NIELS J. ELBERT

FETAL AND INFANT ORIGINS OF CHILDHOOD ECZEMA, ALLERGIC SENSITIZATION AND ALLERGY

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Fetal and infant origins of childhood eczema, allergic sensitization and allergy

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“HIER WERD EEN TOEKOMST GEBOREN, BOUW VOORT.”

THEO VAN REIJN (1884–1954)

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Gazibara T, **Elbert NJ**, den Dekker HT, de Jongste JC, Reiss I, McGrath JJ, Eyles DW, Burne TH, Tiemeier H, Jaddoe VW, Pasmans SG, Duijts L. Associations of maternal and fetal 25-hydroxyvitamin D levels with childhood eczema: The Generation R Study. Pediatr Allergy Immunol. 2016;27(3):283-9.

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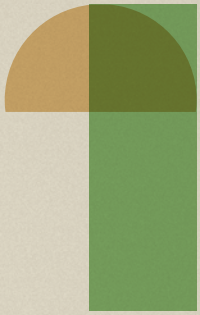
CHAPTER 1



GENERAL INTRODUCTION

BACKGROUND

Eczema is the most common inflammatory skin disease among children of developed countries, with an estimated lifetime prevalence of 15–30%.[1] The prevalence of childhood eczema varies considerably worldwide[2], and seems to increase in the majority of developing countries while leveling off or even decreasing in some developed countries.[3, 4] In the Netherlands, 8.1% of all children aged 13–14 years report symptoms of eczema in the last year.[5] The mean annual societal costs of eczema treatment in the Netherlands are as high as €981–1,409 per child.[6]

Eczema is a complex chronic disease, characterized by intense itching and recurrent eczematous lesions that may affect any part of the body, but typically show a morphology and distribution that varies with age.[7] Also, generalized skin dryness resulting from impaired epidermal barrier function is a common feature. Because no specific laboratory biomarker or histological finding for eczema is known, the diagnosis relies exclusively on clinical features. Several sets of diagnostic criteria have been proposed in the past decades, such as the Hanifin and Rajka criteria, which are useful in clinical settings.[8] In population-based studies, the diagnosis of eczema is often based on a parental-reported physician diagnosis.[9] Originally, the pathogenesis of eczema was mainly attributed to abnormalities in the adaptive immune system, such as a dysregulation of type 1 and type 2 T-helper cells, immunoglobulin E (IgE) production and mast cell hyperactivity.[10] However, recent epidemiological and genetic studies have directed more toward a central role of epidermal barrier dysfunction.[1, 10] Interaction of epidermal barrier impairment and pro-inflammatory cytokines from keratinocytes also promotes percutaneous allergic sensitization.[1, 7]

Eczema is suggested to be the initial manifestation of atopy and the first step of the alleged atopic march that may ultimately lead to inhalant allergy and allergic asthma. Eczema may occur solely or coincide with allergic sensitization and symptoms of allergy as part of an atopic constitution.[11] In the Netherlands, allergic sensitization is present in 26.4% of children aged 7–14 years.[12] In the first 2 years of life, up to two-thirds of children with moderate to severe eczema are sensitized to food allergens[13], but only a minority of these children develop IgE-mediated food allergy.[7] As children grow older, the allergic sensitisation pattern shifts toward inhalant allergens.[14] Approximately 5–10% and 7–20% of Western European children aged 6–7 years and 13–14 years, respectively, report symptoms of inhalant allergy. Furthermore, the prevalence of self-reported symptoms of food allergy among Dutch school-age children is 7.2%.[15]

Childhood eczema may partly has its origin in fetal life and infancy.[16, 17] Environmental exposures that are known to be associated with eczema include housing conditions, urban or rural living, dietary and feeding habits, air pollution, and microbial exposure.[17] However, large population-based observational studies on the role of exposures during critical periods in early life on childhood eczema and related allergic sensitization and

allergy are scarce. This thesis focuses on the associations of detailed fetal and infant exposures with the risk of eczema from birth until school-age, and inhalant and food allergic sensitization and allergy at school-age in children from the general population.

FETAL EXPOSURES

Scientific knowledge on the role of environmental exposures in the developmental origins of health and disease increases rapidly.[18] Because future health care costs are likely to be unaffordable, there is a global imperative to develop and implement powerful prevention strategies. New insights into environmental exposures during fetal life and infancy that affect childhood eczema may provide novel opportunities to develop preventive measures at times when they are most effective.

Maternal psychiatric symptoms during pregnancy, which partly reflect maternal stress, have been associated with an increased risk of asthma-related symptoms, including wheezing, in preschool-age children[19], but exact underlying biological mechanisms are unclear. It is hypothesized that sustained excessive levels of maternal stress-induced hormones, particularly glucocorticoids, may influence T-helper cell phenotype differentiation and, subsequently, atopic inflammation, eczema and allergy in the child.[20] However, associations of maternal psychiatric symptoms during pregnancy with childhood eczema and allergy might be confounded by social, behavioral or environmental factors. Information on both maternal and paternal psychiatric symptoms could disentangle potential intrauterine and confounding mechanisms[19, 21], but has previously not been examined.

Maternal vitamin and fatty acid status during pregnancy, including vitamin E, folate and n-3 or n-6 polyunsaturated fatty acid levels, have been associated with the risk of childhood eczema.[22, 23] Exposure to low vitamin D levels during pregnancy (< 50 nmol/L) may affect the child's developing adaptive and innate immune system in early life and, subsequently, increase the risk of eczema.[24] Vitamin D is suggested to play a role in the cutaneous production of antimicrobial peptides in keratinocytes.[25] Deficient expression of such peptides may increase susceptibility to skin infections with *Staphylococcus aureus*, an important pathogen associated with eczema.[26, 27] In mice, vitamin D receptor activation induces the expression of antimicrobial peptides and epidermal barrier genes, including filaggrin (*FLG*), and improved allergen-triggered eczema.[28] Loss-of-function mutations in the gene encoding filaggrin, an indispensable protein for keratinocyte differentiation and maintenance of an optimal epidermal barrier, are well known to be associated with eczema.[29] However, data from longitudinal population-based studies relating both maternal and fetal vitamin D levels with childhood eczema remain scarce.

INFANT EXPOSURES

Recently, it was observed that structural and functional epidermal barrier characteristics differ between adults of African American, Caucasian and East Asian descent.[30] East Asian skin had the weakest epidermal barrier properties, but without skin dryness and scaliness due to high levels of stratum corneum lipids. African American skin showed a stronger epidermal barrier, but skin dryness due to low levels of stratum corneum lipids and low serine protease activity. Also, the prevalence of eczema varies considerably among children of different ethnic origin[5], which might be explained by environmental and genetic exposures. However, data from multi-ethnic longitudinal studies relating early-life environmental and genetic exposures with eczema in children from the general population are not fully clear.

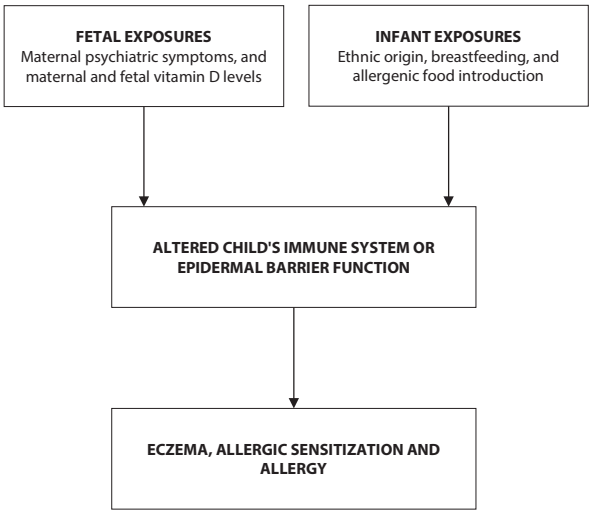
Potential infant dietary exposures associated with the risk of childhood eczema include breastfeeding habits and timing or diversity of allergenic food introduction.[17] Underlying biological mechanisms for the associations of breastfeeding with eczema and allergy are not fully understood. They might involve complex interactions of human milk components, including secretory immunoglobulin A, cytokines, chemokines, long-chain polyunsaturated fatty acids and polyamines, which modulate the child's developing immune system and alter the balance between pro-inflammatory and anti-inflammatory signals.[31, 32] Also, human milk oligosaccharides with prebiotic properties are suggested to influence the development of eczema and allergy by modulating gut microbiota diversity.[33] The effects of breastfeeding on eczema, allergic sensitization and allergy in school-age children are less clear.[34, 35] Also, the effects of disease-related modification of the exposure, meaning that early symptoms of eczema or allergy in the child may encourage a mother to alter breastfeeding habits[36], and the modifying effects of child's atopic susceptibility on these associations are less known.[35, 37, 38]

Timing or diversity of allergenic food introduction in relationship to eczema, allergic sensitization and allergy is a much debated topic. An accumulating body of evidence suggests that at age 4–6 months an early window of immunological opportunity exists to expose children to food proteins and induce immune tolerance.[39, 40] Recently, the LEAP trial showed that peanut introduction in high-risk children aged 4–11 months was associated with a decreased risk of peanut allergy.[41] However, less is known about the effects of introduction practices of allergenic foods in early life on eczema, allergic sensitization and allergy in older children from the general population.

HYPOTHESIS

The hypothesis of this thesis is that fetal and infant exposures modulate the child's developing immune system or impair the epidermal barrier function, potentially leading to eczema, allergic sensitization and allergy (Figure 1).

Figure 1. Overview of the origins of childhood eczema, allergic sensitization and allergy, and the specific fetal and infant exposures studied in this thesis.



OBJECTIVES

The main aims of this thesis are:

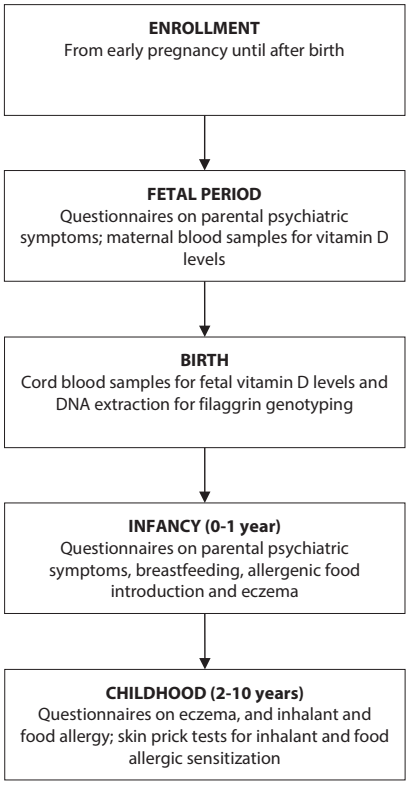
1. To assess the associations of fetal exposures with childhood eczema, allergic sensitization or allergy. The exposures of interest include maternal psychiatric symptoms during pregnancy, and maternal and fetal vitamin D levels.
2. To assess the associations of infant exposures with childhood eczema, allergic sensitization or allergy. The exposures of interest include ethnic origin, breastfeeding duration and exclusiveness, and timing or diversity of allergenic food introduction.

GENERAL DESIGN

The Generation R Study is a population-based prospective cohort study in Rotterdam, The Netherlands, following pregnant women and their children from fetal life onwards (www.generationr.nl).[42] The study was designed to identify early environmental and genetic factors and causal pathways leading to normal and abnormal growth, development and health during fetal life, childhood and adulthood. Enrollment was aimed in first trimester, but was allowed 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 at baseline was 61%. Data collection for this thesis comprised questionnaires for parental psychiatric symptoms during pregnancy and after delivery (Figure 2). Biological samples included maternal and umbilical cord blood for measurement of maternal and fetal 25-hydroxyvitamin D levels in mid-gestation and at birth, respec-

tively. Total maternal and fetal 25-hydroxyvitamin D levels were reported as the sums of 25-hydroxyvitamin D2 and D3 species quantified in plasma using isotope dilution liquid chromatography-tandem mass spectrometry.[43] Child's DNA samples obtained from umbilical cord blood were genotyped by modified Taqman allelic discrimination assays for four common loss-of-function mutations in the *FLG* gene (2282del4, R2447X, R501X, and S3247X).[44] Information on breastfeeding or allergenic food introduction practices was obtained from postal questionnaires at ages 2, 6 and 12 months. Parental questionnaires, including questions adapted from the International Study on Asthma and Allergy in Childhood[45], provided information on physician-diagnosed eczema from birth until age 10 years. At age 10 years, inhalant (house dust mite, 5-grass mixture, birch, cat and dog) and food (hazelnut, cashew nut, peanut and peach) allergic sensitization were measured skin prick tests using the scanned area method[46], and parental questionnaires provided information on physician-diagnosed inhalant and food allergy. Information on covariates, including demographic, socioeconomic, and health-related and lifestyle factors, was mainly obtained by postal questionnaires in the first year of life.

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



OUTLINE OF THIS THESIS

Chapter 2 focuses on associations of fetal exposures with childhood eczema, allergic sensitization or allergy. In *Chapter 2.1*, the associations of maternal psychiatric symptoms during pregnancy with eczema, allergic sensitization and physician-diagnosed inhalant or food allergy in school-age children are presented. The associations of maternal and fetal vitamin D levels and eczema in preschool-age children are explored in *Chapter 2.2*. **Chapter 3** focuses on associations of infant exposures with childhood eczema, allergic sensitization or allergy. In *Chapter 3.1*, the role of environmental exposures and *FLG* genotype on the associations of ethnic origin with eczema in preschool-age children is described. The associations of duration and exclusiveness of breastfeeding with eczema, allergic sensitization and physician-diagnosed inhalant or food allergy in school-age children are presented in *Chapter 3.2*. In *Chapter 3.3*, the associations of timing or diversity of allergenic food introduction with eczema, allergic sensitization and physician-diagnosed inhalant or food allergy are described. **Chapter 4** includes a general discussion that outlines the main findings and discusses the clinical implications of the studies described in this thesis, followed by an English and a Dutch summary in **Chapter 5**.

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CHAPTER 2

FETAL EXPOSURES AND CHILDHOOD ECZEMA, ALLERGIC SENSITIZATION OR ALLERGY

CHAPTER 2.1

MATERNAL PSYCHIATRIC SYMPTOMS DURING PREGNANCY AND ECZEMA, ALLERGIC SENSITIZATION AND ALLERGY IN SCHOOL-AGE CHILDREN

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Clin Exp Allergy. 2017;47(4):509-19

ABSTRACT

Background Maternal psychiatric symptoms during pregnancy might affect the developing immune system and subsequent risk of childhood atopic diseases.

Objective Our aim was to examine the associations of maternal psychiatric symptoms during pregnancy with allergic sensitization, allergy and eczema in children until age 10 years.

