.79 (1.25, 6.23)**	**1.79 (1.07, 3.02)**	2.14 (0.99, 4.66)	**1.88 (1.01, 3.49)**	**5.11 (1.55, 16.86)**
n=89	n=71/89	n=60/89	n=60/89	n=70/89	n=65/89
Persistent	**6.61 (2.69, 16.27)**	**4.54 (1.87, 11.00)**	**9.18 (3.77, 22.37)**	**6.25 (2.83, 13.76)**	**20.41 (6.69, 62.29)**
n=34	n=25/34	n=22/34	n=21/34	n=28/34	n=27/34

Values are odds ratios (95% confidence intervals) from logistic regression models for never/ever eczema and average odds ratios (95% confidence intervals) from logistic regression models after multiple sampling based on 150 imputed datasets for eczema phenotypes. n = number of participants with information on current asthma, allergic sensitization or physician diagnosed allergies and at least 3 eczema measurements. Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.

Supplementary Table 4. Associations of eczema phenotypes with allergic rhinitis in children at age 10 years

	Allergic rhinitis Odds ratio (95% CI)
Never eczema	Reference
Ever eczema	**2.73 (1.93, 3.86)**
Never	Reference
Early transient	**2.30 (1.77, 2.99)**
Mid-transient	**1.43 (1.02, 2.00)**
Late transient	**1.55 (1.16,2.07)**
Persistent	**4.91 (3.14, 7.66)**

Values are odds ratios (95% confidence intervals) from logistic regression models for never/ever eczema and average odds ratios (95% confidence intervals) from logistic regression models after multiple sampling based on 150 imputed datasets for eczema phenotypes. Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.

Supplementary Table 5. Association of eczema phenotypes with combined allergic sensitization and physician-diagnosed allergy groups in children at age 10 years

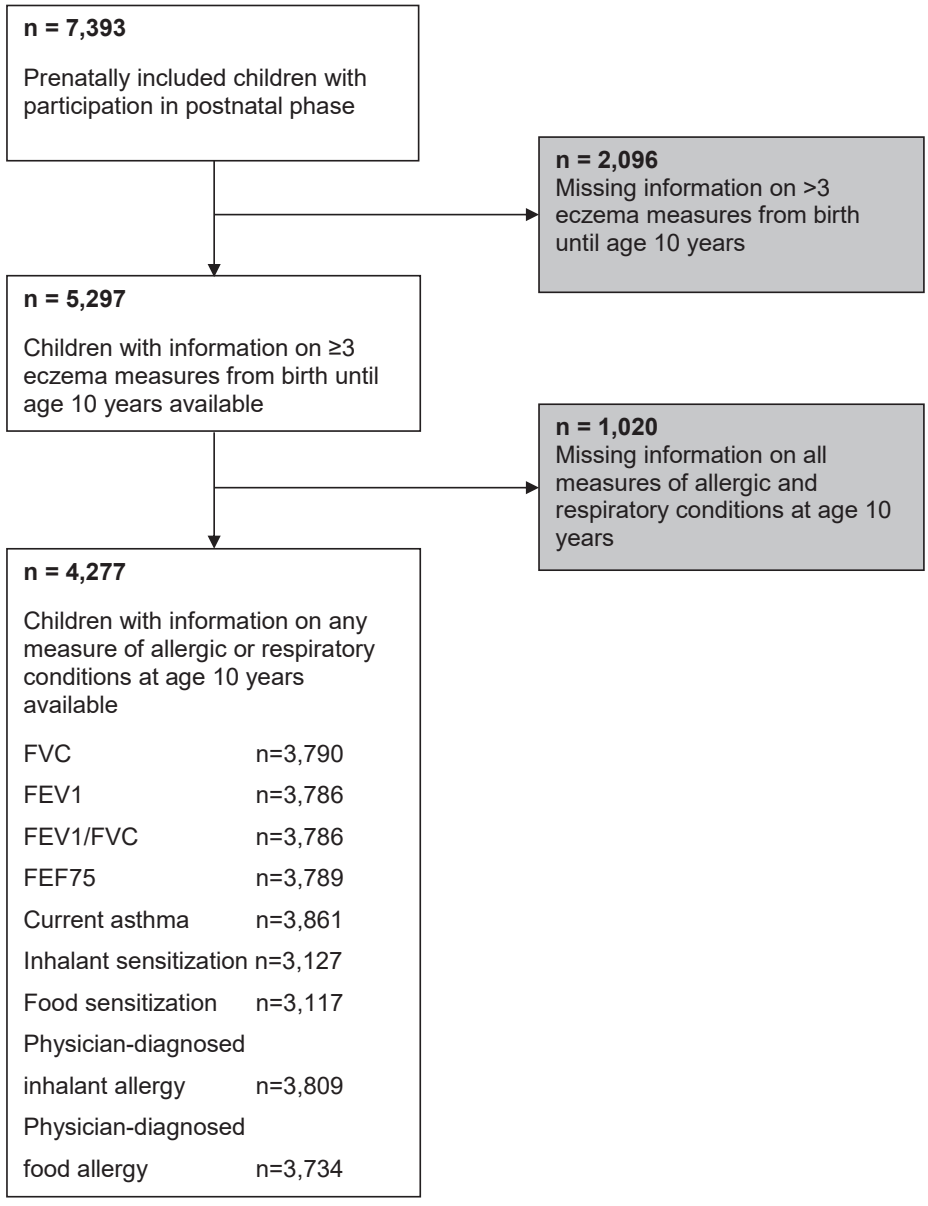
	Any allergic sensitization, but no allergy n= 1,005	**No allergenic sensitization, but any allergy** n= 101	**Any allergic sensitization and any allergy** n= 452
Never eczema	Reference	Reference	Reference
n=2,923	n=637/2,923	n=33/2,923	n=178/2,923
Ever eczema	**2.15 (1.74, 2.64)**	**4.02 (2.17, 7.43)**	**6.28 (4.94, 7.99)**
n=859	n=251/859	n=24/859	n=210/859
Never	Reference	Reference	Reference
n=3,229	n=714/3,229	n=63/3,229	n=223/3,229
Early transient	**2.39 (1.77, 3.24)**	**3.55 (1.62, 7.78)**	**5.95 (4.29, 8.23)**
n=363	n=114/363	n=15/363	n=93/363
Mid-transient	1.44 (0.99,2.08)	2.40 (0.99, 5.79)	**2.97 (1.98, 4.46)**
n=259	n=65/259	n=9/259	n=42/259
Late transient	**1.63 (1.18, 2.25)**	1.80 (0.76, 4.27)	**2.47 (1.68, 3.64)**
n=333	n=96/333	n=10/333	n=45/333
Persistent	**2.24 (1.11, 4.51)**	**4.93 (1.24, 19.56)**	**19.93 (11.42, 34.76)**
n= 93	n=17/93	n=4/93	n=50/93

Values are odds ratios (95% confidence intervals) from logistic regression models for never/ever eczema and average odds ratios (95% confidence intervals) from logistic regression models multiple sampling on 150 imputed datasets for eczema phenotypes. Reference group are children without any allergic sensitization or physician-diagnosed allergy (n =2,719). n = number of participants with information on at least 3 eczema measurements. Missing data on allergic sensitization and physician-diagnosed allergy was imputed. Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex, ethnicity and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.

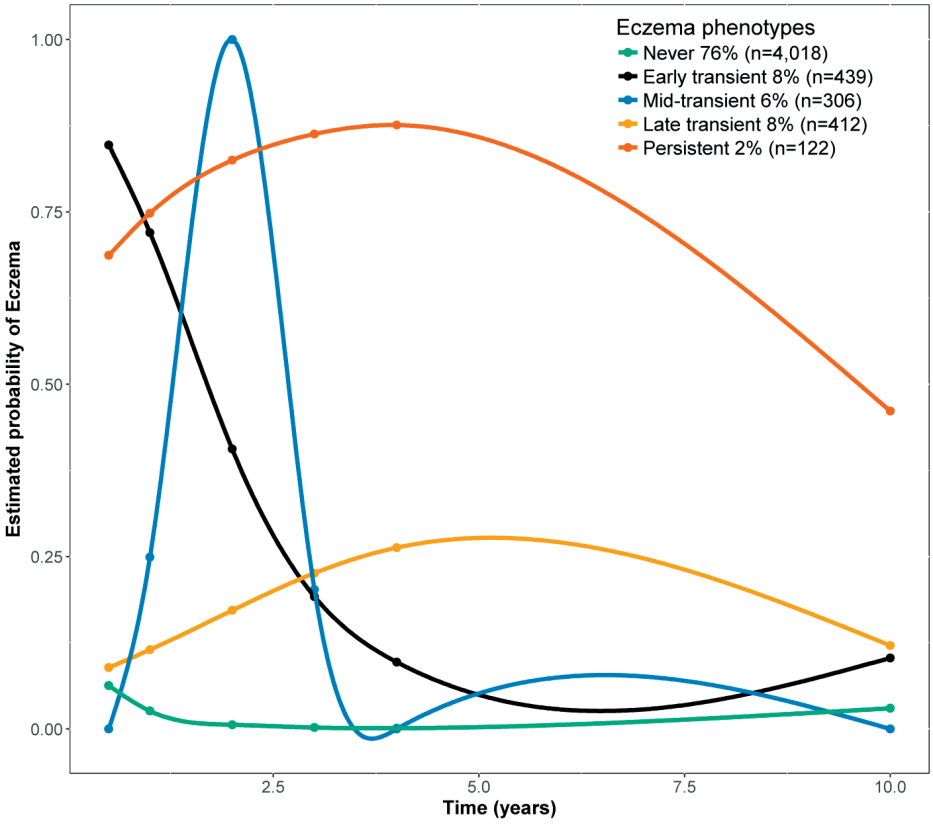
Supplementary Table 6. Association of eczema phenotypes with combined asthma, physician-diagnosed inhalant and food allergy groups in children at age 10 years

	Inhalant allergy, but no asthma and no food allergy n=345	**Food allergy, but no asthma and no inhalant allergy** n=25	**Inhalant and food allergy, but no asthma** n=43	**Asthma, but no inhalant and food allergy** n=97	**Asthma and inhalant allergy, but no food allergy** n=103	**Asthma and food allergy, but no inhalant allergy** n=7	**Asthma, inhalant and food allergy** n=31
Never n=3,229	**Reference**	**Reference**	**Reference**	**Reference**	**Reference**	**Reference**	**Reference**
Early transient n=363	**3.22 (2.27, 4.56)**	**5.72 (1.67,19.60)**	**9.03 (3.61, 22.57)**	**5.36 (1.55, 9.35)**	**6.07 (3.44, 10.71)**	7.14 (0, >100)	**11.84 (3.82, 36.70)**
Mid-transient n=259	**2.37 (1.56, 3.59)**	0 (0,>100)	1.42 (0.19, 10.71)	1.37 (0.45, 4.19)	**4.35 (2.20, 8.61)**	10.15(0.68, >100)	2.82 (0.36, 22.29)
Late transient n=333	1.56 (0.92, 2.31)	2.11 (0.34, 13.13)	**6.08 (2.22, 16.61)**	**2.94 (1.47, 5.90)**	**2.82 (1.34, 5.95)**	0.00 (0, >100)	**7.24 (1.92, 27.24)**
Persistent n=93	**6.69 (3.63,12.33)**	**8.19 (1.04,64.52)**	**67.07(26.13,>100)**	**5.23 (1.55,17.64)**	**17.14 (7.68, 38.24)**	**67.57(4.60, >100)**	**97.86(31.97, >100)**

Values are odds ratios (95% confidence intervals) from logistic regression models for never/ever eczema and average odds ratios (95% confidence intervals) from multinomial regression models after multiple sampling based on 150 imputed datasets for eczema phenotypes. Reference group is no asthma and no physician-diagnosed food and inhalant allergy (n=3,627). n = number of participants with information on at least 3 eczema measurements. Missing data on asthma and physician-diagnosed allergy was imputed. Full models were adjusted for parental history of allergy, asthma or eczema, maternal education, parity, child's sex, ethnicity and breastfeeding. Bold values indicate statistical significance at the α=0.05 level.