Methods This study among 5,205 children was performed in a population-based prospective cohort from fetal life onwards. We assessed maternal and paternal psychiatric symptoms (overall, depressive and anxiety) during pregnancy and at 36 months after delivery, and maternal psychiatric symptoms at 2 and 6 months after delivery using the Brief Symptom Inventory. Inhalant and food allergic sensitization were measured by skin prick tests, and physician-diagnosed inhalant and food allergy or eczema by questionnaires from birth until age 10 years. We used multivariate logistic regression, multinomial logistic regression or generalized estimating equation models where appropriate.

Results We observed no association of maternal psychiatric symptoms during pregnancy with allergic sensitization. Maternal overall psychiatric, depressive and anxiety symptoms during pregnancy were associated with an increased risk of inhalant allergy only (adjusted odds ratio (95% confidence interval): 1.96 (1.44, 2.65), 1.58 (1.25, 1.98), and 1.61 (1.27, 2.03), respectively, per 1-unit increase). Maternal overall psychiatric and anxiety symptoms during pregnancy were associated with an increased risk of eczema (1.21 (1.05, 1.39) and 1.15 (1.02, 1.29), respectively, per 1-unit increase). Effect estimates did not materially change when maternal psychiatric symptoms after delivery, or paternal psychiatric symptoms during pregnancy and after delivery were taken into account.

Conclusions and Clinical Relevance Maternal psychiatric symptoms during pregnancy were associated with increased risks of childhood inhalant allergy and eczema, independent of maternal psychiatric symptoms after delivery and of paternal psychiatric symptoms.

INTRODUCTION

Childhood atopic diseases such as inhalant or food allergy and eczema are suggested to have their origin in fetal life.[1, 2] Maternal stress during pregnancy may increase the risk of inhalant or food allergy and eczema in the child[3], but the exact underlying biological mechanisms are unclear. Animal studies showed that stress during pregnancy can activate the fetal hypothalamic-pituitary-adrenal axis and sympathetic adrenomedullary system leading to sustained excessive glucocorticoids and catecholamines levels, respectively.[4, 5] Glucocorticoids and catecholamines may induce a type 2 T-helper (Th2) cell predominance leading to increased secretion of pro-inflammatory and Th2 cytokines.[6, 7] This may promote immunoglobulin E (IgE) production and, subsequently, atopic inflammation and diseases.[8, 9] Associations of maternal psychiatric symptoms during pregnancy, which partly reflect maternal stress, with childhood inhalant or food allergy and eczema might be confounded by social, behavioral or environmental factors. Information on both maternal and paternal psychiatric symptoms could disentangle potential intrauterine and confounding mechanisms.[10, 11] Stronger effect estimates for the association of maternal than paternal psychiatric symptoms during pregnancy with childhood inhalant or food allergy and eczema suggest underlying intrauterine mechanisms, while similar effect estimates indicate that these associations may result from residual confounding of unmeasured factors. In a previous study, we demonstrated that maternal psychiatric symptoms during pregnancy are associated with an increased risk of childhood wheezing until age 4 years.[11]

We now examined among 5,205 children and their parents participating in a population-based prospective cohort study the associations of maternal psychiatric symptoms during pregnancy with allergic sensitization, inhalant or food allergy and eczema until age 10 years, and assessed whether these associations were independent of paternal psychiatric symptoms during pregnancy, and maternal and paternal psychiatric symptoms after delivery.

METHODS

General design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards.[12, 13] The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, The Netherlands (MEC-2012–165). Written informed consent was obtained from both parents or legal guardians. For the current study, twins (n = 208) were excluded because of high correlation, as well as children without data on maternal psychiatric symptoms during pregnancy (n = 2,381), and allergic sensitization, allergy and eczema (n = 650), leaving a total of 5,205 children for the analyses (Supplementary Figure).

Maternal and paternal psychiatric symptoms

Information on maternal and paternal psychiatric symptoms was obtained by questionnaires, completed in the second trimester of pregnancy and at 36 months after delivery. Maternal psychiatric symptoms were also assessed at 2 and 6 months after delivery. Parents each answered their own questionnaires. We used the Brief Symptom Inventory, a validated 53-item self-report questionnaire covering a broad spectrum of psychiatric symptoms experienced in the last 7 days.[14, 15] A global scale (Global Severity Index) and two symptom scales (depressive and anxiety) were defined.[11] At 6 and 36 months after delivery, only depressive and anxiety symptoms were measured. The Global Severity Index is a measure of the current level or the depth of symptoms and denotes overall psychiatric symptoms. All 53 items were rated on a 5-point unidimensional scale ranging from 0 ('not at all') to 4 ('extremely'). Total scores for each scale were calculated by summing the item scores and dividing this by the number of endorsed symptoms. Based on Dutch cut-off values, mothers and fathers were categorized as having clinically relevant psychiatric symptoms (no; yes) when having a score of ≥ 0.71 or ≥ 0.66 on the global scale, ≥ 0.80 or ≥ 0.71 on the depressive symptom scale and ≥ 0.71 or ≥ 0.65 on the anxiety scale, respectively.[16] We focused primarily on depressive and anxiety symptoms, because these are generally considered the most common psychiatric symptoms during pregnancy.

Allergic sensitization, allergy and eczema

At a median age of 9.7 years (2.5–97.5th percentile: 9.3–10.7), children visited our research center. Allergic sensitization was measured by skin prick tests on the volar surface of the left forearm with single-use sterile 1-mm-tip metal lancets (ALK-Abelló A/S, Hørsholm, Denmark). Histamine dihydrochloride (10 mg/mL) and a saline solution (NaCl 0.9%) served as two positive controls and one negative control, respectively. Inhalant allergens comprised house dust mite (*Dermatophagoides pteronyssinus*), 5-grass mixture (*Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*), birch (*Betula verrucosa*), cat (*Felis catus*) and dog (*Canis familiaris*) (ALK-Abelló B.V., Almere, The Netherlands). Food allergens comprised hazelnut, cashew nut, peanut and peach extracts.[17–19] For all food allergens, appropriate aliquots were stored at −20°C and were defrosted for 1 h prior to the skin prick test. Skin responses were recorded 15 min after applying allergens to the skin by measuring the area of the wheal (in mm^2) using the Precise Automated Area Measurement of Skin Test (PAAMOST) software and considered positive if the area of the wheal was ≥ 40% of the histamine response (i.e., histamine equivalent prick index area ≥ 0.40).[19] A skin prick test was not performed if children could not omit antihistamine intake or corticosteroid ointment ≤ 72 h and ≤ 48 h prior to the test, respectively, used oral prednisone ≥ 10 mg daily or had eczema on the volar surface of the left forearm (n = 51). We grouped children into inhalant or food

allergic sensitization (no; yes). We used questions adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires to obtain information on allergy and eczema.[20] Physician-diagnosed allergy was parental-reported at age 10 years ("Was your child ever diagnosed with an allergy to pollen (hay fever)/house dust mite/cat/dog/cashew nut/peanut?") (no; yes) and categorized into inhalant or food allergy (no; yes). A child was considered to have an inhalant allergic sensitization when sensitized to at least one inhalant allergen and to have inhalant allergy when diagnosed for at least one inhalant allergen, irrespective of a possible food allergic sensitization or food allergy. Similar considerations were made for food allergic sensitization and physician-diagnosed food allergy. Categorization into other groups ('no allergic sensitization', 'inhalant allergic sensitization only', 'food allergic sensitization only', 'inhalant and food allergic sensitization') was not feasible mainly due to the low number of children with food allergic sensitization only (n = 16) or food allergy only (n = 20). However, group sizes allowed categorization into 'no allergic sensitization and no allergy', 'any allergic sensitization, but no allergy', 'no allergic sensitization, but any allergy' and 'any allergic sensitization and any allergy'. Physician-diagnosed eczema was parental-reported at ages 6 months and 1, 2, 3, 4 and 10 years ("Was your child diagnosed with eczema in the last 6 months/last year?") (no; yes). Response rates of questionnaires were 73%, 71%, 76%, 72%, 73% and 75%, respectively.

Covariates

Information on maternal and paternal age, education (primary or secondary; higher), ethnic origin (European; non-European), and history of allergy, eczema or asthma (no; yes), and maternal parity (nulliparous; multiparous) and pet keeping (no; yes) was obtained by questionnaires at enrollment. Maternal and paternal body mass index (BMI) was calculated using weight and height measured at enrollment. Information on maternal and paternal smoking (no; yes) was obtained by postal questionnaires multiple times during pregnancy. Information on child's sex, gestational age at birth and birth weight was obtained from obstetric and midwife records at birth. Delivery reports and postal questionnaires completed by the mother when the child was 2, 6 and 12 months old provided data on ever breastfeeding (no; yes). We obtained information on day care attendance (no; yes) and current asthma (no; yes) by questionnaires at ages 1 year and 10 years, respectively.

Statistical analysis

We compared children included in the analyses and those lost to follow-up using independent samples T-tests, Mann-Whitney U-tests, and Pearson's Chi-square tests. We used logistic regression models to examine the associations of maternal psychiatric symptoms with the risk of allergic sensitization and physician-diagnosed allergy at age

10 years. We used multinomial logistic regression models to examine the associations of maternal psychiatric symptoms with the risk of combined allergic sensitization and physician-diagnosed allergy groups. We used generalized estimating equation models to examine the associations of maternal psychiatric symptoms with the longitudinal odds of eczema at ages 6 months and 1, 2, 3, 4 and 10 years independently and overall, taking into account correlations between repeated measurements of eczema within the same child. First, we adjusted for potential confounders, including maternal age at enrollment, education, ethnic origin, parity, pet keeping, BMI at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight. We considered this the main model. Second, child's ever breastfeeding and day care attendance were considered as intermediates and additionally adjusted for in the model. Third, to disentangle the effects of psychiatric symptoms during pregnancy from the effects of psychiatric symptoms after delivery on the various outcomes, we additionally adjusted for maternal psychiatric symptoms at 2, 6 and 36 months after delivery, and for paternal psychiatric symptoms during pregnancy and 36 months after delivery by adding each variable separately to the models. We also used information on paternal psychiatric symptoms during pregnancy to address residual confounding of unmeasured social, behavioral and environmental factors. We used similar models to assess the associations of paternal psychiatric symptoms during pregnancy with allergic sensitization, physician-diagnosed allergy and eczema, adjusting for maternal psychiatric symptoms during pregnancy. Finally, we tested the interactions between maternal psychiatric symptoms during pregnancy and maternal history of allergy, eczema or asthma, and child's current asthma at age 10 years as proxies for atopic susceptibility. Confounders were included in the models based on previous literature, if they were associated with both the determinant and the outcome, or if they changed the effect estimates with ≥ 10%. Analyses with inhalant or food allergic sensitization or allergy as the outcomes were mutually adjusted for each other. To reduce potential bias and improve efficiency, we performed a multiple imputation analysis of covariates and eczema generating 25 independent datasets using the Markov chain Monte Carlo method, and calculated pooled estimates.[21] No major differences in the magnitude or direction of the effect estimates were observed between analyses with imputed data and complete cases only (data not shown). We present results based on imputed analyses only. Measures of association are presented as adjusted odds ratios (aOR) with their 95% confidence intervals (CI). Statistical analyses were performed using SPSS 21.0.0.1 for Windows (IBM Corp., Armonk, NY, USA) and SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

General

Of the participating mothers, 9.0% (n = 468) had overall psychiatric symptoms, 8.9% (n = 461) depressive symptoms and 9.8% (n = 511) anxiety symptoms during pregnancy (Table 1).The median scores for maternal overall, depressive and anxiety symptoms were 0.15 (0–1.33), 0 (0–1.67) and 0.17 (0–1.50), respectively (Supplementary Table 1). In children, inhalant or food allergic sensitization was present in 32.6% (n = 994) and 7.5% (n = 228), respectively, while physician-diagnosed inhalant or food allergy was present in 12.2% (n = 438) and 2.3% (n = 79), respectively (Table 2 and Supplementary Table 1). The prevalence of eczema declined from 17.2% (n = 895) at age 6 months to 8.5% (n = 444) at age 10 years. Of the children with physician-diagnosed inhalant allergy (n = 438), 29.2% (n = 128) had asthma, 56.6% (n = 248) had hay fever and 15.5% (n = 68) had eczema in the last 12 months before age 10 years. Of the children with physician-diagnosed food allergy (n = 79), 38.0% (n = 30) had asthma, 51.9% (n = 41) had hay fever and 24.1% (n = 19) had eczema at age 10 years. The prevalence of asthma, hay fever and eczema differed between children with and without inhalant or food allergy (*p*-values

Table 1. Characteristics of mothers and fathers.

	n = 5,205	
	Mother	**Father**
Age at enrollment (years)*	30.7 (4.8)	33.2 (5.6)
Education, higher (%)	50.4 (2,624)	50.1 (2,607)
Ethnic origin, European (%)	67.7 (3,470)	65.2 (3,395)
History of allergy, eczema or asthma, yes (%)	38.6 (2,008)	34.3 (1,786)
Parity, ≥ 1 (%)	41.2 (2,142)	-
Pet keeping during pregnancy, yes (%)	34.9 (1,815)	-
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.6)	25.0 (19.6–33.2)
Smoking during pregnancy, yes (%)	24.6 (1,282)	43.4 (2,259)
Overall psychiatric symptoms during pregnancy, yes (%)	9.0 (468)	3.5 (181)
Depressive symptoms during pregnancy, yes (%)	8.9 (461)	3.7 (192)
Anxiety symptoms during pregnancy, yes (%)	9.8 (511)	7.1 (371)
Overall psychiatric symptoms at 2 months after delivery, yes (%)	8.2 (429)	-
Depressive symptoms at 2 months after delivery, yes (%)	8.7 (451)	-
Anxiety symptoms at 2 months after delivery, yes (%)	8.3 (430)	-
Depressive symptoms at 6 months after delivery, yes (%)	8.6 (450)	-
Anxiety symptoms at 6 months after delivery, yes (%)	9.8 (511)	-
Depressive symptoms at 36 months after delivery, yes (%)	5.3 (275)	4.1 (211)
Anxiety symptoms at 36 months after delivery, yes (%)	5.1 (267)	7.4 (387)

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on imputed data.