Supplementary Figure 1. Flow chart of participants included for analysis



Supplementary Figure 2. Previously identified eczema phenotypes trajectories in 5,297 children from latent class growth analysis

3.2

Eczema phenotypes and risk of emotional and behavioural problems from birth until school age

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Chen Hu
Tamar Nijsten
Suzanne G.M.A. Pasmans
Johan C. de Jongste
Pauline W. Jansen
Liesbeth Duijts

ABSTRACT

Background Eczema phenotypes and emotional and behavioural problems are highly prevalent in childhood, but their mutual relationship is not fully clear.

Objective To examine the associations of eczema phenotypes with school-age emotional and behavioural problems, and the bidirectional associations of eczema and emotional and behavioural problems from birth until 10 years.

Methods This study among 5,265 subjects was embedded in a prospective population-based cohort study. Never, early transient, mid-transient, late transient and persistent eczema phenotypes were identified based on parental-reported physician-diagnosed eczema from age 6 months until 10 years. Emotional (internalizing) and behavioural (externalizing) problems were measured repeatedly using the Child Behavior Checklist from age 1.5 to 10 years. Cross-lagged models were applied for bidirectional analyses.

Results All eczema phenotypes were associated with more internalizing problems and attentions problems at age 10 years, compared to never eczema (range Z-score difference (97% confidence interval): 0.14 (0.01,0.27) - 0.39 (0.18,0.60)). Children with early transient eczema had more aggressive behaviour symptoms at age 10 years (0.16 (0.04,0.27)). Bidirectional analysis showed that eczema at 0-2 years was associated with more internalizing and externalizing problems at ages 3-6 and 10 years, while reversely, only internalizing problems at 0-2 years was associated with an increased risk of eczema at age 10 years.

Conclusion Eczema phenotypes are very modestly associated with more somatic symptoms and attention problems at school age. Early transient eczema is associated with more aggressive behaviour symptoms. Directional effects seem from early life eczema to later life internalizing and externalizing problems rather than reversely.

INTRODUCTION

Eczema is a common skin disorder in early life. Both genetic and environmental early life factors affect the risk of childhood eczema.[1, 2] Psychopathological problems seem also to be involved.[3-5] We previously showed that maternal psychiatric symptoms during pregnancy were associated with an increased risk of childhood eczema, independent of maternal psychiatric symptoms after birth or paternal psychiatric symptoms.[6] Stress, as proxy of these problems, could shift the balance towards type 2 T-helper cells via the hypothalamic-pituitary-adrenal axis and sympathetic adrenomedullary system leading to more susceptibility for atopic diseases.[7] If emotional and behavioural problems of the child affect the risk of eczema is not clear. Previous systematic reviews and large survey studies showed that children in all age groups with eczema had more emotional problems, anxiety, depression, attention deficit hyperactivity disorders (ADHD) and conduct disorders, compared to children who never had eczema.[5, 8-10] Taking the onset of eczema into account, it was shown that children with early onset, transient and chronic eczema had increased risks of emotional and behavioural problems at age 10-15 years.[11, 12] Alternatively, previous studies showed that children with emotional and behavioural problems had more severe eczema and eczema exacerbations between ages 3-18 years.[3, 4, 9, 10] Thus, the effects between eczema and emotional and behavioural problems could be in both directions.[9] Eczema phenotypes take into account the variability of eczema onset and persistence within and between individuals over time.[13] Using eczema phenotypes might clarify which children with eczema in early life are most at risk of emotional and behavioural problems later in life. Additionally, bidirectional analyses could reveal whether eczema lead to emotional and behavioural problems or reversely.

Therefore, we aimed to examine the associations of eczema phenotypes from birth until age 10 years with school-age emotional and behavioural problems among 5,265 subjects of a population-based prospective cohort study. Next, we examined the associations of eczema with emotional and behavioural problems from birth until age 10 years bidirectionally.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands. Eligible women were those who were living in Rotterdam and who had an expected delivery data from April 2002 until January 2006, as described previously.[14] The children form a prenatally

recruited birth cohort that will be followed at least until young adulthood. Around the age of 10 years, all children were invited to visit our research centre in the Erasmus MC-Sophia Children's Hospital to participate in hands-on measurements, advanced imaging modalities, behavioural observations and biological sample collection. The study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam, Rotterdam, the Netherlands. Written informed consent was obtained from both parents or legal guardians. For the current analysis, children were included with available information on eczema on at least 3 or more time points to create eczema phenotypes and any assessment of emotional and behavioural problems from birth until the age of 10 years. A total of 5,265 children were included (Supplementary Figure 1).

Eczema phenotypes

Information on physician-diagnosed eczema was obtained from parental-reported questionnaires at the ages of 6 months, and 1, 2, 3, 4 and 10 years ('Was your child diagnosed with eczema in the last 6 months/last year by a general practitioner or physician in the hospital?') (no; yes). In children with available data on physician-diagnosed eczema on at least 3 time points, we previously identified five eczema phenotypes, namely never, early transient, mid-transient, late transient and persistent eczema.[13] Subjects were assigned to the eczema phenotype for which they had the highest posterior probability. Data on ever eczema was collected by parental-reported questionnaires at 10 years of age ('Has your child ever had eczema diagnosed by a doctor?').

Emotional and behavioural problems

The Child Behavior Checklist (CBCL) was used to assess parent-reported child emotional and behavioural problems. This instrument has proven to be reliable and valid in various population samples from different countries and cultures.[15, 16] The symptoms were measured at median ages of 1.5 (interquartile range (IQR): 1.5-1.6), 3.0 (3.0-3.1), 6.0 (5.8-6.2), and 9.7 (9.6-9.8) years. At the ages of 1.5, 3 and 6 years, the preschool CBCL version for children aged 1.5 to 5 years (CBCL/1.5–5) was used. [17] At the age of 10 years, the school age version for children 6 to 18 years of the CBCL was used (CBCL/6–18). The CBCL consists of empirically-derived broadband scales and syndrome scales. We used two broadband scales and five syndrome scales based on the strongest associations with eczema observed in previous studies.[3-5, 8-12] We examined the broadband scale of internalizing problems, a measure of emotional problems, and the three comprising syndrome scales of anxious-depressed, withdrawn-depressed, and somatic symptoms (e.g. constipation, dizziness, headaches). We also examined the broadband scale externalizing problems, a measure of behavioural problems, and the two comprising syndrome scales of attention problems and aggressive behaviour symptoms at age 1.5, 3 and 5 years, and only aggressive behaviour symptoms at age 10 years. In CBCL/6–18

version, attention problems are not part of externalizing problems due to its sizeable factor in both broadband scales in factor analysis.[15] Scale scores were log, quadratic and square-root transformed and then standardized because of non-normal distributions.

Covariates

Information on maternal age, parity (multiparous; nulliparous), education (primary or secondary school; higher than secondary school) and parental history eczema, allergy or asthma (no; yes) was available from questionnaires obtained at enrolment. Maternal psychiatric symptoms during pregnancy was defined using the Global Severity Index (GSI).[18] Child's sex, gestational age at birth and birthweight were obtained from midwives and hospital records, and ethnic origin (European; non-European) was defined based on the parents' country of birth according to Statistics Netherlands.[19] Postnatal questionnaires provided information on breastfeeding at 2, 6 and 12 months after birth, which was combined into never versus ever, and on sleep problems at 2 months after birth (no; yes).

Statistical analysis

We compared characteristics of those included and not included in our study using Pearson's Chi-square, independent sample *t*-, and Mann-Whitney U tests. We examined the associations of eczema phenotypes with internalizing and externalizing problems, and comprising syndrome scales, at age 10 years using linear regression models. Next, cross-lagged models were used to examine bidirectional associations of eczema with internalizing and externalizing problems, and comprising syndrome scales, from birth until 10 years. Cross-lagged models allow associations between two repeatedly measured variables to be examined in both directions simultaneously while accounting for continuity between the repeated measures over time. With this method, we were able to disentangle the predominant direction of the observed association between eczema and emotional and behavioural problems. In order to make a more balanced model, three age categories (0-2, 3-6 and 10 years) were defined based on the prevalence of physician-diagnosed eczema and distribution of CBCL scales. We examined cross-lagged effects, cross-sectional effects, and stability effects. A conceptual model of the studied cross-lagged associations is presented in Supplementary Figure 2. Bidirectional associations of withdrawn/depressed symptoms were not studied because this scale was not assessed by CBCL/1.5–5. All analyses were adjusted for potential confounders, which were selected from literature if they were strongly related to eczema and internalizing and externalizing problems, and were not in the causal pathway. Therefore, maternal age, parity and history of eczema, allergy or asthma, and child's birthweight were not included in the model. We assumed that data were missing at random. Missing data in covariates were ≤ 21% and 125 datasets were created using multiple imputation by

chained equations. The class assignment was sampled using the subject specific posterior class probabilities determined by the latent class growth model in regression modelling based on the posterior probabilities, in order to take into account the uncertainty of eczema phenotypes class assignment. The size and direction of the effect estimates were similar when we used complete-case-analyses, and therefore, we only present the results based on imputed data. For more easy interpretation, we also examined the associations of eczema phenotypes with borderline clinical cut-offs of the internalizing and the externalizing problem scales (< 84^{th} vs ≥ 84^{th} percentile), and syndrome scales (< 80^{th} vs ≥ 80^{th} percentile).[15] All research questions, including defined exposure definitions and outcomes, and statistical analyses, were determined a priori, and were discussed with the principal investigators and study team members, and adapted where appropriate. No post hoc analyses have been performed. We did not adjust for multiple testing, because the used syndrome scales were related to each other, and examined under the same hypothesis. All measures of association are presented as Z-score differences or odds ratios (OR) together with their corresponding 95% confidence intervals (95%CI). Imputation and regression analyses were performed using the packages 'mice' (version 3.3.0) and 'stats' (version 3.5.2) in R version 3.5.2[20, 21] and cross-lagged analyses were performed in Mplus (version 8.2).[22]

RESULTS

Subject characteristics

Characteristics of children and their mothers are shown in Table 1. Physician-diagnosed eczema ranged from 16% at age 6 months to 6% at age 10 years, and 23% of children had ever eczema at age 10 years. Compared with children who were included in the analysis, those not included had mothers who were lower educated and more often had psychiatric symptoms and a history of asthma, allergy and eczema (Supplementary Table 1). Children not included were more often of non-European ethnicity, never breastfed and had a lower gestational age and birth weight.

Eczema phenotypes and internalizing problems in school-age children

Compared with children who never had eczema, those who ever had eczema had more internalizing problems, including more anxious-depressed and somatic symptoms, but not withdrawn-depressed symptoms at age 10 years (Z score difference (95%CI): 0.21 (0.14,0.29), 0.10 (0.02,0.17) and 0.29 (0.22,0.37), respectively) (Table 2). Compared with the never eczema phenotype, all eczema phenotypes were associated with more internalizing problems (range: 0.14 (0.01,0.27) to 0.30 (0.10,0.51)), and had an increased risk of somatic symptoms (range: 0.15 (0.01,0.24) to 0.39 (0.18,0.60)). When we used

borderline clinical cut-offs, children with early transient, late transient and persistent eczema had the highest risks of somatic symptoms at age 10 years (OR (95%CI): 1.61 (1.26,2.04), 1.43 (1.12,1.83) and 2.37 (1.53,3.66), respectively) (Supplementary Table 2).