Table 2. Characteristics of children.

	n = 5,205
Sex, female (%)	50.7 (2,637)
Gestational age at birth (weeks)†	40.1 (36.0–42.4)
Birth weight (grams)*	3,449 (550)
Breastfed ever, yes (%)	91.2 (4,745)
Day care attendance until age 1 year, yes (%)	54.8 (2,853)
Asthma current at age 10 years, yes (%)	5.7 (298)
Allergic sensitization at age 10 years, yes (%)	
Inhalant	32.6 (994)
Food	7.5 (228)
Physician-diagnosed allergy at age 10 years, yes (%)	
Inhalant	12.2 (438)
Food	2.3 (79)
Allergic sensitization and allergy combined at age 10 years (%)	
No allergic sensitization and no allergy	66.2 (1,678)
Any allergic sensitization, but no allergy	22.8 (578)
No allergic sensitization, but any allergy	1.3 (33)
Any allergic sensitization and any allergy	9.7 (246)
Eczema, yes (%)	
Age 6 months	17.2 (895)
Age 1 year	13.6 (710)
Age 2 years	14.7 (763)
Age 3 years	11.0 (574)
Age 4 years	9.3 (485)
Age 10 years	8.5 (444)

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on imputed data. Data on allergic sensitizations and physician-diagnosed allergies are not imputed.

for difference < 0.05; data not shown). Children without follow-up data at age 10 years had younger, lower educated parents who were more often of non-European origin and smoked more during pregnancy, had mothers with higher parity, pet exposure during pregnancy and BMI at enrollment, and were less often female, had a lower gestational age at birth and birth weight and less often attended day care until age 1 year than those children with follow-up data (Supplementary Table 2).

Maternal psychiatric symptoms during pregnancy and childhood atopic diseases

We observed no associations of maternal psychiatric symptoms during pregnancy with food or inhalant allergic sensitization (Table 3). Children of mothers with overall psychiatric, depressive or anxiety symptoms during pregnancy had an increased risk of

Table 3. Associations of maternal and paternal psychiatric symptoms during pregnancy with allergic sensitizations in children at age 10 years.

	Odds ratio (95% confidence interval) for allergic sensitization	
	Inhalant* **n = 3,052**	**Food†** **n = 3,044**
Maternal psychiatric symptoms		
Overall psychiatric symptoms		
No	Reference	Reference
Yes	1.10 (0.82, 1.48)	1.05 (0.58, 1.91)
Per 1-unit increase	1.03 (0.80, 1.32)	1.02 (0.62, 1.66)
Depressive symptoms		
No	Reference	Reference
Yes	1.20 (0.90, 1.61)	0.90 (0.49, 1.65)
Per 1-unit increase	1.08 (0.90, 1.30)	0.96 (0.66, 1.40)
Anxiety symptoms		
No	Reference	Reference
Yes	1.08 (0.81, 1.42)	1.06 (0.59, 1.90)
Per 1-unit increase	1.08 (0.89, 1.32)	0.92 (0.60, 1.41)
Paternal psychiatric symptoms‡		
Overall psychiatric symptoms		
No	Reference	Reference
Yes	1.06 (0.62, 1.82)	1.69 (0.64, 4.48)
Per 1-unit increase	1.16 (0.76, 1.76)	1.50 (0.69, 3.25)
Depressive symptoms		
No	Reference	Reference
Yes	1.01 (0.58, 1.75)	1.10 (0.42, 2.89)
Per 1-unit increase	1.09 (0.78, 1.51)	1.04 (0.57, 1.88)
Anxiety symptoms		
No	Reference	Reference
Yes	0.98 (0.68, 1.42)	1.54 (0.74, 3.20)
Per 1-unit increase	1.21 (0.89, 1.64)	1.33 (0.70, 2.52)

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Parental psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Models are adjusted for maternal age at enrollment, education, ethnic origin, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, and child's sex, gestational age and birth weight. *Additionally adjusted for food allergic sensitization. †Additionally adjusted for inhalant allergic sensitization. ‡Additionally adjusted for maternal psychiatric symptoms during pregnancy.

physician-diagnosed inhalant allergy (aOR (95% CI): 1.96 (1.44, 2.65), 1.58 (1.25, 1.98), and 1.61 (1.27, 2.03), respectively, per 1-unit increase), but not of physician-diagnosed food allergy (Table 4). Children of mothers with overall psychiatric or depressive symptoms, but not with anxiety symptoms during pregnancy, had an increased risk of any

Table 4. Associations of maternal and paternal psychiatric symptoms during pregnancy with physician-diagnosed allergies in children at age 10 years.

	Odds ratio (95% confidence interval) for physician-diagnosed allergy	
	Inhalant* **n = 3,576**	**Food†** **n = 3,503**
Maternal psychiatric symptoms		
Overall psychiatric symptoms		
No	Reference	Reference
Yes	**1.91 (1.32, 2.77)**	0.52 (0.18, 1.49)
Per 1-unit increase	**1.96 (1.44, 2.65)**	0.58 (0.25, 1.33)
Depressive symptoms		
No	Reference	Reference
Yes	**2.07 (1.43, 2.97)**	0.75 (0.29, 1.97)
Per 1-unit increase	**1.58 (1.25, 1.98)**	0.79 (0.41, 1.54)
Anxiety symptoms		
No	Reference	Reference
Yes	**1.51 (1.06, 2.16)**	0.43 (0.14, 1.32)
Per 1-unit increase	**1.61 (1.27, 2.03)**	0.52 (0.25, 1.08)
Paternal psychiatric symptoms‡		
Overall psychiatric symptoms		
No	Reference	Reference
Yes	1.29 (0.69, 2.40)	1.23 (0.26, 5.85)
Per 1-unit increase	1.38 (0.85, 2.23)	0.86 (0.20, 3.73)
Depressive symptoms		
No	Reference	Reference
Yes	1.58 (0.89, 2.80)	0.87 (0.18, 4.14)
Per 1-unit increase	1.31 (0.91, 1.88)	1.11 (0.39, 3.15)
Anxiety symptoms		
No	Reference	Reference
Yes	1.38 (0.89, 2.15)	0.56 (0.14, 2.32)
Per 1-unit increase	1.22 (0.82, 1.83)	0.54 (0.14, 2.18)

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. Parental psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Models are adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight, and mutually for physician-diagnosed inhalant or food allergy. *Additionally adjusted for physician-diagnosed food allergy. †Additionally adjusted for physician-diagnosed inhalant allergy. ‡Additionally adjusted for maternal psychiatric symptoms during pregnancy.

allergic sensitization and any allergy combined (1.60 (1.10, 2.33) and 1.42 (1.07, 1.88), respectively, per 1-unit increase), compared with children without any allergic sensitization and any allergy combined (Table 5). Children of mothers with overall psychiatric

Table 5. Associations of maternal and paternal psychiatric symptoms during pregnancy with combined allergic sensitization and physician-diagnosed allergy groups in children at age 10 years.

	Odds ratio (95% confidence interval) for any allergic sensitization and any physician-diagnosed allergy combined		
	Any allergic sensitization, but no allergy n = 578	**No allergic sensitization, but any allergy n = 33**	**Any allergic sensitization and any allergy n = 246**
Maternal psychiatric symptoms			
Overall psychiatric symptoms	0.74 (0.52, 1.06)	1.58 (0.73, 3.40)	**1.60 (1.10, 2.33)**
Depressive symptoms	0.91 (0.70, 1.18)	1.14 (0.60, 2.17)	**1.42 (1.07, 1.88)**
Anxiety symptoms	0.79 (0.60, 1.04)	1.23 (0.61, 2.46)	1.34 (0.99, 1.80)
Paternal psychiatric symptoms*			
Overall psychiatric symptoms	1.08 (0.65, 1.77)	0.67 (0.12, 3.68)	1.21 (0.64, 2.31)
Depressive symptoms	1.04 (0.70, 1.53)	0.30 (0.02, 4.61)	1.22 (0.76, 1.97)
Anxiety symptoms	1.16 (0.81, 1.68)	1.50 (0.56, 4.04)	1.22 (0.72, 2.08)

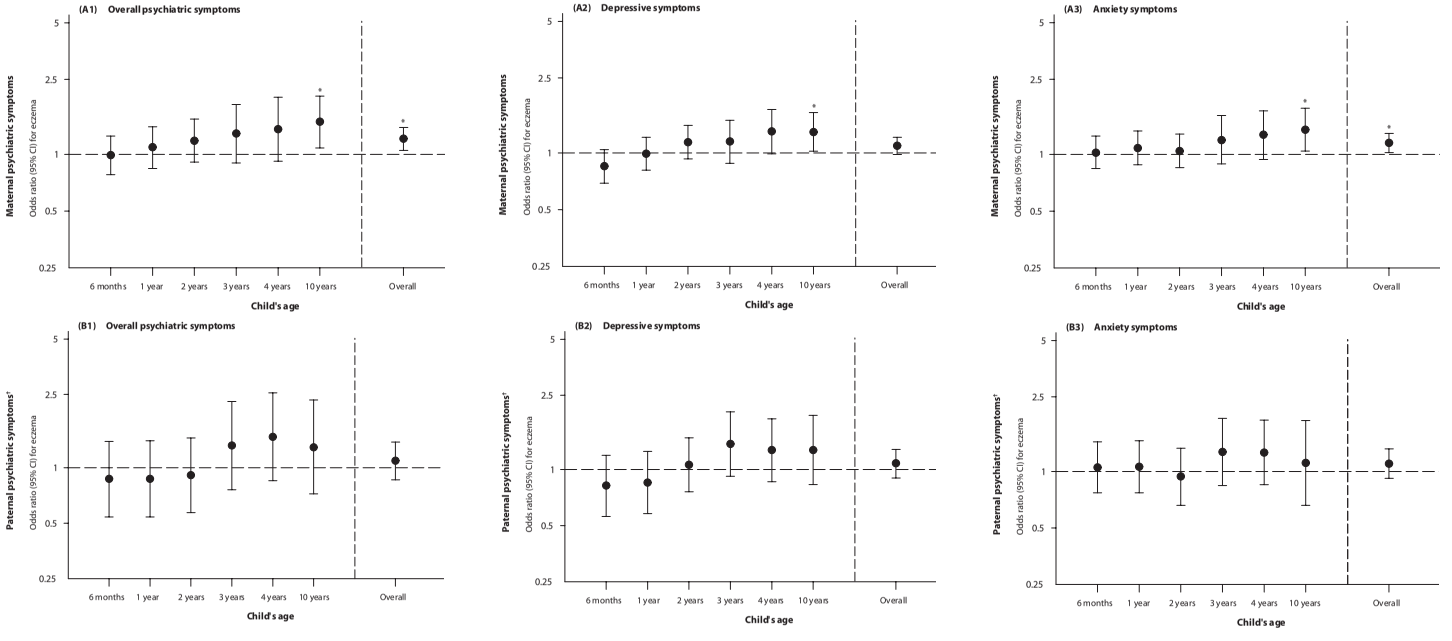
Values are odds ratios (95% confidence interval) from multinomial logistic regression models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. Reference group is children without any allergic sensitization and any physician-diagnosed allergy (n = 1,678). Parental psychiatric symptoms are treated as continuous variables (per 1-unit increase). Models are adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight. *Additionally adjusted for maternal psychiatric symptoms during pregnancy.

or anxiety symptoms, but not with depressive symptoms during pregnancy, had an overall increased risk of eczema until age 10 years (1.21 (1.05, 1.39) and 1.15 (1.02, 1.29), respectively, per 1-unit increase) (Figure and Supplementary Table 3). Additional adjustment for intermediates (data not shown), maternal psychiatric symptoms at 2, 6 and 36 months after delivery, and paternal psychiatric symptoms during pregnancy and at 36 months after delivery separately did not materially affect the size and the direction of the effect estimates (Supplementary Tables 4–9). Paternal psychiatric symptoms during pregnancy (Tables 3 and 4, and Figure), and maternal and paternal psychiatric symptoms after delivery were not associated with childhood atopic diseases (*p*-values > 0.05; data not shown). We observed no modifying effect of maternal history of allergy, eczema or asthma and child's current asthma on the associations of maternal psychiatric symptoms during pregnancy with child's allergic sensitization, physician-diagnosed allergy, or eczema (*p*-values for interaction > 0.05).

DISCUSSION

In this large prospective population-based study, we observed that children of mothers with overall psychiatric, depressive or anxiety symptoms during pregnancy had an

Figure. Associations of maternal overall psychiatric (A1), depressive (A2) and anxiety (A3) symptoms during pregnancy, and paternal overall psychiatric (B1), depressive (B2) and anxiety (B3) symptoms during pregnancy with eczema in children until age 10 years.



Values are odds ratios (95% confidence interval) for eczema per year and overall for children with parental psychiatric symptoms during pregnancy (per 1-unit increase) obtained from generalized estimating equation models based on imputed data. Models are adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight. *P-value < 0.05. †Additionally adjusted for maternal psychiatric symptoms during pregnancy.

increased risk of physician-diagnosed inhalant allergy, and children of mothers with overall psychiatric or anxiety sympt oms during pregnancy an increased risk of eczema. Results were independent of maternal psychiatric symptoms after delivery, and of paternal psychiatric symptoms during pregnancy and after delivery. Maternal psychiatric symptoms during pregnancy were not associated with childhood allergic sensitization and physician-diagnosed food allergy.

Comparison of main findings with other studies

Studies that used IgE levels to identify allergic sensitization observed conflicting results for the association of maternal stress during pregnancy with childhood allergic sensitization.[22–27] Four birth cohort studies showed that maternal stress during pregnancy was associated with elevated umbilical cord blood total or serum allergen-specific IgE[22, 24, 25, 27], while two other birth cohort studies did not find such an association.[23, 26] This discrepancy might be explained by differences in the definition of maternal stress (psychosocial stress, distress, psychiatric symptoms, or adverse life events), time point during pregnancy at which stress was examined, type of allergen-specific IgE that was measured, and age at which IgE levels were measured. Only one previous birth cohort study used skin prick test responses to identify common inhalant and food allergic sensitization, and like our study also observed no association of maternal stress during pregnancy with childhood allergic sensitization.[23] One previous birth cohort study among 516 children combined outcome data on food-specific serum IgE levels and information from a physician-administered questionnaire on food allergy and observed no association of maternal distress during pregnancy with food allergy.[28] Due to the low number of children with food allergic sensitization only (n = 16) or food allergy only (n = 20), a similar approach was not feasible in our study. To our knowledge, there is no literature on the association of maternal psychiatric symptoms during pregnancy with inhalant allergy. Previous birth cohort studies reported inconsistent results regarding the association of maternal stress during pregnancy with childhood eczema.[22, 23, 29–32] Several studies showed that prenatally stressed children had an up to 4.19-fold increased risk of eczema, compared with prenatally unstressed children[22, 29, 31, 32], while only one study observed no association of maternal stress during pregnancy with eczema.[30] Our study aimed to address methodological differences of previous studies and additionally used a well-known definition of psychiatric symptoms commonly used in epidemiological studies, and measured both psychiatric symptoms and eczema at multiple time points.