Table 1. Characteristics of children and their mothers

	All subjects n=5,265	**Never eczema phenotype** n=3,995	**Ever eczema phenotype*** n=1,270
Maternal characteristics			
Age at enrolment, years (mean (SD))	31.5 (4.6)	31.5 (4.6)	31.4 (4.6)
Parity, nulliparous (n (%))	58 (3,072)	57 (2,280)	62 (792)
Maternal education, higher (n (%))	57 (3,001)	57 (2,277)	57 (724)
Maternal psychiatric symptom scale (median (IQR))	0.1 (0.1 - 0.3)	0.1 (0.1 - 0.3)	0.2 (0.1 - 0.3)
Child characteristics			
Sex, female (n (%))	50 (2,652)	51 (2,041)	48 (611)
Gestational age at birth, weeks (median (IQR))	40.1 (39.0 - 41.0)	40.1 (39.0 - 41.0)	40.1 (39.0 - 41.0)
Birth weight, grams (mean (SD))	3446 (567)	3454 (569)	3422 (559)
Ethnicity, non-European (n (%))	25 (1,331)	25 (984)	27 (347)
Breastfeeding, ever(n (%))	92 (4,857)	93 (3,695)	92 (1,162)
Sleep problems at 2 months, yes (n (%))	12 (615)	11 (447)	13 (168)
Eczema phenotypes (n (%))†			
Never	76 (3,995)	100 (3,995)	0 (0)
Early transient	8 (434)	0 (0)	34 (434)
Mid-transient	6 (302)	0 (0)	24 (302)
Late transient	8 (412)	0 (0)	32 (412)
Persistent	2 (122)	0 (0)	10 (122)
Emotional and behavioural problems at age 10 years (median item score (IQR))†			
Internalizing problems	3 (1 - 7)	3 (1 - 6)	4 (2 - 7)
Anxious/depressed symptoms	1 (0 - 3)	1 (0 - 3)	1 (0 - 3)
Withdrawn/depressed symptoms	1 (0 - 2)	0 (0 - 2)	1 (0 - 2)
Somatic symptoms	1 (0 - 2)	1 (0 - 2)	1 (0 - 2)
Externalizing problems	2 (0 - 5)	2 (0 - 5)	3 (1 - 6)
Aggressive behaviour symptoms	2 (0 - 4)	1 (0 - 4)	2 (0 - 5)
Attention problems	2 (1 - 5)	2 (1 - 5)	3 (1 - 6)

Values are percentages (absolute values), mean (SD) or median (interquartile range) after imputation. *Ever eczema phenotype consists of early transient, mid-transient, late transient and persistent eczema phenotypes. †Data was not imputed, and was missing for ever/never eczema at age 10 years (29%), and emotional and behavioural problems at age 1.5 (12%), 3 (12%), 6 (13%) and 10 years (26%).

Table 2. Associations of eczema phenotypes with internalizing problems at age 10 years

	Internalizing problems Z-score (95%CI)	Anxious-depressed symptoms Z-score (95%CI)	Withdrawn-depressed symptoms Z-score (95%CI)	Somatic symptoms Z-score (95%CI)
Never	Reference	Reference	Reference	Reference
Ever	0.21 (0.14,0.29)*	0.10 (0.02,0.17)*	0.05 (-0.02,0.12)	0.29 (0.22,0.37)*
Never	Reference	Reference	Reference	Reference
Early transient	0.20 (0.09,0.31)*	0.10 (-0.02,0.21)	0.09 (-0.02,0.20)	0.23 (0.12,0.34)*
Mid-transient	0.14 (0.01,0.27)*	0.12 (-0.01,0.25)	0.12 (-0.01,0.25)	0.15 (0.02,0.27)*
Late transient	0.14 (0.03,0.25)*	0.07 (-0.04,0.19)	0.09 (-0.03,0.20)	0.13 (0.01,0.24)*
Persistent	0.30 (0.10,0.51)*	0.18 (-0.03,0.38)	0.00 (-0.20,0.21)	0.39 (0.18,0.60)*

Values are average standardized regression coefficients (95% confidence intervals) from linear regression models. Reference group is the never eczema (phenotype) group. Models were adjusted for maternal education and psychiatric symptoms, and child's sex, gestational age, ethnicity, breastfeeding and sleep disturbances. *p-value <0.05.

Eczema phenotypes and externalizing or attention problems in school-age children

Compared with children who never had eczema, those who ever had eczema had more externalizing problems, including more symptoms of aggressive behaviour, and attention problems (Z score difference (95%CI): 0.13 (0.06,0.21), 0.12 (0.05,0.19), and 0.15 (0.08,0.23), respectively) (Table 3). Compared with the never eczema phenotype, only early transient eczema was associated with more externalizing problems, including more symptoms of aggressive behaviour (0.16 (0.04,0.27) and 0.16 (0.05, 0.27)). All eczema phenotypes were associated with more attention problems (range: 0.15 (0.04,0.26) to 0.24 (0.04,0.45)). When we used borderline clinical cut-offs, children with early transient, mid-transient and persistent eczema had the highest risks of attention problems (OR (95%CI) range: 1.36 (1.02,1.81) and 1.74 (1.08,2.79)) (Supplementary Table 3).

Direction of associations between eczema and emotional and behavioural problems

Figure 1 and supplementary Table 4 show the bidirectional associations between eczema and internalizing problems. Cross-lagged effects showed that compared with children without eczema at ages 0-2 years, those with eczema at ages 0-2 years had more internalizing problems at ages 3-6 years and 10 years (Z score difference (95%CI) : 0.08 (0.02,0.15) and 0.09 (0.02,0.16)) (Figure 1a). This included more anxious-depressed symptoms at ages 3-6 years (0.08 (0.02,0.15) (Figure 1b), and more somatic symptoms at ages 3-6 years and 10 years (0.11 (0.04,0.18)) and (0.13 (0.05,0.20)) (Figure 1c). Reversely, cross-lagged effects showed that children with more internalizing problems at ages 0-2 years had an increased risk of eczema at age 10 years only (OR (95%CI): 1.19 (1.00,1.40),

Table 3. Associations of eczema phenotypes with externalizing problems at age 10 years

	Externalizing problems Z-score (95%CI)	Aggressive behaviour symptoms Z-score (95%CI)	Attention problems Z-score (95%CI)
Never	Reference	Reference	Reference
Ever	0.13 (0.06,0.21)*	0.12 (0.05,0.19)*	0.15 (0.08,0.23)*
Never	Reference	Reference	Reference
Early transient	0.16 (0.04,0.27)*	0.16 (0.05,0.27)*	0.20 (0.09,0.31)*
Mid-transient	0.12 (-0.01,0.25)	0.12 (-0.01,0.25)	0.21 (0.08,0.34)*
Late transient	0.08 (-0.03,0.20)	0.10(-0.01,0.22)	0.15 (0.04,0.26)*
Persistent	0.05 (-0.16,0.26)	0.04 (-0.17,0.24)	0.24 (0.04,0.45)*

Values are average standardized regression coefficients (95% confidence intervals) from linear regression models. Reference group is the never eczema (phenotype) group. Models were adjusted for maternal education and psychiatric symptoms, and child's sex, gestational age, ethnicity, breastfeeding and sleep disturbances. *p-value <0.05.

per point increase in internalizing problem scale) (Figure 1a). No associations of anxious-depressed and somatic symptoms with later eczema were observed (Figures 1b and 1c). Figure 2 (Supplementary Table 4) shows the bidirectional associations between eczema and externalizing problems. Cross-lagged effects showed that compared with children without eczema at ages 0-2 years, children with eczema at ages 0-2 years had more externalizing problems at age 10 years only (Z score difference (95%CI): 0.08 (0.01,0.14)) (Figure 2a), including more aggressive behaviour at age 10 years (0.08 (0.01,0.14)) (Figures 2b). Compared to children without eczema at ages 0-2 and 3-6 years, children with eczema at ages 0-2 years and those with eczema at ages 3-6 years had more attention problems at age 10 years (0.09 (0.02,0.15) and 0.10 (0.01,0.19)) (Figures 2c). Reversely no associations of early externalizing problems or the related syndrome scales with later eczema were observed (Figure 2). Results of cross-sectional and stability effects of eczema with internalizing and externalizing problems are presented in Supplementary Table 4.

DISCUSSION

In this population-based prospective cohort study, we observed that children with ever eczema have more emotional (internalizing) and behavioural (externalizing) problems at the age of 10 years, compared to children without eczema. All eczema phenotypes were most consistently but very modestly associated with more somatic symptoms and attention problems at school age. Additionally, children with early transient eczema had more aggressive behaviour symptoms at school age. The direction of effects were predominantly present for eczema until 2 years leading to increased internalizing and externalizing problems at school age rather than reversely.

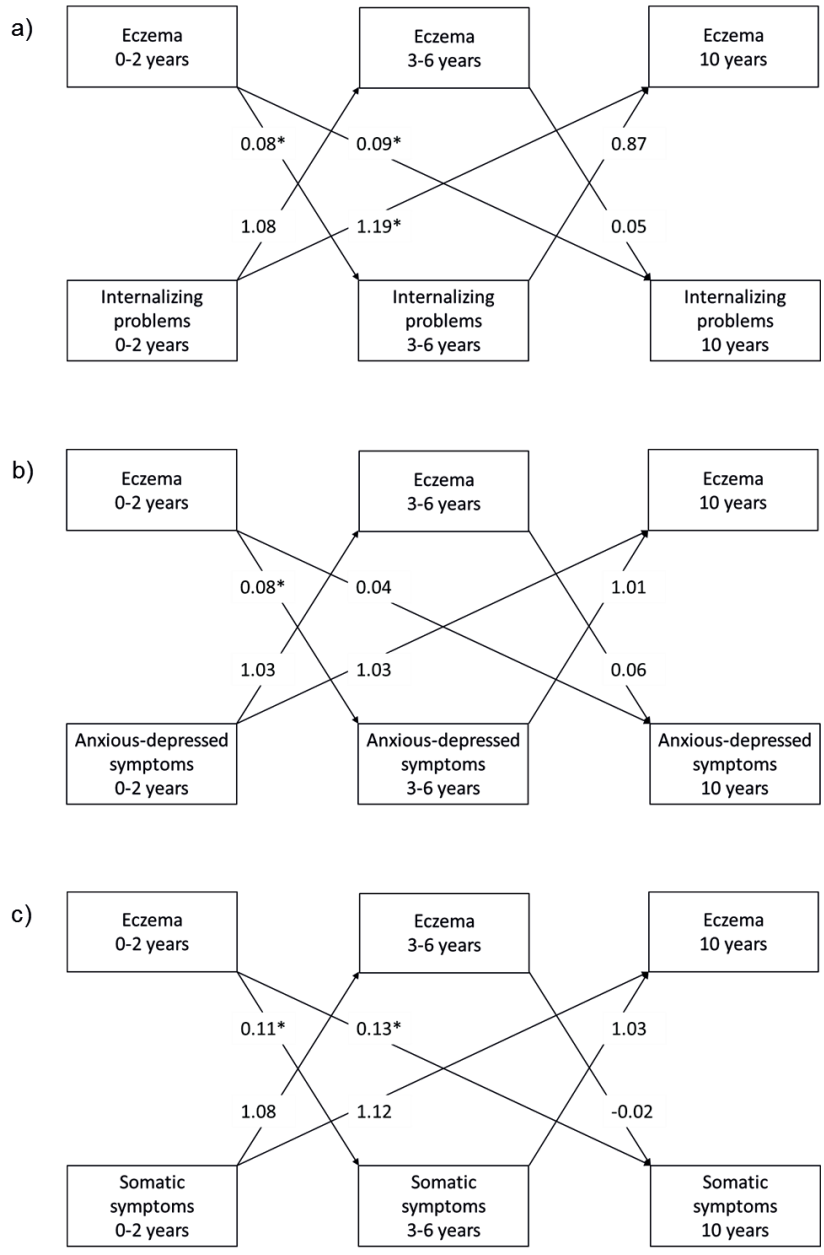

Figure 1. Bidirectional associations between eczema and internalizing problems

Direction of associations between physician-diagnosed eczema and internalizing problems (a), anxious-depressed (b) and somatic symptoms (c) from birth until age 10 years. Arrows indicate the direction of the associations. Values are average standardized regression coefficients for internalizing problems as outcome and odds ratios for eczema as outcome (reference group: no eczema) derived from linear or logistic regression models, using cross-lagged modelling. Models were adjusted for maternal education and psychiatric symptoms, and child's sex, gestational age, ethnicity, breastfeeding and sleep disturbances. *p-value <0.05. The corresponding 95% confidence intervals of the cross-lagged effects, and the effect estimates of the cross-sectional and stability effects are shown in Supplementary Table 4.

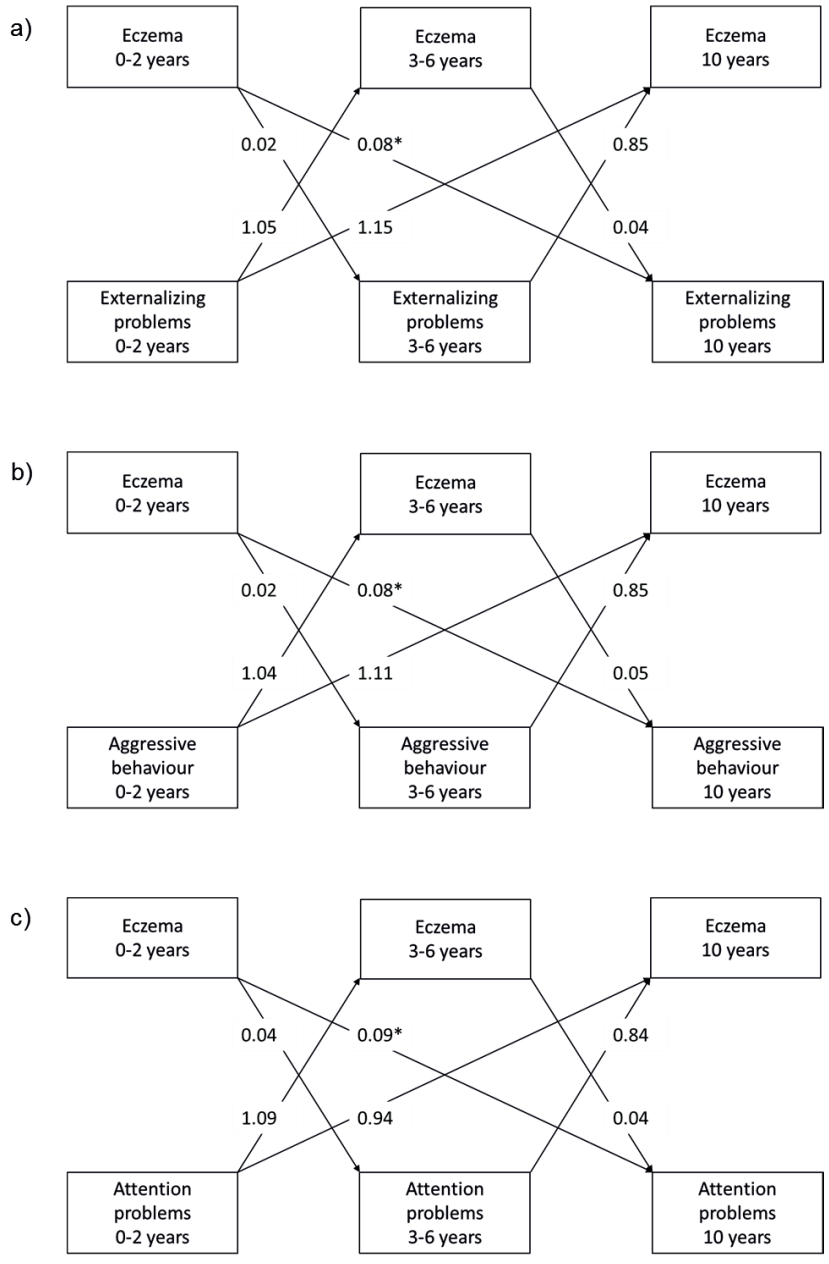

Figure 2. Bidirectional association between eczema and externalizing problems

Direction of associations between physician-diagnosed eczema and externalizing problems (a), aggressive behaviour symptoms (b) and attentions problems (c) from birth until age 10 years. Arrows indicate the direction of the associations. Values are average standardized regression coefficients for externalizing problems as outcome and odds ratios for eczema as outcome (reference group: no eczema) derived from linear or logistic regression models, using cross-lagged modelling. Models were adjusted for maternal education and psychiatric symptoms, and child's sex, gestational age, ethnicity, breast-feeding and sleep disturbances. *p-value <0.05.The corresponding 95% confidence intervals of the cross-lagged effects, and the effect estimates of the cross-sectional and stability effects are shown in Supplementary Table 4.

Comparison with previous studies

We observed that children with ever eczema had more internalizing and externalizing problems at school age. These results are in line with previous meta-analyses.[3-5, 10] Of the internalizing problems, we observed that children with ever eczema had more anxious-depressed symptoms only, but not when we grouped children based on the onset and persistence over time using data-driven analysis. In contrast, previous meta-analysis showed that children with eczema have more anxious and depressed symptoms.[4, 5] Differences in results might partly be explained by our study population, which consists of relatively healthy and young children until age 10 years, compared to previous studies consisting of patients and children until 18 years. They observed stronger effect estimates of eczema with anxious and depressed symptoms in adults as compared to children.[4] Also, differences in sample size, used definition of eczema or measurement tools for anxiety and depression-related symptoms might have played a role. Furthermore, we observed that all eczema phenotypes were most consistently associated with more somatic symptoms and attention problems at school age. Previous cohort studies using non-data driven methods to define eczema phenotypes, such as grouping of children by researchers' experiences, observed that children with early onset and persistent eczema had a higher risk of emotional and behavioural problems in preadolescence.[4, 9-11] While data-driven analyses appear to be less biased, the best method to define eczema phenotypes depends on the study population, data distribution and availability, and specific research aim.

To the best of our knowledge, our study is the first that applied cross-lagged modelling for bidirectional analyses, and observed that eczema until age 2 years was associated with more emotional and behavioural problems at school age more dominantly than reversely. These findings contribute to the disentanglement of the complex bidirectional relationship of eczema with emotional and behavioural problems and suggest that the directional effects seem from early life eczema to later life internalizing and externalizing problems rather than reversely.

The modest effect sizes of the observed associations of eczema phenotypes with emotional and behavioural problems imply that, on a population based level, most children with eczema are mentally healthy. However, results might be different on an individual or hospital-based level. When we used subclinical cut-offs, the effect estimates were greater and consistent for the association of early transient eczema with the risk of emotional and behavioural problems. Therefore, part of our results are in line with the current European guidelines, suggesting that children with moderate or recurrent eczema should receive psychosomatic counselling.[23] Future studies are needed to examine the

role of such eczema treatment on the development of emotional and behavioural problems later in life, while taking severity and genetic susceptibility into account.

Possible mechanisms

Several hypotheses exist on the relationship of eczema with emotional and behavioural problems. Eczema and related symptoms such as chronic itchiness, red patchy skin appearance and disturbed sleep could negatively affect mental health via social isolation, low self-image, lack of concentration and more irritability, and possibly also via low-grade inflammation and blood-brain barrier disruption.[10, 24-26] Reversely, stress, as proxy of these problems, could shift the balance towards type 2 T-helper cells via the hypothalamic-pituitary-adrenal axis and sympathetic adrenomedullary system leading to more susceptibility of atopic inflammation and diseases, and resulting in a vicious cycle.[7] Another explanation is based on shared pathogenesis between eczema and mental health disorders as both skin cells and neurons originate from the ectoderm.[27] Shared common genetic variants were found for skin barrier defects and mental health disorders that are involved in both the histamine and immune response regulation, and the dopaminergic system.[28-32] The immune system has been associated with many mental health disorders in adults, but the underlying pathways remain unclear.[33] Expression of pro-inflammatory factors in adults with atopic diseases and autism spectrum disorders were shown to disrupt the blood-brain barrier.[34] Therefore, chronic inflammation, specifically in early life when maturation of the nervous system and the immune system are still ongoing, might increase susceptibility for mental health disorders.

Strengths and limitations

The strengths of this study are its prospective design with detailed, repeated information on eczema and emotional and behavioural problems, and the use of data-driven defined eczema phenotypes. By using sampling based on class assignment probabilities, we achieved less misclassification bias and more precise effect estimates. However, some methodological limitations need to be addressed. Children not included resulted in a selection towards a healthier and more affluent population. Second, reporting of emotional and behavioural problems by parents of children with eczema might be different than by parents of children without eczema. We tried to minimize this misclassification by using validated questionnaires.[15, 35] Third, residual confounding might be present since not all factors associated with eczema and emotional and behavioural problems were measured or included in the analysis, such as severity of eczema, sleep problems in later life, and other (atopic) comorbidities.[10] No information was available to determine the severity of eczema cases. Fourth, the clinical impact might be minimal due to the observed very modest effect sizes in Z-score differences and the use of subclinical cut-offs for the associations of eczema phenotypes with emotional and behavioural problems in

this population-based study. Last, we cannot exclude that the observations from cross-lagged models might be the result of the natural course of the studied conditions.[2, 36] However, cross-lagged models take the natural course into account by adjusting for the stability effects.

Conclusion

All eczema phenotypes were very modestly associated with more somatic symptoms and attention problems at school age. Children with early transient eczema had more symptoms of aggressive behaviour. The very modest effect sizes of the observed associations of eczema phenotypes with emotional and behavioural problems imply that, on a population-based level, most children with eczema are mentally healthy. Directional effects seem from eczema in early life leading internalizing and externalizing problems in later life rather than reversely. Therefore, future research should focus on the effect of early optimal eczema management on mental health disorders in children later in life.

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Supplementary Table 1. Characteristics of children and their mothers of those included and not included in the analyses

	Included n=5,265	**Not included** n=2,628	**p-value for difference**
Maternal characteristics			
Age at enrollment, years (mean)	31.5 (4.6)	28.3 (5.7)	<0.001
Parity, % (n)			
Nulliparous	59 (2,999)	48 (1,176)	<0.001
Multiparous	41 (2,123)	52 (1,282)	
Maternal education, % (n)			
Primary or secondary	42 (2,147)	79 (1,513)	<0.001
Higher	58 (2,917)	22 (414)	
Parental history of eczema, allergy or asthma, % (n)			
No	50 (2,515)	58 (1,133)	<0.001
Yes, at least one parent	50 (2,522)	42 (819)	
Maternal psychiatric symptom scale (median)	0.1 (0.1,0.3)	0.3 (0.1,0.5)	<0.001
Child characteristics			
Sex, % (n)			
Male	50 (2,613)	52 (1,369)	0.04
Female	50 (2,652)	48 (1,257)	
Gestational age at birth, weeks (median)	40.1 (39.1 - 41.0)	40 (38.9 - 40.9)	<0.001
Birth weight, grams (mean)	3446 (567)	3332 (574)	<0.001
Ethnicity, % (n)			
European	75 (3,905)	41 (906)	<0.001
Non-European	25 (1,333)	59 (1,305)	
Breastfeeding, % (n)			
Never	8 (395)	10 (166)	0.00
Ever	92 (4,728)	90 (1,496)	
Sleep problems, % (n)			0.88
No	89 (3,724)	89 (800)	
Yes	11 (477)	11 (100)	

Values are percentages (absolute values), mean (SD) or median (interquartile range) based on observed data.

Supplementary Table 2. Associations of eczema phenotypes with borderline clinical cut-offs of internalizing problems at age 10 years

	Internalizing problems OR (95% CI)	Anxious-depressed symptoms OR (95% CI)	Withdrawn-depressed symptoms OR (95% CI)	Somatic symptoms OR (95% CI)
Never	Reference	Reference	Reference	Reference
Ever	1.21 (0.98,1.48)	1.14 (0.95,1.37)	1.01 (0.85,1.21)	1.78 (1.52,2.10)*
Never	Reference	Reference	Reference	Reference
Early transient	1.08 (0.79,1.48)	1.37 (1.05 1.79)*	1.13 (0.87,1.46)	1.61 (1.26,2.04)*
Mid-transient	1.11 (0.78,1.58)	1.10 (0.80,1.51)	1.28 (0.95,1.71)	1.28 (0.97,1.70)
Late transient	1.19 (0.88,1.63)	1.20 (0.90 1.58)	1.22 (0.94,1.59)	1.43 (1.12,1.83)*
Persistent	1.16 (0.67,2.01)	1.11 (0.67,1.85)	1.02 (0.63,1.64)	2.37 (1.53,3.66)*

Values are odds ratios (95% confidence intervals) from logistic regression models. Reference group is the never eczema (phenotype) group and children with a lower score of internalizing problems (<84th for internalizing problems or <80th percentile for specific syndrome scales). Models were adjusted for maternal education and psychiatric symptoms, and child's sex, gestational age, ethnicity, breastfeeding and sleep disturbances. *p-value <0.05.

Supplementary Table 3. Associations of eczema phenotypes with borderline clinical cut-offs of externalizing problems at age 10 years

	Externalizing problems OR(95% CI)	**Aggressive behaviour symptoms OR (95%CI)**	**Attention problems OR (95%CI)**
Never	Reference	Reference	Reference
Ever	1.20 (0.97,1.47)	1.19 (0.99,1.43)	1.22 (0.99,1.45)
Never	Reference	Reference	Reference
Early transient	1.51 (1.13,2.00)*	1.35 (1.04,1.75)*	1.42 (1.08,1.87)*
Mid-transient	1.05 (0.72,1.51)	1.17 (0.86,1.60)	1.56 (1.14,2.13)*
Late transient	1.13 (0.82,1.55)	1.18 (0.90,1.56)	1.36 (1.02,1.81)*
Persistent	1.04 (0.59,1.83)	0.97 (0.58,1.62)	1.74 (1.08,2.79)*

Values are odds ratios (95% confidence intervals) from logistic regression models. Reference group is the never eczema (phenotype) group and children with a lower score of externalizing problems (<84th for externalizing problems or <80th percentile for specific syndrome scales). Models were adjusted for maternal education and psychiatric symptoms, and child's sex, gestational age, ethnicity, breastfeeding and sleep disturbances. *p-value <0.05.