Interpretation of results

Our results showed that maternal psychiatric symptoms during pregnancy were associated with an increased risk of physician-diagnosed inhalant allergy, with mostly current hay fever, and eczema in children until age 10 years, but not with allergic sensitization

and physician-diagnosed food allergy. Differences in observed associations of maternal psychiatric symptoms during pregnancy with eczema and allergic sensitizations or physician-diagnosed allergies might be due to differences in the timing of these outcome measurements. Eczema was measured longitudinally, while allergic sensitizations and physician-diagnosed allergy were assessed at one time point only. Parents of children who were sensitized to inhalant allergens did not report a physician-diagnosed inhalant allergy for 25% of children. This may suggest that the skin prick test method at the population level is less sensitive, showing children who are sensitized but do not experience symptoms of inhalant allergy. This could explain the differences in observed associations of maternal psychiatric symptoms during pregnancy with physician-diagnosed inhalant allergy but not with inhalant allergic sensitization, and with allergic sensitization and physician-diagnosed allergy combined but not separately. With 80.5% of participating mothers reported living together with their partner during pregnancy, paternal psychiatric symptoms during pregnancy were not associated with childhood atopic diseases, although size and direction of the effect estimates were similar as for associations of maternal psychiatric symptoms with childhood atopic diseases. Still, an independent effect of maternal psychiatric symptoms on childhood atopic diseases could be considered because maternal and paternal psychiatric symptoms after delivery were not associated with childhood atopic diseases, and adjustment for a large set of potential confounding factors and additional adjustment for maternal and paternal psychiatric symptoms after delivery and for paternal psychiatric symptoms during pregnancy did not materially affect the results. Potential early-life underlying pathophysiological mechanisms for associations of maternal psychiatric symptoms with the child's risk of developing atopic diseases may include fetal growth[33, 34], altered intestinal microbiota[35, 36], oxidative stress[22, 36], and epigenetic programming.[37] Specifically, maternal psychiatric symptoms during pregnancy might impair fetal growth[33], and children of low and high birth weight are associated with a higher and lower risk of eczema, respectively.[34] However, our results did not change after adjusting for birth weight and gestational age at birth. Recently, maternal stress during pregnancy was found to be associated with the child's intestinal microbiota composition and colonization pattern, which appeared to predispose the child to allergic reactions.[35] The intestinal microbiota may influence the metabolism of the antioxidant glutathione[36], which could play a mediating role in the association of maternal stress during pregnancy with eczema.[22] Finally, maternal depressive or anxiety symptoms during pregnancy are associated with increased neonatal methylation of the glucocorticoid receptor gene *NR3C1* and altered stress reactivity of the hypothalamic-pituitary-adrenal axis during infancy.[37] Future studies are needed to explore potential microbiomic, oxidative and epigenetic mechanisms for the association of maternal psychiatric symptoms during pregnancy with childhood atopic diseases.

Strengths and limitations

The major strengths of this study are the use of a population-based prospective study design from fetal life onwards with a large number of participants, the assessment of maternal and paternal psychiatric symptoms during pregnancy and after delivery using the same instrument at similar time points, and detailed information on allergic sensitization, allergy and eczema. Also, we adjusted for multiple social, behavioral and environmental factors. However, some methodological limitations should be considered. First, characteristics of non-responders for phase 3 of the Generation R Study and those lost to follow-up differed from those included in the study. Non-included subjects were more often of non-European origin and non-affluent background, and less healthy. This selective loss to follow-up might have led to bias if the associations of maternal psychiatric symptoms during pregnancy with allergic sensitization, allergy and eczema were different between those included and not included in the study. It is unlikely that these differences led to selection bias but this cannot be excluded.[38] However, these differences should be taken into account regarding the generalizability of our results. Second, the presence of maternal psychiatric symptoms may have influenced the recognition and reporting of allergies and eczema in the child despite unawareness of our specific research question, which could have potentially lead to differential misclassification of the self-reported outcomes and, subsequently, might have led to overestimation of our results. Maternal psychiatric symptoms were measured only once during pregnancy and, therefore, their intensity and persistence may have varied throughout pregnancy. We obtained information on parental psychiatric symptoms using the Brief Symptom Inventory, a widely used self-report questionnaire that has been validated in the Dutch population.[39] However, whether used clinical cut-off values also apply to pregnant women should be carefully considered before our results could be used for prevention strategies. For allergic sensitization, we used an advanced software tool to calculate the histamine equivalent prick index, which is considered to be more accurate than measuring the average wheal diameter and corrects for interobserver variability and ethnic differences in skin response to histamine.[19] We selected a panel of common inhalant and food allergens relevant to children at age 10 years. Food allergens such as milk and egg were not taken into account because of low sensitization rates at this age.[40] We were not able to use an accepted and validated allergy questionnaire but used questions adapted from the ISAAC core questionnaires instead.[20] Information on eczema was obtained by parental questionnaires, consisting widely accepted and commonly used questions that reliably reflect the prevalence of eczema in young children at the population level.[20, 41] Furthermore, self-reported diagnosis of eczema in the last year based on a single question seems sufficiently valid for studying current childhood eczema.[42] We had no additional information on eczema from medical records or physical examinations.

Finally, we did not have data on other possible confounding and mediating factors, such as housing conditions or current air pollution.[22, 35, 43]

In conclusion, our results suggest a possible intrauterine programming effect of maternal psychiatric symptoms that increases the child's risk of developing atopic diseases. Therefore, future studies are needed to examine potential underlying mechanisms and potential primary prevention strategies focused on reducing maternal psychiatric symptoms during pregnancy to reduce development of atopic diseases in children.

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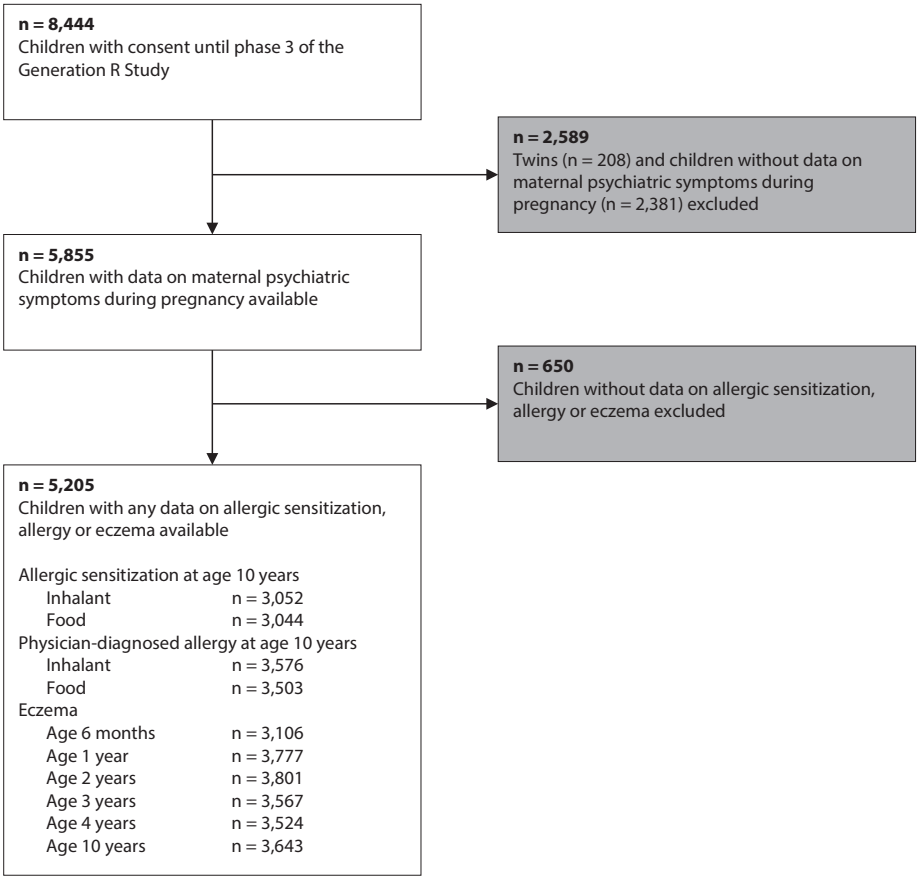
SUPPLEMENTARY MATERIAL

Supplementary Figure. Flowchart of participants.



Supplementary Table 1. Characteristics of mothers, fathers and children (n = 5,205).

	Observed	Imputed
Maternal characteristics		
Age at enrollment (years)*	30.7 (4.8)	30.7 (4.8)
Missing	*0 (0)*	*0 (0)*
Education (%)		
Primary or secondary	48.9 (2,460)	49.6 (2,581)
Higher	51.1 (2,575)	50.4 (2,624)
Missing	*3.3 (170)*	*0 (0)*
Ethnic origin (%)		
European	67.2 (3,467)	67.7 (3,470)
Non-European	32.8 (1,691)	33.3 (1,735)
Missing	*0.9 (47)*	*0 (0)*
History of allergy, eczema or asthma (%)		
No	60.6 (2,659)	61.4 (3,197)
Yes	39.4 (1,728)	38.6 (2,008)
Missing	*15.7 (818)*	*0 (0)*
Parity (%)		
0	59.1 (3,061)	58.8 (3,063)
≥ 1	40.9 (2,120)	41.2 (2,142)
Missing	*0.5 (24)*	*0 (0)*
Pet keeping during pregnancy (%)		
No	65.6 (3,020)	65.1 (3,390)
Yes	34.4 (1,585)	34.9 (1,815)
Missing	*11.5 (600)*	*0 (0)*
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.6)	23.7 (18.8–35.6)
Missing	*0.6 (33)*	*0 (0)*
Smoking during pregnancy (%)		
No	75.4 (3,600)	75.4 (3,923)
Yes	24.6 (1,172)	24.6 (1,282)
Missing	*8.3 (433)*	*0 (0)*
Overall psychiatric symptoms during pregnancy (%)		
No	91.0 (4,732)	91.0 (4,737)
Yes	9.0 (467)	9.0 (468)
Continuous†	0.15 (0–1.33)	0.15 (0–1.33)
Missing	*0.1 (6)*	*0 (0)*
Depressive symptoms during pregnancy (%)		
No	91.2 (4,738)	91.1 (4,744)
Yes	8.8 (457)	8.9 (461)
Continuous†	0 (0–1.67)	0 (0–1.67)
Missing	*0.2 (10)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers, fathers and children (n = 5,205). (continued)

	Observed	Imputed
Anxiety symptoms during pregnancy (%)		
No	90.2 (4,689)	90.2 (4,694)
Yes	9.8 (507)	9.8 (511)
Continuous†	0.17 (0–1.50)	0.17 (0–1.50)
Missing	*0.2 (9)*	*0 (0)*
Overall psychiatric symptoms at 2 months after delivery (%)		
No	92.7 (3,448)	91.8 (4,776)
Yes	7.3 (272)	8.2 (429)
Continuous†	0.12 (0–1.23)	0.13 (0–1.33)
Missing	*28.5 (1,485)*	*0 (0)*
Depressive symptoms at 2 months after delivery (%)		
No	92.4 (3,432)	91.3 (4,754)
Yes	7.6 (282)	8.7 (451)
Continuous†	0 (0–1.50)	0 (0–1.67)
Missing	*28.6 (1,491)*	*0 (0)*
Anxiety symptoms at 2 months after delivery (%)		
No	92.5 (3,443)	91.7 (4,775)
Yes	7.5 (278)	8.3 (430)
Continuous†	0 (0–1.50)	0 (0–1.50)
Missing	*28.5 (1,484)*	*0 (0)*
Depressive symptoms at 6 months after delivery (%)		
No	92.0 (3,046)	91.4 (4,755)
Yes	8.0 (264)	8.6 (450)
Continuous†	0 (0–1.67)	0 (0–1.83)
Missing	*36.4 (1,895)*	*0 (0)*
Anxiety symptoms at 6 months after delivery (%)		
No	90.7 (3,005)	90.2 (4,694)
Yes	9.3 (307)	9.8 (511)
Continuous†	0.17 (0–1.67)	0.17 (0–1.83)
Missing	*36.4 (1,893)*	*0 (0)*
Depressive symptoms at 36 months after delivery (%)		
No	95.8 (3,458)	94.7 (4,930)
Yes	4.2 (152)	5.3 (275)
Continuous†	0 (0–1.00)	0 (0–1.17)
Missing	*30.6 (1,595)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers, fathers and children (n = 5,205). (continued)

	Observed	Imputed
Anxiety symptoms at 36 months after delivery (%)		
No	95.7 (3,455)	94.9 (4,938)
Yes	4.3 (154)	5.1 (267)
Continuous†	0 (0–1.00)	0 (0–1.17)
Missing	*30.7 (1,596)*	*0 (0)*
Paternal characteristics		
Age at enrollment (years)*	33.3 (5.4)	33.2 (5.6)
Missing	*18.9 (986)*	*0 (0)*
Education (%)		
Primary or secondary	45.4 (1,758)	49.9 (2,598)
Higher	54.6 (2,111)	50.1 (2,607)
Missing	*25.7 (1,336)*	*0 (0)*
Ethnic origin (%)		
European	66.9 (3,362)	65.2 (3,395)
Non-European	33.1 (1,666)	34.8 (1,810)
Missing	*3.4 (177)*	*0 (0)*
History of allergy, eczema or asthma (%)		
No	67.1 (2,426)	65.7 (3,419)
Yes	32.9 (1,188)	34.3 (1,786)
Missing	*30.6 (1,591)*	*0 (0)*
Body mass index at enrollment (kg/m^2)†	24.9 (19.6–32.7)	25.0 (19.6–33.2)
Missing	*19.1 (994)*	*0 (0)*
Smoking during pregnancy (%)		
No	56.9 (2,691)	56.6 (2,946)
Yes	43.1 (2,037)	43.4 (2,259)
Missing	*9.2 (477)*	*0 (0)*
Overall psychiatric symptoms during pregnancy (%)		
No	97.2 (3,663)	96.5 (5,024)
Yes	2.8 (106)	3.5 (181)
Continuous†	0.06 (0–0.73)	0.08 (0–0.80)
Missing	*27.6 (1,436)*	*0 (0)*
Depressive symptoms during pregnancy (%)		
No	97.0 (3,651)	96.3 (5,013)
Yes	3.0 (113)	3.7 (192)
Continuous†	0 (0–0.83)	0 (0–1.00)
Missing	*27.7 (1,441)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers, fathers and children (n = 5,205). (continued)