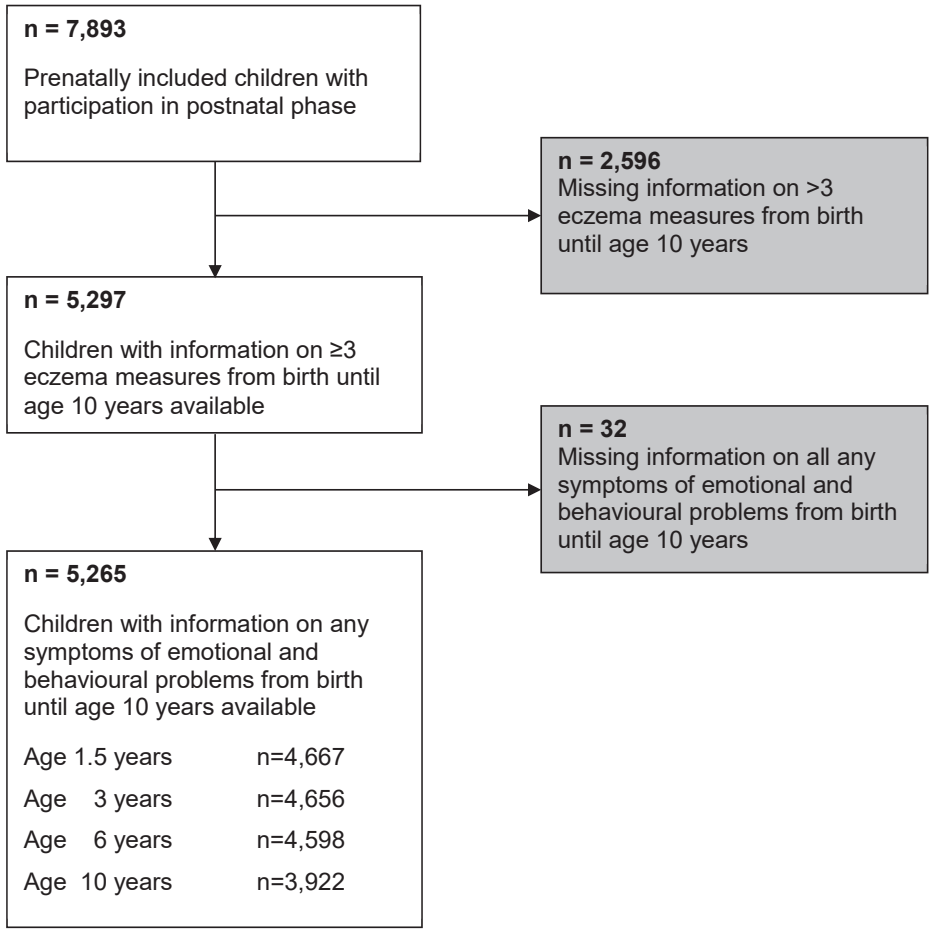
Supplementary Table 4. Direction of associations between eczema and emotional and behavioural problems from birth until age 10 years

	Internalizing problems	Anxious-depressed symptoms	Somatic symptoms	Externalizing problems	Aggressive behaviour symptoms	Attention problems
Cross-lagged effects						
Eczema 0-2y → emotional and behavioural problems 3-6y	0.08 (0.02,0.15)*	0.08 (0.02,0.15)*	0.11 (0.04,0.18)*	0.02 (-0.04,0.08)	0.02 (-0.04,0.08)	0.04 (-0.02,0.11)
Eczema 0-2y → emotional and behavioural problems 10y	0.09 (0.02,0.16)*	0.04 (-0.04,0.11)	0.13 (0.05,0.20)*	0.08 (0.01,0.14)*	0.08 (0.01,0.14)*	0.09 (0.02,0.15)*
Eczema 3-6y → emotional and behavioural problems 10y	0.05 (-0.04,0.13)	0.06 (-0.03,0.16)	-0.02(-0.12,0.08)	0.04 (-0.05,0.13)	0.05 (-0.04,0.14)	0.10 (0.01,0.19)*
Emotional and behavioural problems 0-2y → eczema 3-6y	1.08 (0.99,1.19)	1.03 (0.94,1.13)	1.06 (0.97,1.16)	1.05 (0.95,1.15)	1.04 (0.95,1.14)	1.09 (1.00,1.20)
Emotional and behavioural problems 0-2y → eczema 10y	1.19 (1.00,1.40)*	1.03 (0.89,1.20)	1.12 (0.96,1.30)	1.15 (0.97,1.36)	1.11 (0.94,1.31)	1.09 (0.94,1.28)
Emotional and behavioural problems 3-6y → eczema 10y	0.89 (0.75,1.05)	1.01 (0.87,1.17)	1.03 (0.89,1.21)	0.85 (0.71,1.01)	0.85 (0.72,1.02)	0.99 (0.84,1.16)
Cross-sectional effects						
Eczema ↔ emotional and behavioural problems 0-2 y	0.02 (-0.04,0.09)	0.03 (-0.03,0.09)	0.03 (-0.03,0.10)	0.04 (-0.03,0.10)	0.02 (-0.04,0.08)	0.05 (-0.02,0.11)
Eczema ↔ emotional and behavioural problems 3-6 y	0.07 (-0.01,0.15)	0.08 (0.00,0.17)	0.09 (0.00,0.18)*	0.12 (0.05,0.20)*	0.13 (0.05,0.20)*	0.07 (-0.01,0.15)
Eczema ↔ emotional and behavioural problems 10 y	0.21 (0.10,0.32)*	-0.03(-0.16,0.10)	0.42 (0.28,0.55)	-0.11(-0.23,0.01)	-0.12(-0.23,0.00)	-0.02(-0.14,0.10)
Stability effects						
Emotional and behavioural problems 0-2y → 3-6y	0.50 (0.47,0.52)*	0.39 (0.36,0.42)*	0.35 (0.32,0.38)*	0.55 (0.52,0.57)*	0.53 (0.50,0.56)*	0.47 (0.44,0.50)*

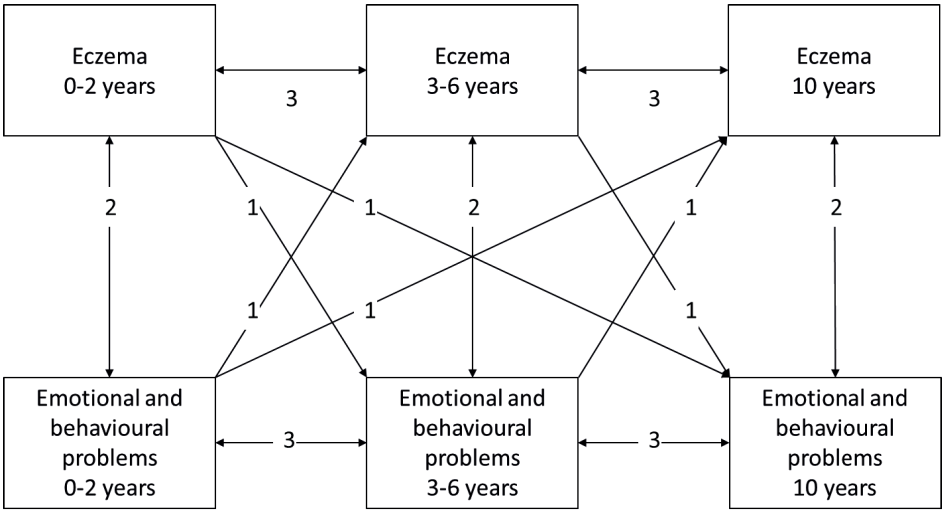
Supplementary Table 4. Direction of associations between eczema and emotional and behavioural problems from birth until age 10 years (continued)

	Internalizing problems	Anxious-depressed symptoms	Somatic symptoms	Externalizing problems	Aggressive behaviour symptoms	Attention problems
Emotional and behavioural problems 3-6y → 10y	0.47 (0.44,0.50)*	0.37 (0.33,0.40)*	0.29 (0.25,0.32)*	0.53 (0.50,0.56)*	0.52 (0.50,0.55)*	0.49 (0.46,0.51)*
Eczema 0-2y → 3-6 y	5.05 (4.26,5.99)*	5.05 (4.26,5.99)*	5.05 (4.26,6.05)*	5.05 (4.26,5.99)*	5.05 (4.26,5.99)*	5.05 (4.26,5.99)*
Eczema 3-6y → 10 y	4.10 (3.03,5.47)*	4.06 (3.03,5.42)*	4.01 (3.00,5.37)*	4.14 (3.10,5.58)*	4.18 (3.10,5.58)*	4.01 (3.00,5.37)*

Values are average standardized regression coefficients when internalizing problems, and their specific symptoms, were the outcome (reference group: no eczema) and odds ratios when eczema was the outcome (per point increased of internalizing problem scale) derived from linear or logistic regression models, respectively, using cross-lagged modelling. Models were adjusted for maternal education and psychiatric symptoms, and child's sex, gestational age, ethnicity, breastfeeding and sleep disturbances. *p-value <0.05. Abbreviation used: years (y).



Supplementary Figure 1. Flow chart of participants included for analysis



Supplementary Figure 2. Conceptual model of cross-lagged model of eczema and emotional and behavioural problems

Arrows indicate the direction of the associations. (1) Cross-lagged effects, (2) Cross-sectional effects, and (3) stability effects.

4

General discussion

INTRODUCTION

Childhood eczema is a common chronic disorder worldwide, characterized by recurrent red itchy skin, and variable age of onset and persistence.[1, 2] In order to develop more effective prevention and management strategies, it is important to understand the origins and consequences of eczema in more detail. Eczema phenotypes, that takes into account the age of onset and persistence, and thereby better reflecting the natural course of eczema, may enable better identification of specific risk factors and consequences. Genetic, early life environmental and microbial factors could potentially be most influential risk factors of eczema phenotypes. Consequently, eczema phenotypes could help identifying which children have higher risks of asthma, allergies, and emotional and behavioural problems.

The aim of this thesis was to identify eczema phenotypes, and to examine the associations of specific genetic, early environmental, and microbial factors with eczema phenotypes, and consequently the associations of eczema phenotypes with asthma, allergies, and emotional and behavioural problems in later life. The main findings, strengths and limitations of the manuscripts that form the basis of this thesis have been discussed in detail in previous chapters. This chapter provides a general overview and discussion of main findings, methodological considerations, and suggestions for future research directions.

INTERPRETATION OF MAIN FINDINGS

Eczema phenotypes

In clinical practice, eczema is diagnosed by a physician and/or dermatologist based on the patient's history, and the morphological and distributional features of the skin lesions. In large-scale population studies, a questionnaire-based definition of eczema is often used for practicality and efficiency reasons.[3] The extensive follow-up periods of large prospective cohort studies enable the identification of more homogenous subgroups for time-varying outcomes based on age of onset and persistence over time, such as eczema phenotypes. Two previous cohorts that identified eczema phenotypes included children from birth until the age of 6, and 11-16 years, but were mostly of European ethnicity.[4, 5] Therefore, an unique aspect of the studies presented in this thesis is that we included children from European and non-European ethnicity participating in the Generation R study localized in Rotterdam, a city in the Netherlands with the most non-western citizens.[6, 7] Findings showed that the identified eczema phenotypes sup-

port the hypothesis that also in a multi-ethnic population eczema phenotypes better fit the natural course of eczema than a cross-sectional dichotomous definition of eczema.

Other definitions that take the variability of eczema into account, such as atopic or allergic phenotypes, combine eczema, allergies, and asthma (or wheezing) into subgroups.[8, 9] We only included eczema in our phenotypes model for a simpler and cleaner model, which enables better identification of early life risk factors and later life consequences for eczema considering the different pathophysiology of atopic diseases. Future research should investigate if including more parameters, such as severity of eczema and other atopic diseases, leads to a better fitted and more practical model. Furthermore, a combined model of external (phenotypes) and internal parameters (endotypes), such as serum biomarkers, may lead to an even better reflection of the natural variability of eczema.