	Observed	Imputed
Anxiety symptoms during pregnancy (%)		
No	93.5 (3,524)	92.9 (4,834)
Yes	6.5 (245)	7.1 (371)
Continuous†	0 (0–0.83)	0 (0–1.00)
Missing	*27.6 (1,436)*	*0 (0)*
Depressive symptoms at 36 months after delivery (%)		
No	97.3 (2,851)	95.9 (4,994)
Yes	2.7 (80)	4.1 (211)
Continuous†	0 (0–0.83)	0 (0–1.17)
Missing	*43.7 (2,274)*	*0 (0)*
Anxiety symptoms at 36 months after delivery (%)		
No	94.0 (2,757)	92.6 (4,818)
Yes	6.0 (177)	7.4 (387)
Continuous†	0 (0–0.83)	0 (0–1.00)
Missing	*43.6 (2,271)*	*0 (0)*
Child characteristics		
Sex (%)		
Male	49.3 (2,568)	49.3 (2,568)
Female	50.7 (2,637)	50.7 (2,637)
Missing	*0 (0)*	*0 (0)*
Gestational age at birth (weeks)†	40.1 (36.0–42.4)	40.1 (36.0–42.4)
Missing	*0 (0)*	*0 (0)*
Birth weight (grams)*	3,450 (549)	3,449 (550)
Missing	*0.1 (4)*	*0 (0)*
Breastfed ever (%)		
No	8.1 (369)	8.8 (460)
Yes	91.9 (4,161)	91.2 (4,745)
Missing	*13.0 (675)*	*0 (0)*
Day care attendance until age 1 year (%)		
No	39.8 (1,407)	45.2 (2,352)
Yes	60.2 (2,131)	54.8 (2,853)
Missing	*32.0 (1,667)*	*0 (0)*
Asthma ever at age 10 years (%)		
No	90.3 (3,217)	88.6 (4,611
Yes	9.7 (346)	11.4 (594)
Missing	*31.5 (1,642)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers, fathers and children (n = 5,205). (continued)

	Observed	Imputed
Allergic sensitization at age 10 years – inhalant (%)		
No	67.4 (2,058)	67.4 (2,058)
Yes	32.6 (994)	32.6 (994)
Missing	*41.4 (2,153)*	*41.4 (2,153)*
Allergic sensitization at age 10 years – food (%)		
No	92.5 (2,816)	92.5 (2,816)
Yes	7.5 (228)	7.5 (228)
Missing	*41.5 (2,161)*	*41.5 (2,161)*
Physician-diagnosed allergy at age 10 years – inhalant (%)		
No	87.8 (3,138)	87.8 (3,138)
Yes	12.2 (438)	12.2 (438)
Missing	*31.3 (1,629)*	*31.3 (1,629)*
Physician-diagnosed allergy at age 10 years – food (%)		
No	97.7 (3,424)	97.7 (3,424)
Yes	2.3 (79)	2.3 (79)
Missing	*32.7 (1,702)*	*32.7 (1,702)*
Allergic sensitization and allergy combined at age 10 years (%)		
No allergic sensitization and no allergy	66.2 (1,678)	66.2 (1,678)
Any allergic sensitization, but no allergy	22.8 (578)	22.8 (578)
No allergic sensitization, but any allergy	1.3 (33)	1.3 (33)
Any allergic sensitization and any allergy	9.7 (246)	9.7 (246)
Missing	*51.3 (2,670)*	*51.3 (2,670)*
Eczema last 6 months at age 6 months (%)		
No	83.7 (2,599)	82.8 (4,310)
Yes	16.3 (507)	17.2 (895)
Missing	*40.3 (2,099)*	*0 (0)*
Eczema last 6 months at age 1 year (%)		
No	87.1 (3,289)	86.4 (4,495)
Yes	12.9 (488)	13.6 (710)
Missing	*27.4 (1,428)*	*0 (0)*
Eczema last 12 months at age 2 years (%)		
No	86.2 (3,278)	85.3 (4,442)
Yes	13.8 (523)	14.7 (763)
Missing	*27.0 (1,404)*	*0 (0)*
Eczema last 12 months at age 3 years (%)		
No	90.4 (3,225)	89.0 (4,631)
Yes	9.6 (342)	11.0 (574)
Missing	*31.5 (1,638)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers, fathers and children (n = 5,205). (continued)

	Observed	Imputed
Eczema last 12 months at age 4 years (%)		
No	92.2 (3,248)	90.7 (4,720)
Yes	7.8 (276)	9.3 (485)
Missing	*32.3 (1,681)*	*0 (0)*
Eczema last 12 months at age 10 years (%)		
No	92.9 (3,383)	91.5 (4,761)
Yes	7.1 (260)	8.5 (444)
Missing	*30.0 (1,562)*	*0 (0)*

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed and imputed data. Data on allergic sensitizations and physician-diagnosed allergies are not imputed.

Supplementary Table 2. Characteristics of mothers, fathers and children included and not included in the study.

	Included n = 5,205	Not included n = 3,239	*P*-value for difference
Maternal characteristics			
Age at enrollment (years)*	30.7 (4.8)	29.5 (5.8)	**< 0.001**
Missing	*0 (0)*	*0.03 (1)*	
Education (%)			**< 0.001**
Primary or secondary	48.9 (2,460)	67.4 (1,711)	
Higher	51.1 (2,575)	32.6 (826)	
Missing	*3.3 (170)*	*21.7 (702)*	
Ethnic origin (%)			**< 0.001**
European	67.2 (3,467)	48.9 (1,424)	
Non-European	32.8 (1,691)	51.1 (1,490)	
Missing	*0.9 (47)*	*10.0 (325)*	
History of allergy, eczema or asthma (%)			0.25
No	60.6 (2,659)	62.1 (1,400)	
Yes	39.4 (1,728)	37.9 (856)	
Missing	*15.7 (818)*	*30.3 (983)*	
Parity (%)			**< 0.001**
0	59.1 (3,061)	49.0 (1,456)	
≥ 1	40.9 (2,120)	51.0 (1,518)	
Missing	*0.5 (24)*	*8.2 (265)*	

Supplementary Table 2. Characteristics of mothers, fathers and children included and not included in the study. (continued)

	Included n = 5,205	Not included n = 3,239	*P*-value for difference
Pet keeping during pregnancy (%)			**< 0.01**
No	65.6 (3,020)	69.8 (1,304)	
Yes	34.4 (1,585)	30.2 (565)	
Missing	*11.5 (600)*	*42.3 (1,370)*	
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.6)	24.4 (18.6–37.4)	**< 0.001**
Missing	*0.6 (33)*	*25.5 (826)*	
Smoking during pregnancy (%)			**< 0.001**
No	75.4 (3,600)	71.2 (1,768)	
Yes	24.6 (1,172)	28.8 (715)	
Missing	*8.3 (433)*	*23.3 (756)*	
Paternal characteristics			
Age at enrollment (years)*	33.3 (5.4)	32.0 (6.1)	**< 0.001**
Missing	*18.9 (986)*	*56.3 (1,822)*	
Education (%)			**< 0.001**
Primary or secondary	45.4 (1,758)	59.1 (556)	
Higher	54.6 (2,111)	40.9 (385)	
Missing	*25.7 (1,336)*	*70.9 (2,298)*	
Ethnic origin (%)			**< 0.001**
European	66.9 (3,362)	50.8 (1,282)	
Non-European	33.1 (1,666)	49.2 (1,240)	
Missing	*3.4 (177)*	*22.1 (717)*	
History of allergy, eczema or asthma (%)			0.58
No	67.1 (2,426)	66.3 (841)	
Yes	32.9 (1,188)	33.7 (428)	
Missing	*30.6 (1,591)*	*60.8 (1,970)*	
Body mass index at enrollment (kg/m^2)†	24.9 (19.6–32.7)	25.0 (19.2–34.0)	0.21
Missing	*19.1 (994)*	*56.5 (1,830)*	
Smoking during pregnancy (%)			**< 0.001**
No	56.9 (2,691)	50.1 (965)	
Yes	43.1 (2,037)	49.9 (960)	
Missing	*9.2 (477)*	*40.6 (1,314)*	
Child characteristics			
Sex (%)			**< 0.01**
Male	49.3 (2,568)	52.3 (1,695)	
Female	50.7 (2,637)	47.7 (1,543)	
Missing	*0 (0)*	*0.03 (1)*	

Supplementary Table 2. Characteristics of mothers, fathers and children included and not included in the study. (continued)

	Included n = 5,205	**Not included n = 3,239**	***P*-value for difference**
Gestational age at birth (weeks)†	40.1 (36.0–42.4)	39.7 (34.0–42.1)	**< 0.001**
Missing	*0 (0)*	*2.2 (70)*	
Birth weight (grams)*	3,450 (549)	3,316 (611)	**< 0.001**
Missing	*0.1 (4)*	*0.8 (27)*	
Breastfed ever (%)			0.67
No	8.1 (369)	8.5 (148)	
Yes	91.9 (4,161)	91.5 (1,598)	
Missing	*13.0 (675)*	*46.1 (1,493)*	
Day care attendance until age 1 year (%)			**< 0.001**
No	39.8 (1,407)	46.4 (411)	
Yes	60.2 (2,131)	53.6 (475)	
Missing	*32.0 (1,667)*	*72.6 (2,353)*	

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed data. *P*-values for difference are calculated by independent samples T-test for continuous variables with a normal distribution, the Mann-Whitney U-test for continuous variables with a skewed distribution, and Pearson's Chi-square test for categorical variables. Bold values indicate statistical significance at the $\alpha = 0.05$ level.

Supplementary Table 3. Associations of maternal psychiatric symptoms during pregnancy with eczema per year and overall in children until age 10.

	Odds ratio (95% confidence interval) for eczema						
	6 months	**1 year**	**2 years**	**3 years**	**4 years**	**10 years**	**Overall**
Maternal psychiatric symptoms							
Overall psychiatric symptoms							
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.90 (0.67, 1.19)	1.07 (0.80, 1.44)	1.27 (0.95, 1.72)	1.29 (0.87, 1.91)	1.39 (0.91, 2.13)	**1.54 (1.05, 2.57)**	**1.20 (1.04, 1.40)**
Per 1-unit increase	0.99 (0.78, 1.25)	1.09 (0.84, 1.40)	1.18 (0.91, 1.54)	1.29 (0.90, 1.84)	1.36 (0.92, 2.01)	**1.49 (1.08, 2.04)**	**1.21 (1.05, 1.39)**
Depressive symptoms							
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.79 (0.57, 1.09)	0.95 (0.69, 1.32)	1.20 (0.88, 1.64)	1.18 (0.78, 1.78)	1.39 (0.90, 2.13)	**1.53 (1.06, 2.21)**	1.12 (0.96, 1.32)
Per 1-unit increase	0.85 (0.69, 1.04)	0.99 (0.81, 1.21)	1.14 (0.93, 1.40)	1.15 (0.88, 1.49)	1.30 (0.99, 1.70)	**1.29 (1.02, 1.64)**	1.09 (0.98, 1.21)
Anxiety symptoms							
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.95 (0.70, 1.28)	1.08 (0.80, 1.44)	1.08 (0.80, 1.45)	1.27 (0.85, 1.89)	1.31 (0.86, 1.99)	1.44 (0.99, 2.08)	1.15 (1.00, 1.34)
Per 1-unit increase	1.02 (0.84, 1.25)	1.08 (0.88, 1.33)	1.04 (0.85, 1.28)	1.19 (0.89, 1.61)	1.27 (0.94, 1.70)	**1.35 (1.04, 1.76)**	**1.15 (1.02, 1.29)**
Paternal psychiatric symptoms*							
Overall psychiatric symptoms							
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.75 (0.40, 1.40)	0.89 (0.52, 1.53)	0.94 (0.57, 1.57)	1.29 (0.71, 2.34)	1.50 (0.79, 2.87)	1.36 (0.66, 2.79)	1.08 (0.84, 1.40)
Per 1-unit increase	0.87 (0.54, 1.39)	0.87 (0.54, 1.40)	0.91 (0.57, 1.45)	1.32 (0.76, 2.29)	1.47 (0.85, 2.55)	1.29 (0.72, 2.33)	1.09 (0.86, 1.38)
Depressive symptoms							
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.70 (0.39, 1.27)	0.85 (0.50, 1.45)	1.02 (0.61, 1.72)	1.42 (0.77, 2.62)	1.41 (0.76, 2.60)	1.30 (0.65, 2.59)	1.06 (0.82, 1.36)
Per 1-unit increase	0.82 (0.56, 1.19)	0.85 (0.58, 1.25)	1.06 (0.76, 1.48)	1.37 (0.92, 2.04)	1.27 (0.86, 1.87)	1.27 (0.83, 1.95)	1.08 (0.90, 1.28)

Supplementary Table 3. Associations of maternal psychiatric symptoms during pregnancy with eczema per year and overall in children until age 10. (continued)

	Odds ratio (95% confidence interval) for eczema						
	6 months	**1 year**	**2 years**	**3 years**	**4 years**	**10 years**	**Overall**
Anxiety symptoms							
No	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.00 (0.70, 1.44)	1.07 (0.74, 1.54)	0.96 (0.66, 1.40)	1.31 (0.82, 2.10)	1.17 (0.73, 1.85)	1.33 (0.81, 2.18)	1.12 (0.91, 1.37)
Per 1-unit increase	1.05 (0.77, 1.44)	1.06 (0.77, 1.46)	0.94 (0.66, 1.33)	1.27 (0.84, 1.92)	1.26 (0.85, 1.88)	1.11 (0.66, 1.87)	1.10 (0.92, 1.32)

Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. Maternal psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Models are adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight. *Additionally adjusted for maternal psychiatric symptoms during pregnancy.

Supplementary Table 4. Associations of maternal psychiatric symptoms during pregnancy with inhalant allergic sensitization in children at age 10 years, adjusted for maternal psychiatric symptoms at 2, 6 and 36 months after delivery and for paternal psychiatric symptoms during pregnancy and at 36 months after delivery.

	Odds ratio (95% confidence interval) for inhalant allergic sensitization					
		Model 1 + maternal psychiatric symptoms after delivery			Model 1 + paternal psychiatric symptoms	
	Model 1	**2 months**	**6 months**	**36 months**	**Pregnancy**	**36 months**
Overall psychiatric symptoms*						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.10 (0.82, 1.48)	1.13 (0.81, 1.58)	-	-	1.10 (0.82, 1.48)	-
Per 1-unit increase	1.03 (0.80, 1.32)	1.07 (0.76, 1.52)	-	-	1.00 (0.77, 1.30)	-
Depressive symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.20 (0.90, 1.61)	1.19 (0.86, 1.61)	1.16 (0.85, 1.59)	1.20 (0.88, 1.63)	1.20 (0.89, 1.62)	1.20 (0.89, 1.61)
Per 1-unit increase	1.08 (0.90, 1.30)	1.07 (0.85, 1.35)	1.03 (0.83, 1.28)	1.08 (0.88, 1.33)	1.07 (0.88, 1.29)	1.07 (0.89, 1.30)
Anxiety symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.08 (0.81, 1.42)	1.16 (0.85, 1.57)	1.04 (0.77, 1.41)	1.06 (0.79, 1.42)	1.08 (0.81, 1.43)	1.06 (0.80, 1.40)
Per 1-unit increase	1.08 (0.89, 1.32)	1.21 (0.94, 1.55)	1.09 (0.86, 1.37)	1.06 (0.86, 1.32)	1.06 (0.87, 1.29)	1.06 (0.87, 1.30)

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Maternal psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Model 1 is adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight, and food allergic sensitization. *Not available at 6 and 36 months after delivery.

Supplementary Table 5. Associations of maternal psychiatric symptoms during pregnancy with food allergic sensitization in children at age 10 years, adjusted for maternal psychiatric symptoms at 2, 6 and 36 months after delivery and for paternal psychiatric symptoms during pregnancy and at 36 months after delivery.

	Odds ratio (95% confidence interval) for food allergic sensitization					
		Model 1 + maternal psychiatric symptoms after delivery			**Model 1 + paternal psychiatric symptoms**	
	Model 1	**2 months**	**6 months**	**36 months**	**Pregnancy**	**36 months**
Overall psychiatric symptoms*						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.05 (0.58, 1.91)	1.20 (0.61, 2.37)	-	-	1.01 (0.55, 1.85)	-
Per 1-unit increase	1.02 (0.62, 1.66)	1.02 (0.53, 1.98)	-	-	0.96 (0.58, 1.60)	-
Depressive symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.90 (0.49, 1.65)	0.94 (0.47, 1.87)	0.87 (0.45, 1.70)	0.89 (0.46, 1.72)	0.89 (0.48, 1.65)	0.87 (0.47, 1.61)
Per 1-unit increase	0.96 (0.66, 1.40)	0.92 (0.56, 1.51)	0.98 (0.62, 1.54)	0.93 (0.60, 1.44)	0.96 (0.65, 1.40)	0.94 (0.64, 1.38)
Anxiety symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.06 (0.59, 1.90)	1.11 (0.59, 2.11)	1.04 (0.54, 2.01)	1.23 (0.67, 2.25)	1.02 (0.57, 1.84)	1.02 (0.56, 1.86)
Per 1-unit increase	0.92 (0.60, 1.41)	0.90 (0.54, 1.51)	0.90 (0.55, 1.48)	1.03 (0.65, 1.66)	0.90 (0.58, 1.38)	0.90 (0.58, 1.41)

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Maternal psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Model 1 is adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight, and inhalant allergic sensitization. *Not available at 6 and 36 months after delivery.

Supplementary Table 6. Associations of maternal psychiatric symptoms during pregnancy with physician-diagnosed inhalant allergy in children at age 10 years, adjusted for maternal psychiatric symptoms at 2, 6 and 36 months after delivery and for paternal psychiatric symptoms during pregnancy and at 36 months after delivery.

	Odds ratio (95% confidence interval) for physician-diagnosed inhalant allergy					
		Model 1 + maternal psychiatric symptoms after delivery			**Model 1 + paternal psychiatric symptoms**	
	Model 1	**2 months**	**6 months**	**36 months**	**Pregnancy**	**36 months**
Overall psychiatric symptoms*						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	**1.91 (1.32, 2.77)**	**1.82 (1.17, 2.82)**	-	-	**1.87 (1.28, 2.72)**	-
Per 1-unit increase	**1.96 (1.44, 2.65)**	**1.52 (1.01, 2.29)**	-	-	**1.85 (1.35, 2.54)**	-
Depressive symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	**2.07 (1.43, 2.97)**	**1.89 (1.25, 2.84)**	**1.96 (1.30, 2.95)**	**2.02 (1.37, 2.97)**	**1.97 (1.35, 2.85)**	**2.05 (1.42, 2.97)**
Per 1-unit increase	**1.58 (1.25, 1.98)**	**1.35 (1.02, 1.81)**	**1.49 (1.12, 1.99)**	**1.56 (1.20, 2.02)**	**1.51 (1.19, 1.92)**	**1.57 (1.24, 2.00)**
Anxiety symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	**1.51 (1.06, 2.16)**	1.33 (0.89, 1.97)	1.29 (0.87, 1.92)	**1.52 (1.05, 2.20**)	1.33 (0.96, 1.85)	**1.47 (1.02, 2.10)**
Per 1-unit increase	**1.61 (1.27, 2.03)**	**1.44 (1.07, 1.93)**	**1.49 (1.11, 2.01)**	**1.64 (1.27, 2.14)**	**1.57 (1.24, 2.00)**	**1.57 (1.23, 2.00)**

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. Maternal psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Model 1 is adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight, and physician-diagnosed food allergy. *Not available at 6 and 36 months after delivery.

Supplementary Table 7. Associations of maternal psychiatric symptoms during pregnancy with physician-diagnosed food allergy in children at age 10 years, adjusted for maternal psychiatric symptoms at 2, 6 and 36 months after delivery and for paternal psychiatric symptoms during pregnancy and at 36 months after delivery.

	Odds ratio (95% confidence interval) for physician-diagnosed food allergy					
		Model 1 + maternal psychiatric symptoms after delivery			Model 1 + paternal psychiatric symptoms	
	Model 1	**2 months**	**6 months**	**36 months**	**Pregnancy**	**36 months**
Overall psychiatric symptoms*						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.52 (0.18, 1.49)	0.66 (0.19, 2.31)	-	-	0.51 (0.18, 1.48)	-
Per 1-unit increase	0.58 (0.25, 1.33)	0.79 (0.25, 2.44)	-	-	0.59 (0.25, 1.41)	-
Depressive symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.75 (0.29, 1.97)	0.97 (0.33, 2.84)	0.92 (0.30, 2.87)	0.84 (0.29, 2.45)	0.77 (0.28, 2.06)	0.80 (0.30, 2.16)
Per 1-unit increase	0.79 (0.41, 1.54)	0.85 (0.36, 1.98)	0.94 (0.40, 2.18)	0.69 (0.31, 1.56)	0.77 (0.38, 1.56)	0.84 (0.41, 1.73)
Anxiety symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	0.43 (0.14, 1.32)	0.54 (0.15, 1.88)	0.49 (0.14, 1.71)	0.44 (0.13, 1.45)	0.45 (0.15, 1.40)	0.46 (0.15, 1.43)
Per 1-unit increase	0.52 (0.25, 1.08)	0.63 (0.27, 1.48)	0.60 (0.25, 1.44)	0.54 (0.24, 1.23)	0.55 (0.26, 1.17)	0.55 (0.26, 1.16)

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Maternal psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Model 1 is adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight, and physician-diagnosed inhalant allergy. *Not available at 6 and 36 months after delivery.

Supplementary Table 8. Associations of maternal psychiatric symptoms during pregnancy with eczema overall in children until age 10 years, adjusted for maternal psychiatric symptoms at 2, 6 and 36 months after delivery and for paternal psychiatric symptoms during pregnancy and at 36 months after delivery.

	Odds ratio (95% confidence interval) for eczema overall					
		Model 1 + maternal psychiatric symptoms after delivery			**Model 1 + paternal psychiatric symptoms**	
	Model 1	**2 months**	**6 months**	**36 months**	**Pregnancy**	**36 months**
Overall psychiatric symptoms*						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	**1.20 (1.04, 1.40)**	**1.16 (0.98, 1.38)**	-	-	**1.20 (1.03, 1.39)**	-
Per 1-unit increase	**1.21 (1.05, 1.39)**	1.10 (0.92, 1.31)	-	-	**1.19 (1.03, 1.37)**	-
Depressive symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.12 (0.96, 1.32)	1.08 (0.91, 1.29)	1.07 (0.89, 1.27)	1.14 (0.97, 1.35)	1.12 (0.95, 1.32)	1.12 (0.95, 1.32)
Per 1-unit increase	1.09 (0.98, 1.21)	1.03 (0.91, 1.16)	1.04 (0.91, 1.18)	1.09 (0.98, 1.22)	1.08 (0.97, 1.20)	1.09 (0.98, 1.21)
Anxiety symptoms						
No	Reference	Reference	Reference	Reference	Reference	Reference
Yes	1.15 (1.00, 1.34)	1.11 (0.95, 1.31)	1.13 (0.96, 1.34)	**1.17 (1.00, 1.36)**	1.14 (0.98, 1.33)	1.15 (0.99, 1.33)
Per 1-unit increase	**1.15 (1.02, 1.29)**	1.09 (0.95, 1.25)	1.13 (0.98, 1.31)	**1.17 (1.03, 1.32)**	**1.13 (1.01, 1.27)**	**1.13 (1.01, 1.27)**

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. Maternal psychiatric symptoms are treated as dichotomous variables based on clinical cut-off values (no; yes) and as continuous variables (per 1-unit increase). Model 1 is adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight. *Not available at 6 and 36 months after delivery.

Supplementary Table 9. Associations of maternal psychiatric symptoms during pregnancy with combined allergic sensitization and physician-diagnosed allergy groups in children at age 10 years, adjusted for maternal psychiatric symptoms at 2, 6 and 36 months after delivery and for paternal psychiatric symptoms during pregnancy and at 36 months after delivery.

	Odds ratio (95% confidence interval) for any allergic sensitization and any physician-diagnosed allergy combined					
		Model 1 + maternal psychiatric symptoms after delivery			**Model 1 + paternal psychiatric symptoms**	
	Model 1	**2 months**	**6 months**	**36 months**	**Pregnancy**	**36 months**
			Any allergic sensitization, but no allergy n = 578			
Overall psychiatric symptoms*	0.74 (0.52, 1.06)	0.71 (0.45, 1.12)	-	-	0.73 (0.51, 1.05)	-
Depressive symptoms	0.91 (0.70, 1.18)	0.84 (0.61, 1.15)	0.85 (0.63, 1.15)	0.92 (0.69, 1.21)	0.90 (0.69, 1.18)	0.90 (0.69, 1.18)
Anxiety symptoms	0.79 (0.60, 1.04)	0.84 (0.61, 1.16)	0.78 (0.57, 1.07)	0.79 (0.59, 1.05)	0.77 (0.59, 1.02)	0.78 (0.59, 1.03)
			No allergic sensitization, but any allergy n = 33			
Overall psychiatric symptoms*	1.58 (0.73, 3.40)	1.35 (0.49, 3.73)	-	-	1.66 (0.75, 3.68)	-
Depressive symptoms	1.14 (0.60, 2.17)	0.91 (0.41, 2.03)	1.05 (0.49, 2.25)	1.16 (0.57, 2.36)	1.25 (0.64, 2.43)	1.21 (0.62, 2.34)
Anxiety symptoms	1.23 (0.61, 2.46)	1.16 (0.50, 2.70)	1.16 (0.51, 2.62)	1.45 (0.68, 3.07)	1.17 (0.58, 2.38)	1.21 (0.59, 2.46)
			Any allergic sensitization and any allergy n = 246			
Overall psychiatric symptoms*	**1.60 (1.10, 2.33)**	1.34 (0.79, 2.24)	-	-	**1.55 (1.04, 2.29)**	-
Depressive symptoms	**1.42 (1.07, 1.88)**	1.23 (0.86, 1.76)	1.26 (0.90, 1.77)	1.35 (0.99, 1.86)	**1.37 (1.02, 1.84)**	**1.39 (1.04, 1.86)**
Anxiety symptoms	1.34 (0.99, 1.80)	1.23 (0.84, 1.80)	1.19 (0.83, 1.71)	1.33 (0.96, 1.84)	1.30 (0.96, 1.77)	1.29 (0.95, 1.75)

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. Reference group is children without any allergic sensitization and any physician-diagnosed allergy (n = 1,678). Maternal psychiatric symptoms are treated as continuous variables (per 1-unit increase). Model 1 is adjusted for maternal age at enrollment, education, ethnic origin, parity, pet keeping, body mass index at enrollment, smoking and history of allergy, eczema or asthma, and child's sex, gestational age and birth weight. *Not available at 6 and 36 months after delivery.

CHAPTER 2.2

MATERNAL AND FETAL 25-HYDROXYVITAMIN D LEVELS AND ECZEMA IN PRESCHOOL-AGE CHILDREN

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ABSTRACT

Background Exposure to low levels of vitamin D in fetal life might affect the developing immune system and, subsequently, the risk of childhood eczema. We examined whether 25-hydroxyvitamin D levels in mid-gestation and at birth were associated with the risk of eczema until age 4 years.

Methods In a population-based prospective cohort study of 3,019 mothers and their children, maternal blood samples in mid-gestation and umbilical cord blood samples at birth were used to determine 25-hydroxyvitamin D levels (severely deficient (< 25.0 nmol/L), deficient (25.0–49.9 nmol/L), sufficient (50.0–74.9 nmol/L) and optimal (≥ 75.0 nmol/L)). Eczema was prospectively assessed by annual questionnaires until age 4 years. Eczema patterns included never, early (age ≤ 1 year only), late (age > 1 year only) and persistent (age ≤ and > 1 year) eczema. Data were analyzed using generalized estimating equation and multinomial logistic regression models.

Results Compared with optimal 25-hydroxyvitamin D levels, sufficient, deficient and severely deficient 25-hydroxyvitamin D levels in mid-gestation were not associated with the overall risk of eczema (adjusted odds ratio (95% confidence interval): 0.94 (0.81, 1.10), 1.04 (0.87, 1.25) and 1.09 (0.82, 1.43), respectively) (*p*-values for trend > 0.05), nor with the risk of eczema per year or eczema patterns in children until age 4 years. Similarly, we observed no association of 25-hydroxyvitamin D levels at birth with any eczema outcome.

Conclusions Our results suggest that levels of 25-hydroxyvitamin D in mid-gestation and at birth are not associated with the overall risk of eczema, nor with the risk of eczema per year or eczema patterns in children until age 4 years.