Risk factors

In the studies presented in this thesis, we examined the associations of several genetic, early life environmental and microbial factors with eczema phenotypes in a multi-ethnic paediatric population (Table 1).

Genetic and early life environmental factors

We observed that most genetic and early life environmental factors poorly differentiate between eczema phenotypes, except for sex and ethnicity. This partly implies that the underlying pathophysiological mechanisms of each eczema phenotype is comparable. Only two previous studies have examined environmental and/or genetic risk factors with eczema phenotypes, but were performed in a paediatric population of mainly European ethnicity.[4, 5] Interestingly, the results from these studies showed that the main early life environmental risk factors for childhood eczema, such as parental education and history of atopic diseases, siblings, breastfeeding, day care attendance, and pet exposure were not consistently associated with all eczema phenotypes, nor did they differentiate between eczema phenotypes. In line with previous results, we observed that the included environmental factors combined only explained a small propotion of the variability of eczema phenotypes (p-value of Hosmer Lemeshow test $<10^{-12}$). We observed stronger effect estimates for genetic factors, such as filaggrin (*FLG*) mutations, with eczema phenotypes, and an even better model fit when environmental and genetic factors were combined. However, still much unexplained variability remained (p-value of Hosmer Lemeshow test $<10^{-5}$). A possible explanation is that the established risk factors that we included in our studies may be more strongly associated with having eczema (yes/no, ever/never), or severity of eczema, and less associated with the age of onset and/or persistence of eczema, since most previous large observational studies

on identification of eczema risk factors have defined eczema as a dichotomous trait, or examined the severity of eczema (often based on drug exposure).[4, 10-20] There is a need to identify unknown risk factors that better explain the variability of eczema onset and persistence.

Our observations and previous studies suggest that sex- or ethnicity related factors have an important role on the development and persistence of eczema. Boys more often have early onset, but transient eczema, and children of non-European ethnicity more often have persistent eczema.[4, 5, 21] A birth cohort study in the United States (US) identified sex-specific predictors, such as paternal asthma and child's ethnicity, for wheezing phenotypes, which were associated with eczema phenotypes in our study.[22] Another US cohort study in children until age 7 years showed that African and Hispanic ethnicity was associated with an increased risk of persistent eczema compared to European ethnicity.[23] The global prevalence of eczema varies, and is highest in Africa and Oceania.[24] Europeans and non-Europeans also differ in eczema-related genetic mutations, inflammatory cytokines, and T-cell activation levels.[19, 25] However, since sex and ethnicity are based on socioenvironmental, behavioural and genetic components, it is difficult to establish the contribution of each component on the effect size. For some diseases, such as specific X- linked syndromes like Turner's syndrome, the genetic aspects of sex explains all effects. However, for eczema and eczema phenotypes, genetic factors only explain part of the effects. A large US paediatric and adult cohort study showed that genetic ancestry does not explain the increased prevalence and worse disease control of eczema in individuals that self-identified as African American.[26] Therefore, detailed socioenvironmental and behavioural factors underlying the idientified risk factors age and ethnicity seem to play a more important role, and further studies on the relation between ethnicity, full exposome, and eczema phenotypes might uncover novel differentiating factors for eczema phenotypes.[27] Though, with the increasing globalization and diversity within regions, studying ethnicity will become more complex, and in the very far future, it might even become not significant if globalization leads to a scenario of a homogenous global culture.[28, 29]

Microbial factors

One of the most well-known bacteria associated with more severe eczema is *Staphylococcus aureus.*[30] However, a recent systematic review showed insufficient evidence that *Staphylococcus aureus (S. aureus)* reducing treatments on the skin or nose are beneficial for eczema. In line with these findings, results of our current study did not show longitudinal associations of bacterial nasal carriage of *S. aureus* with eczema. Recently, instead of single bacteria, the human microbiota has gained much interest in the development of atopic diseases. The human microbiota consists of 5000-1000 different bacterial spe-

cies at any time, that all together contain more genes than the human genome. The gut microbiota has been associated with an increasing number of diseases, such as obesity, inflammatory bowel disease, and rheumatoid arthritis.[31] The gut microbiota is also suggested to play an important role in the development of eczema.[32] During infancy, a lower diversity of gut microbiota is associated with an increased risk of eczema, and other atopic diseases in later life.[32] The role of gut microbiota at later age on atopic diseases is less clear, and therefore we examined the associations of stool microbiota with atopic diseases in children aged 10 years. We observed that a lower diversity, but not composition or functional pathways of stool microbiota was associated with eczema in children aged 10 years. This is in line with the weak and conflicting evidence from clinical trials of preventive and therapeutic probiotics interventions for children with eczema.[33-35] These findings suggest that the role of gut microbiota on atopic diseases in later childhood is limited. However, due to the complex and high-dimensionality of the microbiota data and the relative low prevalence of atopic diseases in our study, we cannot exclude that weak associations with eczema and other atopic diseases were not detected. Future large-scale studies using different microbiota body sites such as the skin, different definitions such as eczema phenotypes, and different study population such as more severe eczema in a hospital population, are needed to replicate and build upon the observations from our study. Current –omics approaches and advanced statistical tools for analyses are developing rapidly.[36] Therefore, stronger collaboration between clinicians, epidemiologists, fundamental researchers, statisticians and bioinformatics should be established in order to advance clinically relevant –omics research.

Consequences

The observations from our studies on eczema phenotypes and consequences suggests that children with early onset eczema are at risk of developing comorbidities such as asthma, allergy, and emotional and behavioural problems at school age (Table 2). Children with early transient and persistent eczema had the highest risk for asthma, and food and inhalant allergies. Children with any eczema phenotype had more somatic symptoms, and attention problems at school age, and children with early transient eczema also had more aggressive behaviour symptoms. In addition, having eczema at ages 0-2 years was associated with more internalizing and externalizing problems in later childhood. Our observations support the hypothesis that in this early life period (age <2 years), upregulated type 2 inflammation might disrupt early life maturation processes of the skin immune, and nervous system, and leads to increased risk of other diseases related to these systems, such as allergy, asthma and emotional and behavioural problems.[37-39] Eczema in children and adults has also been associated with increased risk of cardiovascular, neuropsychiatric, and malignant diseases, which suggests that eczema may be a more systemic inflammation condition, rather than a local inflammatory skin condition,

Table 1. Overview of results of studies on risk factors and eczema phenotypes from birth until age 10 years and eczema at age 10 years as presented in this thesis

	Eczema phenotypes				
	Early transient eczema	Mid-transient eczema	Late transient eczema	Persistent eczema	Eczema at 10 years
Early life environmental factors					
Parity (nulliparous)	↑	=/↑	=	↑	n.a.
Maternal education (higher)	=	=	=	=	n.a.
Parental history of eczema, allergy or asthma (yes)	↑	=	=/↑	↑	n.a.
Sex (male)	↑	=	=	=	n.a.
Ethnicity (non-European)	=	=	↑	↑	n.a.
Ethnicity (Mediterranean)	=	=	=	=	n.a.
Ethnicity (Asian)	=	=	↑	↑	n.a.
Ethnicity (African)	=	=	=/↑	=/↑	n.a.
Breastfeeding (ever)	=	=	=	=	n.a.
Child day care(yes)	=	=	=/↑	=	n.a.
Pet exposure (yes)	=	=	=	=	n.a.
Wheezing pattern (early)	=	=	=	=	n.a.
Wheezing pattern (late)	↑	=	=	↑	n.a.
Wheezing pattern (persistent)	↑	=	↑	↑	n.a.
Genetic factors					n.a.
FLG genotype (≥ 1 mutations)	↑	=	↑	=	n.a.
Genetic risk score (per additional allele)	↑	=/↑	=/↑	↑	n.a.
Microbial factors					
Bacterial nasal carriage					
S. aureus carriage at 6 weeks	=	=	=	=	=
S. aureus carriage at 6 months	↑	=	=	↑	↑
S. aureus carriage at age 1-6 years	=	=	=	=	=
H. influenzae, M. catarrhalis or S. pneumonia At ages 6 weeks to 6 years	=	=	=	=	=
Stool microbiota					
Diversity	n.a.	n.a.	n.a.	n.a.	↓
Differential relative abundance	n.a.	n.a.	n.a.	n.a.	↓
Functional pathways	n.a.	n.a.	n.a.	n.a.	=

Arrows represent the direction of the associations; arrows pointing upwards represent positive associations, while arrows pointing downwards represent negative associations. Equal signs represent null associations. n.a. = not applicable.

Table 2. Overview of results of studies on eczema phenotypes and consequences at age 10 years as presented in this thesis

	Food allergy	Inhalant allergy	Lung function	Asthma	Internalizing problems	Externalizing symptoms
Eczema phenotypes						
Early transient	↑	↑	=	↑	↑	↑
Mid-transient	↑	↑	=	↑	↑	=
Late transient	↑	↑	=	↑	↑	=
Persistent	↑↑	↑	=	↑↑	↑	=

Arrows represent the direction of the associations; arrows pointing upwards represent positive associations, while arrows pointing downwards represent negative associations. Equal signs represent null associations.

but might also be associated with specific lifestyle choices.[40] The systemic inflammatory hypothesis is supported by elevated serum inflammatory biomarkers in patients with eczema.[41] Children that develop eczema in early life may already be more at risk of developing other systemic inflammatory diseases due to changes in immune system during the preconception and/or pregnancy phase via (epi)genetic and environmental factors. Another hypothesis is that eczema related symptoms such as itch, and disturbed sleep might negatively affect mental health via low self-image, lack of concentration and more irritability. Having eczema could lead to poorer health decisions such as more sedentary behaviour and smoking, and subsequently cardiovascular and malignant diseases.[42, 43] Reversely, the immune system is hypothesized to play an important role in brain and central nervous system development in the perinatal phase.[38, 39] Therefore, it is speculated that disruptions in this phase might also contribute to poor health behaviour in later life. The aetiology of eczema and its associated comorbidities remain complex. Future studies should examine the interplay between (epi)genetic, microbial and environmental factors during preconception and early pregnancy, to uncover the origins and persistence of eczema, and examine whether early lifestyle interventions could prevent eczema and its associated comorbidities.[44-46] Recently, the Generation R Next study, a population-based prospective cohort study, has started to include women with a desire to have children (preconception) or who are pregnant, and follows them and their children until adulthood, which will enable the examination of the before mentioned aims.

The consequences of eczema phenotypes for asthma, allergy, and emotional and behavioural problems should not be overestimated, and reflects the difference between relative and absolute risks. Our observations showed that children with persistent eczema phenotype have an up to 35-fold risk to develop asthma and allergies at school age. However, the absolute number of children that have persistent eczema, allergy, and asthma at school age were relatively small in the population-based cohort (8 of 4277 children). For children with any eczema phenotypes, we observed very modest increased somatic symptoms and attention problems at age 10 years, implying that, on a population-based level, most children with eczema are mentally healthy. These results might be different on an individual or hospital-based population level. Previous systematic reviews of observational studies showed that children with eczema, especially those with higher disease severity, had more emotional problems, anxiety, depression, and attention deficit hyperactivity disorders.[47, 48] While we did not have available information to determine the severity of eczema, we can assume that children with eczema of the general population tend to have more mild eczema symptoms compared to those of a hospital-based population. A large US survey found that the prevalence of childhood eczema was 13%, and of those only 7% had severe disease, and 26% had moderate disease.[49] Therefore, considering the population under study is very important in interpreting the results (i.e. external validation).

In addition, it is also important to realize that positive publication bias might make the potential comorbidities and negative consequences of eczema appear worse than they really are. Null or negative findings might have less appealing content, but must equally be considered for publication to provide an objective view of the outcome of interest. The current solutions to minimize positive publication bias, such as clinical trials registries, have not fully addressed the problem, especially not for observational studies.[50] Continuous active awareness and shifting focus from statistically significant results to well-defined research aims and high methodological quality are needed.

METHODOLOGICAL CONSIDERATIONS

The studies presented in this thesis were embedded in the Generation R study, a multi-ethnic prospective population-based cohort study with follow-up from foetal life onwards in Rotterdam, The Netherlands.[6] Detailed methodological considerations for each individual study have been presented in the previous chapters. In the following paragraphs, the general methodological issues regarding the internal and external validity of epidemiological studies are discussed.

Selection bias

Selection bias is possible when the association of the exposure with the outcome is different between participants included in the study and those not included in the study but were eligible to be included. As a consequence, the observed results may not be representative for the population of interest. Overall, from all eligible children at birth, the participation rate was 61% for the Generation R Study.[6] This response is most likely not random as the non-included participants were more often of non-European ethnicity and lower socio-economic status, suggesting a selection towards a healthier study population, and possibly lower disease rates and less severe diseases.[51]

Selection bias is also possible when the association of the exposure with the outcome is different between participants included in the study and those lost to follow-up. From all live born children enrolled in the Generation R Study (n = 9,749), 75.8 % (n = 7,393) still participated at age 10 years, of whom 71.6 % (n = 5,297) had information available on at least three eczema measurements.[6] Mothers who did not answer questionnaires related to our exposures and outcomes of interest, and who did not visit the research centre with their children for measurements were more often of non-European ethnicity, lower educated, and less often had a history of eczema, allergy or asthma. This leads to a selection towards a healthier study population, and might have biased the observations.

Collider stratification bias (or index event bias) is a special form of selection bias, and can occur when the presence of a particular event is needed for inclusion in a study, often with survival or recurrence as outcome of interest. For example, it is possible that the children included in our study already had less risk factors of eczema, and therefore the established risk factors for eczema were not consistently and not differentially associated with eczema phenotypes. We used multiple imputation for missing values of covariates to minimize the risk of selection bias.[52]

Information bias

Information bias is a systematic difference from reality, and can be differential or non-differential. Non-differential misclassification occurs when the misclassification error is similar for participants with and without the exposure or outcome of interest, and may lead to an underestimation of the true effect. Differential misclassification (non-random) occurs when the misclassification is different between participants with and without the exposure or outcome of interest, and is more problematic as it may result in an over- or underestimation of the true effect. Differential misclassification in our studies are limited, since the exposures in this thesis were collected longitudinally, and often before assessment of the outcomes. Parents and researchers involved with the data collection were not aware of specific research questions, and we mostly used

validated questionnaires. However, non-differential misclassification is still possible due to under- or over-reporting in self-reported questionnaires on eczema, allergy, asthma, and emotional and behavioural problems. Especially emotional and behavioural problems might have been influenced due to socially desirable responding. Furthermore, the used definition of ethnicity might have led to children of European ancestry being non-European if one of their parents was born in a foreign country, but their grandparents had European origins, and reversely, that third generation non-European migrants were defined as European. Allergic sensitization, lung function measures, and nasal, and nasopharyngeal bacterial carriage were measured using validated methods, and therefore unlikely to include bias. However, it cannot be excluded that human error in processing the data might have led to non-differential misclassification errors. We used the most novel techniques available for stool microbiome analyses. Microbiome analyses are a fast-evolving field, and therefore non-differential misclassification might be present in stool microbiota data due the limitations of the currently used analyzing techniques.

Confounding

Confounders are factors that are associated with both the exposure and the outcome, and may not be intermediators in the causal pathway. Adjustment for confounders is needed to prevent biased effect estimates. Selecting which confounders to include in the study remains a topic of discussion. Directed acyclic graphs (DAGs) enables the visualisation of causal relations between exposure and outcome and may help in selecting which confounders to include in the analyses. However, especially in studies with relatively small power including many confounders can be problematic for the degrees of freedom in statistical models. Therefore, we used a more pragmatic approach, and selected confounders based on the literature, and further examined if they were associated with exposure and outcome, or if they changed the effect estimates with ≥10%. While we adjusted for many potential confounders in all studies presented in this thesis, residual confounding remains possible by variables not measured or included, such as housing conditions, air pollution, and diet. This may have resulted in an overestimation of the effect estimates.

External validity

External validity is the extent to which results of a study can be applied to other populations. The Generation R Study is based on the general population of Rotterdam, The Netherlands. Rotterdam has approximately 39% inhabitants with a non-European background and the largest groups are of Turkish, Moroccan and Surinamese origin.[7] In the Generation R study, Turkish, Moroccan and Surinamese ethnicities were also the largest non-European ethnic groups, but were underrepresented, especially children of Moroccan origin. Our study population leans towards a higher socioeconomic status based

on the household income and degree of highest education obtained. This selection towards a more affluent and healthier population is similar in our follow-up assessments until age 10 years and might weaken the external validity of our findings to other less ethnically diverse, and less affluent populations.

CAUSALITY

We examined associations, not causal relations, of risk factors and consequences of eczema phenotypes, because the studies in this thesis were embedded in an observational study. Causality between exposures and outcomes can be determined by the Bradford Hill criteria, which includes strength, consistency, specificity, and temporality of the observed association.[53] In addition, causality is strengthened by a biological dose-response effect, plausible mechanism, coherence with current biological knowledge, experimental evidence, and similarity with comparable exposures. When we apply the Bradford Hill criteria on the studies in our thesis, we observed small to moderate effect estimates of early life environmental, genetic and microbial factors with eczema phenotypes, and small effect estimates of eczema phenotypes with emotional and behavioural problems, making causality unlikely. We observed modest to high effect estimates and a dose-response effect of eczema phenotypes with allergy and asthma, making causality more likely, but this might also be explained by the mutual systemic inflammation and/or comparable pathophysiology of atopic diseases. Temporality (fourth criteria) is essential to causal inference, and we studied this by using cross-lagged models when repeated measures of exposure and outcome were available, and showed that the relation between eczema and *S. aureus* nasal carriage was more likely to be cross-sectional, and that the direction between eczema and emotional and behavioural problems was more from early life eczema to emotional and behavioural problems than reversely. Plausible underlying mechanisms are available for most risk factors and consequences with eczema, but much is still unknown.[2] Randomized controlled trials (RCTs) could provide the experimental evidence, since mouse models and in vivo experiments have limited translatability.[54] For microbial factors, systematic reviews, including RCTs, showed insufficient evidence for *S. aureus* reducing treatments and probiotics for improving eczema symptoms. Consequences of eczema phenotypes are more difficult to study in experimental settings, since eczema is not inducible (and not ethical).

In summary, our epidemiological studies suggests limited causal relationships of early life environmental, genetic and microbial risk factors with childhood eczema phenotypes based on the Bradford Hill criteria. Our studies provide moderate evidence for causal

relationships of eczema phenotypes with allergies and asthma, and with emotional and behavioural problems.

Continuous technical advances in the 21st century has led to an immense growth of data generated each year, and novel concepts such as big data, machine learning and artificial intelligence.[55] Similarly, more and increasing follow-up of prospective cohort studies, and expansion of the –omics research field have increased the demand for more advanced statistical tools that take into account repeated measures, many variables, and high-dimensional data. This demand is being supplied by many open-source statistical tools that continue to be developed, such as the statistical packages in R, which at the moment of this writing, features 16,850 available packages, and many codes available from GitHub, a repository hosting platform with millions of contributors. The advances in statistical tools has also enabled the possibility to infer causality from observational studies/big data, rather than only from RCTs.[56] An example of a causal inference tool is structural equation modelling, which includes the cross-lagged models and latent growth models that we have used in our studies. Modern medicine practices includes more focus on personalized medicine, and therefore person-centred analyses approaches have been increasingly recognised for their usefulness. Latent growth modelling allows for the identification of meaningful groups and homogenous subpopulation within the larger heterogeneous population, and enables the investigation of interindividual differences in intraindividual change while taking into account the unobserved heterogeneity within a larger population.[57] Structural equation models have been used by behavioural scientists to study the time-varying social behaviour, but are also applicable to other longitudinal outcomes of interest, such as eczema in our case. However, while these causal inference tools are readily available, they are not often used by researchers outside the behavioural and social sciences. Therefore, active collaboration and knowledge sharing across conventional borders, with the biostatistics department and also with other research groups, are indispensable for optimal use of the many currently available statistical tools, and successful advancement in research.[58, 59]

FUTURE PERSPECTIVES

We identified eczema phenotypes in multi-ethnic children from birth until school age. As the follow-up period of prospective cohort continues, eczema phenotypes can be updated by including longer periods of time, and ideally, be established in subjects from birth until elderly age. Next, further validation of eczema phenotypes in other birth cohort studies, non-Europeans, and in hospital-based populations is needed. Also, future studies should examine if expanding the eczema phenotypes model with other atopic/

allergic conditions, and disease severity leads to a better and more practical model. Additionally, eczema endotypes based on serum biomarkers could be combined with eczema phenotypes.[60]

Our studies on risk factors and eczema phenotypes showed that most well-known risk factors for eczema were not strongly and differentially associated with eczema phenotypes, except for sex and ethnicity. Therefore, studying sex- and/or ethnicity specific socioenvironmental, behavioural, (epi)genetic, and microbial and other –omics factors in a multilayer data integration analysis approach and starting from preconception onwards might uncover novel risk factors for eczema that are differentially associated with eczema phenotypes. One of the potential differentiating factors that has not been studied in this thesis is diet, which is highly correlated with ethnicity, and has been associated with eczema.[61] A recent cohort study in Canada showed interaction between maternal diet and ethnicity, and risk of eczema, and showed that a maternal plant based diet during pregnancy was associated with a decreased risk of eczema at age 1 year.[62] The relation between ethnicity, diet and eczema may be further clarified by examining certain diet patterns, such as a pro-inflammatory diet, and the interaction with gut microbiota.[63] The microbiota at different body sites at various time points from early age onwards should be examined repeatedly to assess the interplay between microbial communities within an individual, and age-specific microbial risk factors. Also, the associations between the microbiota of the persons of interest and the microbiota of other household members, and environment could clarify the developmental dynamics between microbial communities and its environment, and the impact of that relation on child's health and development. Due to the high-dimensionality of microbial and meta-exposome data, collaborations are needed between large studies to increase the power for detection. Furthermore, uniform and harmonized methodological approaches, especially for –omics data, are needed for better identification and comparison of results between studies.

Disruptions in the early life period, when the immune system is still developing, has potential negative consequences in later life. Early eczema onset was most consistently associated with higher risks of asthma, allergy and emotional and behavioural problems. Examining the meta-exposome during preconception and early pregnancy in more detail may expand the body of knowledge on the origins and persistence of eczema, and may help identify early lifestyle interventions for prevention of eczema onset, persistence and/or worsening. Also, developing a prediction model would be useful to identify children at risk for developing eczema, and to predict the age of onset, if eczema will be persistent, and the risk of associated diseases.

CONCLUSIONS

We identified five eczema phenotypes in multi-ethnic children from birth until age 10 year. The main genetic and early life environmental risk factors for eczema as a binary definition were not strongly and differently associated with eczema phenotypes, except for sex and ethnicity. While early-life *Staphylococcus aureus* nasal carriage, not nasopharyngeal bacterial carriage, was associated with early transient and persistent eczema, the associations were cross-sectional not longitudinal. We observed that stool microbiota at school-age was not strongly associated with eczema, suggesting a limited role of gut microbiota on eczema in later childhood. Children with early transient and persistent eczema had higher risks of asthma and allergies at school age, and might benefit from more intense follow-up for early identification and treatment of these diseases. The minimal increase of emotional and behavioural problems in later childhood in children with eczema phenotypes implies that most children with eczema are mentally healthy.

The eczema phenotypes model may be improved by longer follow-up, adding other parameters such as other atopic diseases, disease severity, and serum biomarkers, and further validation in hospital-based populations. Future research should identify the sex- and ethnicity specific risk factors associated with eczema. Gut microbiota should be examined from early life onwards, and repeatedly, to uncover age-specific effects on eczema. Examining the meta-exposome in early life might provide suggestions for early lifestyle interventions to prevent eczema and its associated disorders. Stronger collaboration with biostatical and other research departments are important to advance in high-dimensional data research by using the most novel statistical techniques.

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5

Summary

5.1

Summary

Summary Childhood eczema is a major common chronic health problem. Due to its variable age of onset and persistence over time, using eczema phenotypes instead of the dichotomous eczema definition is suggested to better reflect the natural course of eczema. Eczema phenotypes may enable better identification of specific genetic, early life environmental and microbial risk factors, and consequences on other atopic diseases and emotional and behavioural problems in later childhood. This could provide new insights for better prevention and treatment strategies focused on early life to reduce the occurrence and burden of childhood eczema.

Chapter 1 describes the background, hypothesis and main objectives of this thesis. Briefly, we aimed to identify eczema phenotypes, and assess its association with genetic, early environmental and microbial factors, and its consequences on asthma and allergies, and emotional and behavioural problems. Our studies were embedded in the Generation R Study, a population-based prospective cohort in Rotterdam, the Netherlands.