INTRODUCTION

Eczema is a common relapsing, inflammatory, pruritic skin condition with an estimated prevalence of up to 22.5% among children at age 7 years.[1] It has been suggested that its origin is partly in pregnancy and early infancy.[2] Maternal vitamin and fatty acid status during pregnancy, including vitamin E, folate and n-3 or n-6 polyunsaturated fatty acid levels, have been associated with the risk of childhood eczema.[3, 4] Also, exposure to low levels of vitamin D in fetal life might affect the developing immune system, leading to unbalanced inflammatory processes and skin barrier impairment and, subsequently, an increased risk of eczema.[5] Animal studies have suggested that vitamin D has a role in regulating the innate and adaptive immune system.[6] *In vitro* studies have shown that the treatment of various human cell lines with 1,25-dihydroxyvitamin D, the active form of 25-hydroxyvitamin D, promotes the development and maturation of type 2 T-helper cells and inhibits inflammatory activity such as that of immunoglobulin E (IgE)-activated mast cells.[6–8] These processes might have a role in initiation and amplification of inflammatory process underlying the development of eczema. Also, systemic administration of vitamin D receptor agonists improved allergen-triggered eczema of mice and showed induction of skin barrier and antimicrobial peptide gene expression.[9] Previous studies in humans reported inconsistent results for the associations of maternal 25-hydroxyvitamin D levels during pregnancy and at birth with the risk of childhood eczema.[5, 10–17] Differences in results might be explained by different methods of vitamin D measurement or adjustment for different confounders. We hypothesized that lower 25-hydroxyvitamin D levels in early life lead to an increased risk of eczema at a young age.

Therefore, we examined among 3,019 mothers and their children participating in a population-based prospective cohort study whether 25-hydroxyvitamin D levels in mid-gestation and at birth were associated with eczema from birth until age 4 years.

METHODS

General design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood in Rotterdam, The Netherlands.[18] The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, The Netherlands, approved the study protocol (MEC 217.595/2002/202). This study was performed in participants of Dutch origin only to prevent confounding or effect modification by the large heterogeneity of other small ethnic groups with different skin types and diet. Written informed consent was obtained from 3,840 parents of participating children until age 4 years (Supplementary Figure). Twins (n = 119) and children of whom we had no data on vitamin D levels in mid-gestation and at birth (n = 597) and on eczema at any time point (n = 105) were excluded, leaving a total of 3,019 children for the analyses.

25-hydroxyvitamin D

We collected maternal venous blood samples in mid-gestation (median (2.5–97.5th percentile): 20.4 (18.8–22.9) weeks of gestation) and umbilical cord blood samples at birth (40.3 (37.0–42.3) weeks of gestation). Measurements of 25-hydroxyvitamin D levels from maternal and umbilical cord blood were conducted at the Eyles Laboratory at the Queensland Brain Institute, University of Queensland, Australia.[19] Details are provided in the Supplementary Methods. Levels of 25-hydroxyvitamin D were categorized as 'severely deficient' (< 25.0 nmol/L (< 10.0 mg/L)), 'deficient' (25.0–49.9 nmol/L (10.0–19.9 mg/L)), 'sufficient' (50.0–74.9 nmol/L (20.0–29.9 mg/L)) and 'optimal' (≥ 75.0 nmol/L (≥ 30.0 mg/L)).[20]

Eczema

Information on physician-diagnosed eczema was obtained by parental questionnaires at ages 6 months and 1, 2, 3 and 4 years based on questions adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) ("Was your child diagnosed with eczema in the last 6 months/last year?") (not as far as I know; yes, did not go to the doctor; yes, went to the general practitioner; yes, went to the hospital).[21] Answers were categorized into 'no' (not as far as I know; yes, did not go to the doctor) or 'yes' (yes, went to the general practitioner; yes, went to the hospital). The response rates at 6 months and 1, 2, 3 and 4 years of follow-up were 73%, 71%, 76%, 72% and 73%, respectively. Based on annual data on eczema, we defined the following eczema patterns: 'never' (no eczema from birth until age 4 years), 'early' (age ≤ 1 year only), 'late' (age > 1 year only), and 'persistent' (age ≤ and > 1 year) eczema.[22]

Covariates

We obtained information on maternal age, education (primary; secondary; higher), history of eczema, allergy or asthma (no; yes), parity (nulliparous; multiparous), and pet keeping (no; yes) by a questionnaire at enrollment. Maternal body mass index (BMI) was calculated using weight and height measured at enrollment. Information on maternal smoking (no; yes) was obtained by postal questionnaires multiple times during pregnancy. Maternal folate level was measured in the first trimester of pregnancy (12.9 (9.6–17.2) weeks of gestation), as previously described.[23] Maternal psychiatric symptoms were assessed in the second trimester of pregnancy using the Global Severity Index of the Brief Symptom Inventory[24], denoting overall psychiatric symptoms. Season of blood sampling was recorded at time of the blood sampling for 25-hydroxyvitamin D levels. We obtained information on child's sex, gestational age at birth and birth weight from obstetric and midwife records at birth. Information on breastfeeding (never; non-exclusive for 4 months; exclusive for 4 months) was collected by questionnaires at ages 2, 6 and 12 months. Information on vitamin D supplementation (no; yes) was collected by

questionnaire at age 2 months. The Dutch Health Council recommends a daily vitamin D supplement of 10 µg for all children until age 4 years.[25]

Statistical analysis

We used generalized estimating equation (GEE) models to examine the associations of 25-hydroxyvitamin D levels in mid-gestation and at birth with the longitudinal odds of eczema at ages 6 months and 1, 2, 3 and 4 years independently and overall. GEE models take into account correlations between repeated measurements of eczema within the same child. We used multinomial logistic regression models for the associations of 25-hydroxyvitamin D levels in mid-gestation and at birth with eczema patterns. Models were adjusted for maternal age at enrollment, education, history of eczema, allergy or asthma, parity, pet keeping, BMI at enrollment, smoking, folate level, psychiatric symptoms, season of blood sampling in mid-gestation, and child's sex, gestational age, birth weight, breastfeeding, vitamin D supplementation at age 2 months, season of blood sampling at birth, and mutually for mid-gestational and umbilical cord blood 25-hydroxyvitamin D levels. Tests for trends were performed by including the categorized 25-hydroxyvitamin D levels in mid-gestation and at birth as continuous variables in the models. The prevalence of missing data of covariates was ≤ 20.0%, except for maternal psychiatric symptoms during pregnancy (31.4%) and folate level (22.2%). To reduce potential bias from missing data and to increase precision, we performed a multiple imputation analysis of covariates, 25-hydroxyvitamin D levels in mid-gestation and at birth, and eczema outcomes generating 10 independent datasets using the Markov chain Monte Carlo method, and calculated pooled estimates.[26] We present effect estimates based on imputed analyses only. Measures of association are presented as adjusted odds ratios (aOR) with their 95% confidence intervals (CI). Statistical analyses were performed using SPSS 21.0.0.1 for Windows (IBM Corp., Armonk, NY, USA) and SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

General

Maternal and child characteristics are presented in Table 1. Observed data showed a median 25-hydroxyvitamin D level in mid-gestation of 66.3 nmol/L (19.4–125.4) and at birth 39.6 nmol/L (11.3–89.1) (p-value for difference < 0.05). The prevalence of eczema declined from 15.1% (n = 319) at age 6 months to 7.2% (n = 177) at age 4 years. Early, late or persistent eczema was present in 12.1% (n = 210), 12.9% (n = 222) and 13.1% (n = 225) of children, respectively. Because we imputed missing values of all subject characteristics, we present the imputed values in comparison with the observed values (Supplementary Table 1). Compared with those included in the study, all maternal and

Table 1. Characteristics of mothers and their children.

	n = 3,019
Maternal characteristics	
Age at enrollment (years)*	31.8 (4.1)
Education, higher (%)	64.0 (1,909)
History of eczema, allergy or asthma, yes (%)	34.7 (951)
Parity, ≥ 1 (%)	40.2 (1,209)
Pet keeping during pregnancy, yes (%)	41.6 (1,122)
Body mass index at enrollment (kg/m^2)†	23.3 (18.9–35.0)
Smoking during pregnancy, yes (%)	23.7 (656)
Folate level in first trimester (nmol/L)†	19.7 (6.4–38.7)
Psychiatric symptoms during pregnancy†	0.12 (0–0.77)
Season of blood sampling in mid-gestation (%)	
Spring	28.4 (787)
Summer	23.3 (646)
Autumn	23.4 (649)
Winter	24.9 (691)
25-hydroxyvitamin D level in mid-gestation (nmol/L)†	66.3 (19.4–125.4)
Child characteristics	
Sex, female (%)	49.1 (1,505)
Gestational age at birth (weeks)†	40.3 (36.0–42.4)
Birth weight (grams)*	3,513 (542)
Breastfeeding, exclusive for 4 months (%)	27.2 (696)
Vitamin D supplementation at age 2 months, yes (%)	71.0 (1,850)
Season of blood sampling at birth (%)	
Spring	24.1 (727)
Summer	26.4 (797)
Autumn	27.9 (842)
Winter	21.6 (653)
25-hydroxyvitamin D level at birth (nmol/L)†	39.6 (11.3–89.1)
Eczema, yes (%)	
Age 6 months	15.1 (319)
Age 1 year	18.2 (518)
Age 2 years	12.8 (340)
Age 3 years	8.1 (201)
Age 4 years	7.2 (177)
Eczema patterns (%)	
Never	61.9 (1,066)
Early	12.1 (210)
Late	12.9 (222)
Persistent	13.1 (225)

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed data.

child characteristics of those lost to follow-up differed, except for maternal history of eczema, allergy or asthma, BMI at enrollment and psychiatric symptoms, child's sex and season of blood sampling of 25-hydroxyvitamin D levels (Supplementary Table 2).

25-hydroxyvitamin D and eczema

Compared with optimal 25-hydroxyvitamin D levels, sufficient, deficient and severely deficient 25-hydroxyvitamin D levels in mid-gestation were not associated with the overall risk of eczema in children until age 4 years (aOR (95% CI): 0.94 (0.81, 1.10), 1.04 (0.87, 1.25) and 1.09 (0.82, 1.43), respectively) (*p*-values for trend > 0.05) (Table 2). Also, per year analysis showed no association of 25-hydroxyvitamin D levels in mid-gestation with the risk of childhood eczema (Table 2). We observed no association of sufficient, deficient and severely deficient 25-hydroxyvitamin D levels at birth with the overall risk of eczema, compared with optimal 25-hydroxyvitamin D levels (0.93 (0.70, 1.23), 0.92 (0.71, 1.20) and 0.89 (0.69, 1.17), respectively) (*p*-values for trend > 0.05). Similarly, per year analysis showed no association of 25-hydroxyvitamin D levels at birth with childhood eczema (Table 2). Levels of 25-hydroxyvitamin D in mid-gestation and at birth were not associated with eczema patterns (Table 3).

DISCUSSION

The results of this population-based prospective cohort study suggested that levels of 25-hydroxyvitamin D in mid-gestation and at birth were not associated with the overall risk of eczema, nor with the risk of eczema per year or eczema patterns in children until age 4 years.

Comparison of main findings with other studies

Studies that explored the association of maternal 25-hydroxyvitamin D levels during pregnancy with childhood eczema reported inconsistent results.[10, 14, 15] Two studies suggested that levels of 25-hydroxyvitamin D in pregnancy or at birth were not associated with the risk of eczema at age 2 or 7 years.[10, 14] Other studies reported that higher maternal 25-hydroxyvitamin D levels in late pregnancy were associated with an increased risk of visible eczema at age 9 months or with a decreased risk of eczema at age 4 years.[14, 15] Differences in results might be explained by the period of pregnancy when 25-hydroxyvitamin D was measured, method of eczema measurement such as the presence of visible eczema on physical examination or parental reporting of eczema by questionnaires, or adjustment for different confounding factors. Additional to these studies, we observed that levels of 25-hydroxyvitamin D in mid-gestation were not associated with the overall risk of eczema, nor with the risk of eczema per year or eczema patterns in children until age 4 years., taking also 25-hydroxyvitamin D levels at birth

Table 2. Associations of 25-hydroxyvitamin D levels with eczema in children until age 4 years.

	Odds ratio (95% confidence interval) for eczema					
	6 months	**1 year**	**2 years**	**3 years**	**4 years**	**Overall**
25-hydroxyvitamin D in mid-gestation*						
Optimal (n = 1,187)	Reference	Reference	Reference	Reference	Reference	Reference
Sufficient (n = 925)	1.03 (0.75, 1.43)	0.92 (0.72, 1.17)	0.81 (0.61, 1.07)	0.90 (0.62, 1.30)	0.93 (0.67, 1.30)	0.94 (0.81, 1.10)
Deficient (n = 752)	1.15 (0.72, 1.85)	0.98 (0.74, 1.31)	0.95 (0.70, 1.29)	0.99 (0.62, 1.58)	0.93 (0.61, 1.43)	1.04 (0.87, 1.25)
Severely deficient (n = 155)	1.05 (0.54, 2.05)	0.91 (0.55, 1.49)	1.05 (0.60, 1.83)	0.97 (0.50, 1.87)	1.33 (0.76, 2.32)	1.09 (0.82, 1.43)
P-value for trend	0.30	0.94	0.77	0.98	0.81	0.87
25-hydroxyvitamin D at birth†						
Optimal (n = 206)	Reference	Reference	Reference	Reference	Reference	Reference
Sufficient (n = 775)	0.83 (0.50, 1.39)	0.90 (0.57, 1.41)	0.95 (0.57, 1.57)	0.85 (0.45, 1.61)	0.98 (0.56, 1.71)	0.89 (0.69, 1.17)
Deficient (n = 1,375)	0.79 (0.47, 1.33)	1.01 (0.66, 1.57)	1.01 (0.65, 1.59)	1.03 (0.57, 1.88)	0.98 (0.52, 1.87)	0.92 (0.71, 1.20)
Severely deficient (n = 663)	0.69 (0.35, 1.36)	0.99 (0.64, 1.55)	0.96 (0.57, 1.62)	1.16 (0.58, 2.32)	1.23 (0.59, 2.56)	0.93 (0.70, 1.23)
P-value for trend	0.30	0.94	0.77	0.98	0.81	0.87

Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Levels of 25-hydroxyvitamin D are categorized as 'severely deficient' (< 25.0 nmol/L (< 10.0 mg/L)), 'deficient' (25.0–49.9 nmol/L (10.0–19.9 mg/L)), 'sufficient' (50.0–74.9 nmol/L (20.0–29.9 mg/L)) and 'optimal' (≥ 75.0 nmol/L (≥ 30.0 mg/L)). Models are adjusted for maternal age at enrollment, education, history of eczema, allergy or asthma, parity, pet keeping, body mass index at enrollment, folate level, smoking, psychiatric symptoms, season of blood sampling in mid-gestation, and child's sex, gestational age, birth weight, breastfeeding, vitamin D supplementation at age 2 months and season of blood sampling at birth. *Additionally adjusted for 25-hydroxyvitamin D levels at birth. †Additionally adjusted for 25-hydroxyvitamin D levels in mid-gestation.

Table 3. Associations of 25-hydroxyvitamin D levels with eczema patterns in children until age 4 years.