Chapter 2 describes the associations of specific risk factors with eczema and eczema phenotypes. In *Chapter 2.1*, we identified five eczema phenotypes in a multiethnic pediatric population from birth until school-age. Children who were first-born, had persistent wheezing, or had a filaggrin mutation or additional risk alleles had increased risks of transient and persistent eczema. Previously known eczema risk factors had limited differentiating capabilities for eczema phenotypes, except for male sex with early transient eczema, and Asian ethnicity with late transient and persistent eczema. In *Chapter 2.2*, we concluded that early life *Staphylococcus aureus* nasal carriage, not nasopharyngeal carriage with other bacteria, was associated with increased risks of early transient and persistent eczema. Bidirectional analyses showed that the associations between *Staphylococcus aureus* nasal carriage and eczema were mostly cross-sectional, and thus showed no specific direction of longitudinal effects. The findings in *Chapter 2.3* suggests that gut microbiota has a limited role on the risk of eczema at school age. The diversity, relative abundance and functional pathways of stool microbiota were most consistently associated with inhalant allergy outcomes only, and not with eczema, food allergy, or asthma in school-aged children.

Chapter 3 describes the consequences of eczema phenotypes on asthma and allergies, and emotional and behavioural problems. In *Chapter 3.1*, we observed that eczema phenotypes were differentially associated with risks of respiratory and allergic conditions in school-aged children. Children with early transient and persistent eczema phenotypes had a very high odds of asthma, allergic sensitization, and physician-diagnosed allergies, including allergic rhinitis. Results were similar for children of European and non-European ethnicity. From *Chapter 3.2,* we concluded that eczema phenotypes were very

modestly associated with more somatic symptoms and attention problems at school age compared to no eczema. Early transient eczema was associated with more aggressive behaviour symptoms. Directional effects seem from early life eczema leading to later life emotional and behavioural problems rather than reversely.

In **Chapter 4**, we present a general overview of the main findings, methodological considerations, the clinical implications of the main findings, and give suggestions for the direction of future research.

5.2

Samenvatting

Samenvatting Eczeem is van de meest voorkomende ziekten met een chronisch beloop op kinderleeftijd. De leeftijd waarop het eczeem ontstaat en de duur van de klachten zijn variabel. Eczeem fenotypen in plaats van de dichotome definitie van eczeem lijkt daarom een betere weerspiegeling te zijn van het natuurlijk beloop. Hierdoor is het mogelijk om beter de specifieke genetische, omgevings- en microbiële risicofactoren en de gevolgen voor andere atopische ziekten en emotionele en gedragsproblemen te bestuderen. Hieruit kunnen uiteindelijk effectievere preventie maatregelen en behandelmogelijkheden voortkomen.

Hoofdstuk 1 geeft de achtergrond, de hypothese en de belangrijkste doelstellingen weer waarop dit proefschrift is gebaseerd. We stelden ons ten doel om de bovengenoemde eczeem fenotypen te identificeren en de relaties tussen deze en specifieke genetische, vroege omgevings- en microbiologische factoren, alsmede de relatie tussen deze en gevolgen op andere atopische ziekten en emotionele- en gedragsproblemen te onderzoeken. De beschreven studies maken deel uit van een grootschalig bevolkingsonderzoek in Rotterdam, genaamd de Generation R Studie.

Hoofdstuk 2 beschrijft de relaties tussen specifieke risicofactoren en eczeem fenotypen. In *Hoofdstuk 2.1* identificeerden we tot en met de schoolleeftijd vijf verschillende eczeem fenotypen bij kinderen van verschillende etnische achtergrond. Kinderen met een vroege voorbijgaand en persisterend eczeem fenotype zijn vaker de eerstgeborene, hebben vaker een persisterende vorm van piepende ademhaling, en tonen vaker filaggrine mutaties en andere genetische risico allelen. De onderzochte risicofactoren van eczeem maken niet goed onderscheid tussen de verschillende eczeem fenotypen. Uitzonderingen hierop zijn het mannelijke geslacht en een Aziatische achtergrond dat vaker voorkomt bij kinderen met respectievelijk vroege voorbijgaande en persisterend eczeem. Uit het onderzoek beschreven in *Hoofdstuk 2.2* kunnen we concluderen dat neusdragerschap met *Staphylococcus aureus* op vroege leeftijd geassocieerd is met een hoger risico op vroege voorbijgaande en persisterend eczeem. Neusdragerschap met *Staphylococcus aureus* leidt niet tot een hoger risico op eczeem dan in omgekeerde richting. We vonden geen relatie tussen dragerschap in de nasofarynx van andere bacteriën en eczeem fenotypen. De resultaten van *Hoofdstuk 2.3* laten zien dat de diversiteit, relatieve hoeveelheid en functies van het microbioom in de ontlasting het meest consistent zijn geassocieerd met inhalatie allergieën, maar niet met eczeem, voedselallergieën of astma bij kinderen van 10 jaar. Dit suggereert dat het darmmicrobioom niet een grote rol heeft bij de ontwikkeling van het eczeem op de schoolleeftijd.

Hoofdstuk 3 beschrijft de gevolgen van eczeem fenotypen op andere atopische ziekten en emotionele en gedragsproblemen. In *Hoofstuk 3.1* laten we zien dat ec-

zeem fenotypen verschillend gerelateerd zijn aan long- en allergische uitkomsten op schoolleeftijd. Kinderen met vroege voorbijgaand en persisterend eczeem hadden vaker astma, allergische sensibilisatie, en allergieën, inclusief allergische rinitis. Er werd geen verschil in resultaten gevonden tussen kinderen van Europese en niet-Europese afkomst. In *Hoofdstuk 3.2* concluderen we dat kinderen met eczeem fenotypen meer somatische klachten en aandachtsproblemen hebben op de schoolleeftijd dan kinderen zonder eczeem, maar dat de verschillen klein zijn. Kinderen met vroege voorbijgaand eczeem vertonen daarnaast ook iets meer agressieve gedragsproblemen. Daarnaast lijkt de relatie van eczeem op vroege leeftijd met meer emotionele en gedragsproblemen op latere leeftijd sterker te zijn dan andersom.

In **Hoofdstuk 4** geven we een overzicht van de belangrijkste resultaten uit bovengenoemde studies, de methodologische beperkingen en de klinische betekenis van onze bevindingen en doen we suggesties voor toekomstig onderzoek.

6

Appendices

LIST OF PUBLICATIONS

Hu C, Duijts L, Erler NS, Elbert NJ, Piketty C, Bourdes V, Blanchet-Réthoré S, de Jongste JC, Pasmans SGMA, Felix JF, Nijsten T. Most associations of early-life environmental exposures and genetic risk factors poorly differentiate between eczema phenotypes: the Generation R Study. *Br J Dermatol. 2019 Dec;181(6):1190-1197*.

Hu C, Nijsten T, van Meel ER, Erler NS, Piketty C, de Jong NW, Pasmans SGMA, de Jongste JC, and Duijts L. Eczema phenotypes and risk of allergic and respiratory conditions in school age children. *Clin Transl Allergy*. 2020 Feb 19;10:7. doi: 10.1186/s13601-020-0310-7.

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Hu C, van Meurs T, van Dooren MF, Mooyaart AL, Munte K. Multipele plaveiselcelcarcinomem van het Ferguson-Smith syndroom. *Nederlands Tijdschrift voor Dermatologie en Verereologie, 2021 Jan*.

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Hu C, van Meel ER, Medina-Gomez C, Kraaij R, Barroso M, Kiefte-de Jong JC, Radjabzadeh D, Pasmans SGMA, de Jong NW, de Jongste JC, Moll HA, Nijsten T, Rivadeneira F, Pardo LM, and Duijts L. A population-based study on associations of stool microbiota with atopic diseases in school-age children. *J Allergy Clin Immunol, 2021 Apr 15;S0091-6749(21)00563-7*.

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Thyssen JP, Ahluwalia TS, Paternoster L, Ballardini N, Bergstrom A, Melen E, Chawes B, Stokholm J, Hourihane J, O'Sullivan D, Bager P, Melbye M, Bustamante M, Torrent M, Esplugues A, Duijts L, **Hu C** et al., Interaction between filaggrin mutations and neonatal cat exposure in atopic dermatitis. *Allergy*. *2019 Dec 26. doi: 10.1111/all.14162*.

ABOUT THE AUTHOR

Chen Hu was born on January 11th 1991 in Zwijndrecht, the Netherlands. In 2009, she finished high school at Johan de Witt gymnasium in Dordrecht and the Junior Med School, a pre-university program at Erasmus Medical Center (Erasmus MC) in Rotterdam. During Medical School at Erasmus MC in Rotterdam, she joined the Honours Class, and enrolled in the Clinical Research master of the Netherlands Institute for Health Sciences. After graduating in 2016, she worked as a resident (ANIOS) at the Department of Internal Medicine of the Albert Schweitzer Hospital in Dordrecht, until she got the opportunity to start her PhD in 2017 at the Department of Dermatology and the Generation R Study, under supervision of Prof. T.E.C. Nijsten and Dr. L. Duijts, which resulted in this thesis. During her PhD-project, Chen has joined the Erasmus MC Women's Academic Network (VENA) to contribute to a more gender diverse and inclusive environment. From January 2021 onwards, she has started her training in Dermatology (AIOS) at Erasmus MC, Rotterdam. Chen lives in Rotterdam with her partner Jeroen Jansen and their cats Mowgli and Coco.

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: Chen Hu
Erasmus MC Department: Dermatology
Research School: Netherlands Institute of Health Sciences

PhD period: May 1st 2017 – June 21st 2021
Promotor(s): Prof. dr. T.E.C. Nijsten
Supervisor: Dr. L. Duijts

1. PhD training	Year	Workload (Hours/ECTS)
Courses		
- Endnote course, Erasmus Medical Center, Rotterdam, The Netherlands	2017	0.25 ECTS
- The Course on R, Erasmus Postgraduate School Molecular Medicine, Rotterdam, The Netherlands	2017	1.8 ECTS
- The course on Gene expression data analysis using R: How to make sense out of y our RNA-Seq/microarray data, Erasmus Postgraduate School Molecular Medicine, Rotterdam, The Netherlands	2017	2.0 ECTS
- Research Integrity	2018	0.3 ECTS
- Repeated Measurements	2018	1.4 ECTS
- Missing Values	2018	1.4 ECTS
- Mendelian Randomisation	2019	0.9 ECTS
- Microbiomics	2019	0.6 ECTS
Seminars, workshops and presentations		
- Research meetings, Generation R, Erasmus Medical Center, Rotterdam, The Netherlands	2017-2020	1.0 ECTS
- Maternal & Child Health meetings, Generation R, Erasmus Medical Center,Rotterdam, The Netherlands	2017-2020	1.0 ECTS
- Research meetings and journal clubs, Department of Dermatology, Erasmus Medical Center, Rotterdam, The Netherlands	2017-2020	1.0 ECTS
- Skintermezzo, Department of Dermatology, Erasmus Medical Center, Rotterdam, The Netherlands	2017-2020	1.0 ECTS
- PhD weekend, 's-Hertogenbosch the Netherlands	2017	1.0 ECTS
- PhD weekend, Scheveningen, the Netherlands	2019	1.0 ECTS
(Inter)national conferences		
- 26th European Academy of Dermatology and Venereology Congress, Geneva, Switzerland	2017	1.0 ECTS
- 10th World Congress on Developmental Origins of Health and Disease, Rotterdam, The Netherlands	2017	1.0 ECTS

- 5th Skin Allergy Club of The European Academy of Allergy and Clinical Immunology, Zurich, Switzerland	2018	1.0 ECTS
- Dutch Society for Experimental Dermatology (NVED)	2019	1.0 ECTS
- European Society of Pediatric Dermatology (ESPD)	2019	1.0 ECTS
- International Society of Atopic Dermatitis (ISAD)	2020/2021	1.0 ECTS
Other		
- NIHES, master of clinical research	2016	120.0 ECTS
- GALDERMA	2017	10 hours
- GALDERMA	2018	10 hours
2. Teaching		
	Year	**Workload (Hours/ECTS)**
- Research education 'Missing values and imputations'	2019	5 hours
- Research education 'Cross lagged models'	2020	5 hours
- Generation R Training Days	2017-2020	20 hours
- Peer review of articles for journals (Clinical Experimental Allergy, British Journal of Dermatology)	2019-2020	6 hours

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湿疹

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