	Odds ratio (95% confidence interval) for eczema patterns		
	Early	**Late**	**Persistent**
25-hydroxyvitamin D in mid-gestation*			
Optimal (n = 1,187)	Reference	Reference	Reference
Sufficient (n = 925)	1.23 (0.78, 1.94)	0.88 (0.58, 1.34)	0.89 (0.58, 1.37)
Deficient (n = 752)	1.02 (0.37, 3.06)	1.12 (0.69, 1.84)	0.90 (0.54, 1.50)
Severely deficient (n = 155)	0.99 (0.33, 2.92)	0.75 (0.28, 2.00)	0.90 (0.35, 2.26)
P-value for trend	0.62	0.71	0.66
25-hydroxyvitamin D at birth†			
Optimal (n = 206)	Reference	Reference	Reference
Sufficient (n = 775)	1.28 (0.54, 3.05)	1.21 (0.48, 3.01)	0.53 (0.25, 1.15)
Deficient (n = 1,375)	0.92 (0.38, 2.22)	0.96 (0.36, 2.30)	0.67 (0.31, 1.43)
Severely deficient (n = 663)	1.00 (0.36, 2.80)	0.98 (0.35, 2.74)	0.63 (0.25, 1.56)
P-value for trend	0.30	0.78	0.79

Values are odds ratios (95% confidence interval) from multinomial logistic regression models based on imputed data. Reference group is children with optimal 25-hydroxyvitamin D levels who never (n = 1,557) had eczema. Levels of 25-hydroxyvitamin D are categorized as 'severely deficient' (< 25.0 nmol/L (< 10.0 mg/L)), 'deficient' (25.0–49.9 nmol/L (10.0–19.9 mg/L)), 'sufficient' (50.0–74.9 nmol/L (20.0–29.9 mg/L)) and 'optimal' (≥ 75.0 nmol/L (≥ 30.0 mg/L)). Models are adjusted for maternal age at enrollment, education, history of eczema, allergy or asthma, parity, pet keeping, body mass index at enrollment, folate level, smoking, psychiatric symptoms, season of blood sampling in mid-gestation, and child's sex, gestational age, birth weight, breastfeeding, vitamin D supplementation at age 2 months and season of blood sampling at birth. *Additionally adjusted for 25-hydroxyvitamin D levels at birth. †Additionally adjusted for 25-hydroxyvitamin D levels in mid-gestation.

into account. We hypothesized that lower 25-dihydroxyvitamin D levels in early life lead to an increased risk of eczema at a young age, but did not find such associations. We observed that levels of 25-hydroxyvitamin D levels at birth were not associated with the risk of childhood eczema. Our results are in contrast to a previous study that reported that higher 25-hydroxyvitamin D3 levels at birth were associated with a decreased risk of eczema at age 6 months.[5] Differences in results might be explained by differences in sample size and type of vitamin D level measurement. Therefore, further studies are needed to replicate our findings before any strong conclusions can be drawn. Several prospective cohort studies examined associations of 25-hydroxyvitamin D levels at birth with the risk of childhood eczema at later ages and reported conflicting results.[11–13] One study did not observe an association of 25-hydroxyvitamin D levels at birth with the risk of eczema, allergic sensitization and total IgE levels until age 7 years[11], but others suggested that higher 25-hydroxyvitamin D levels at birth were associated with a lower risk of eczema in children until age 5 years.[12, 13] Differences in results may be attributed to different sample sizes of the studies[12, 13], use of 25-hydroxyvitamin D3 only[13], or dif-

ferent definitions of eczema patterns.[12] Furthermore, a previous study that measured eczema at multiple time points during follow-up did not take repeated measurements of eczema within the same child into account nor adjustment for 25-hydroxyvitamin D levels during pregnancy.[12]

Interpretation of results

Although the effects of 25-hydroxyvitamin D on the immune system have been well recognized[6], its effects do not seem to have a role in the occurrence and persistence of eczema through childhood. *In vivo* experiments in mice suggested that the treatment of eczema with vitamin D receptor agonists results in improvement of allergen-associated eczema.[9] However, findings in animal models indicated that vitamin D3 or its analogues display different effects on expression of molecules in mouse skin as opposed to human or primate skin.[27] Namely, expression of thymic stromal lymphopoietin was observed in keratinocytes of mice after stimulation with vitamin D3, but not in keratinocytes of humans or primates.[28] We observed no differences in results for the associations of 25-hydroxyvitamin D during pregnancy or at birth with eczema outcomes. Levels of 25-hydroxyvitamin D in mid-gestation and at birth might have been highly correlated due to similar lifestyle and dietary habits of mother during pregnancy. However, we observed a lower median 25-hydroxyvitamin D level at birth than in mid-gestation (39.6 vs. 66.3 nmol/L). This difference in 25-hydroxyvitamin D levels was not expected because pregnant women in the Netherlands are advised to take 10 µg of vitamin D supplement per day throughout pregnancy.[25] Our study lacked information on adherence of vitamin D intake which could have explained the discrepancy between 25-hydroxyvitamin D levels in mid-gestation and at birth. Placental function or vitamin D receptor expression, and genetic susceptibility might have also affected the difference in 25-hydroxyvitamin D levels.[29–32] We did not observe differences in results when we included or excluded supplementation of vitamin D in childhood (data not shown). The potential intermediating and protective role of child's vitamin D supplementation for the associations of 25-hydroxyvitamin D levels in mid-gestation and at birth with eczema is difficult to study in countries, such as the Netherlands, where most preschool-age children take a vitamin D supplement because it is recommended.[25] We did not observe associations of either maternal or fetal 25-hydroxyvitamin D levels with any eczema outcome, and we therefore speculate that other factors, such as genetic or other environmental factors, might have a more important role in the onset of childhood eczema. Eczema appears to have a complex genetic background. Mutations of the gene encoding filament-aggregating protein, or filaggrin, have been strongly associated with the development of eczema.[33] Also, a recent study suggested that microRNAs influence the inhibition of innate immune response in chronic skin inflammation.[27] For environmental factors, climate and housing conditions, urban or rural living, dietary and feeding habits, air

pollution, microbial exposure, and socioeconomic status have been suggested to be associated with childhood eczema. Also, exposure to higher ultraviolet (UV) levels seems associated with higher 25-hydroxyvitamin D levels and a lower prevalence of eczema, compared with exposure to lower UV levels.[34–36] Therefore, future studies are needed to explore the role of multiple genetic and environmental factors, and their interaction, in the development of eczema in childhood.

Strengths and limitations

The strength of this study is its population-based prospective design. Participants were followed up from fetal life onwards, and a large number of potential confounding factors were recorded. Also, 25-hydroxyvitamin D levels were objectively measured in maternal blood in mid-gestation and in cord blood at birth, and categorized according to clinical cut-off values that would allow the comparison with other studies. However, selection bias in cohort studies arises from non-response or missing data of the exposure, and loss to follow-up. Subjects included in the current study were more affluent and healthier than those not included. This could have led to biased effect estimates if the associations of mid-gestational and umbilical cord blood 25-hydroxyvitamin D levels with eczema were different between those included and not included in the analyses. We used the same cut-off values for 25-hydroxyvitamin D levels in pregnant women as the documented levels for the general population, although there is no consensus on optimal 25-hydroxyvitamin D levels.[20] Information on eczema was collected by parental questionnaires, and this method is commonly used in large epidemiological studies.[37, 38] However, some misclassification of eczema might still be present because the questionnaires were not validated against medical records or physical examination in our study population. We took many covariates into account, but lacked data on potential modifying environmental factors, such as UV exposure or dietary patterns of the children, which could have affected our results. Our population for analysis comprised children of Dutch origin only and this may limit the generalizability of our findings to children of other ethnic origin or with heterogeneous skin types. Finally, we cannot exclude residual confounding from unmeasured exposures such as other vitamin or supplement intake, as in any prospective cohort study.

In conclusion, our results suggest that levels of 25-hydroxyvitamin D in mid-gestation and at birth are not associated with the overall risk of eczema, nor with the risk of eczema per year or eczema patterns in children until age 4 years. Future studies focusing on other risk factors, such as environmental and genetic factors, are needed to explore the development of eczema in childhood.

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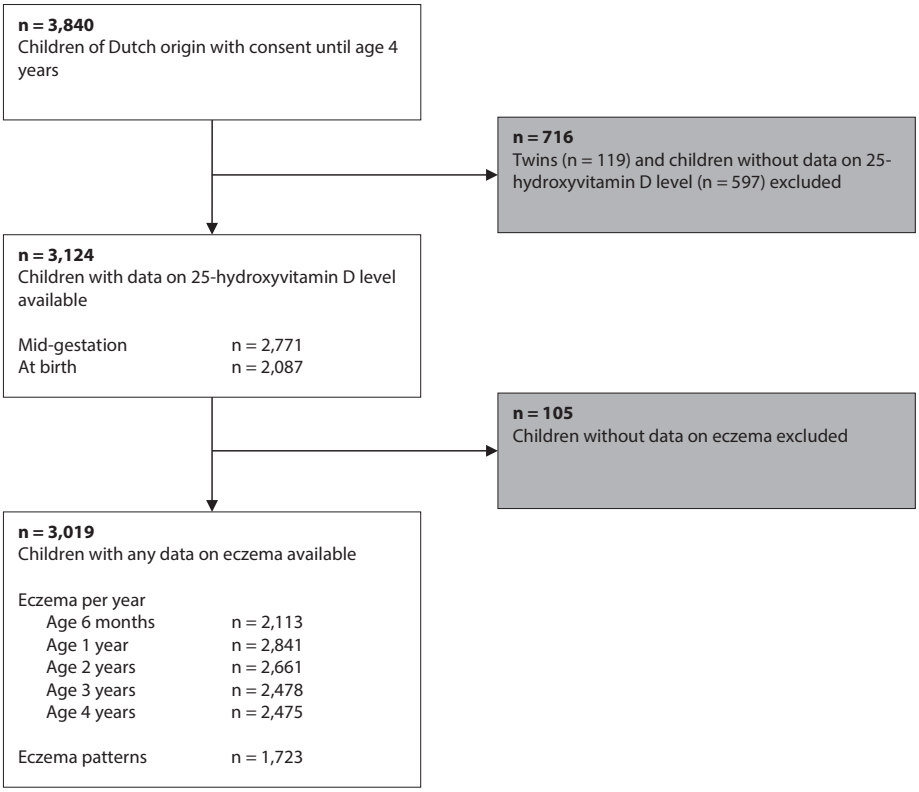
SUPPLEMENTARY MATERIAL

Supplementary Methods

The total 25-hydroxyvitamin D level was reported as the sum of 25-hydroxyvitamin D2 and D3 species measured in plasma using a modification of a method previously described.[19] Briefly, 50 µL milli-Q water and 500 µL of acetonitrile (ACN) containing 6,19,19-(2H3)-25-hydroxyvitamin D2 and 6,19,19-(2H3)-25-hydroxyvitamin D3 at 10 nmol/L each were added to 3 µL plasma, sonicated, vortexed and centrifuged. The supernatant was filtered using a TiO2/ZrO2 filter plate (Glygen Corp., Columbia, MD, USA) and evaporated to dryness. Samples were derivatized using 4-phenyl-1,2,4-triazoline-3,5-dione and reconstituted in ACN:H2O (1:3) prior to analysis. Samples were quantified using isotope dilution liquid chromatography-tandem mass spectrometry. The analytical system was comprised of a Shimadzu Nexera UPLC coupled to an AbSciex 5500 QTRAP equipped with an APCI source. Chromatographic separation was achieved using a Kinetex XB-C18 column (50 x 2.1 mm, 1.7 µm; Phenomenex, Torrance, CA, USA), and 72% acetonitrile/32% aqueous 0.1% formic acid at a flow rate of 0.5 mL/min. Linearity was assessed using matrix-matched calibration standards, with R2 values of > 0.99 across the calibration range (10–125 nmol/L). Inter-assay inaccuracy and imprecision were assessed at four concentration levels for 25-hydroxyvitamin D3 (48.3, 49.4, 76.4 and 139.2 nmol/L) and a single level for 25-hydroxyvitamin D2 (32.3 nmol/L) using certified reference materials purchased from the National Institute of Standards and Technology (NIST SRM 972a Levels 1–4), and were excellent at all concentration levels tested. Inter-assay inaccuracy and imprecision were both < 10% for 25-hydroxyvitamin D3 and < 17% for 25-hydroxyvitamin D2. Assay repeatability was assessed via replicate analysis of an independent reference material (NIST SRM1950, 61.9 nmol/L 25-hydroxyvitamin D3), with inter-assay inaccuracy and imprecision both < 11% (n = 343). The limit of quantification was 1 and 5 nmol/L for 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2, respectively.

Supplementary Figure. Flowchart of participants.



Supplementary Table 1. Characteristics of mothers their children (n = 3,019).

	Observed	Imputed
Maternal characteristics		
Age at enrollment (years)*	31.8 (4.1)	31.8 (4.1)
Missing	*0 (0)*	*0 (0)*
Education (%)		
Primary	2.3 (69)	3.0 (91)
Secondary	33.7 (1,006)	33.6 (1,014)
Higher	64.0 (1,909)	63.4 (1,914)
Missing	*1.2 (35)*	*0 (0)*
History of allergy, eczema or asthma (%)		
No	65.3 (1,793)	65.2 (1,969)
Yes	34.7 (951)	34.8 (1,050)
Missing	*9.1 (275)*	*0 (0)*
Parity (%)		
0	59.8 (1,804)	59.9 (1,807)
≥ 1	40.2 (1,209)	40.1 (1,212)
Missing	*0.2 (6)*	*0 (0)*
Pet keeping during pregnancy (%)		
No	58.4 (1,578)	58.5 (1,765)
Yes	41.6 (1,122)	41.5 (1,254)
Missing	*10.6 (319)*	*0 (0)*
Body mass index at enrollment (kg/m^2)†	23.3 (18.9–35.0)	23.3 (18–9-34.9)
Missing	*0.5 (16)*	*0 (0)*
Smoking during pregnancy (%)		
No	76.3 (2,113)	76.3 (2,303)
Yes	23.7 (656)	23.7 (716)
Missing	*8.3 (250)*	*0 (0)*
Folate level in first trimester (nmol/L)†	19.7 (6.4–38.7)	19.6 (6.3–38.6)
Missing	*22.2 (671)*	*0 (0)*
Psychiatric symptoms during pregnancy†	0.12 (0–0.77)	0.12 (0–0.77)
Missing	*31.4 (947)*	*0 (0)*
Season of blood sampling in mid-gestation (%)		
Spring	28.4 (787)	28.6 (864)
Summer	23.3 (646)	23.1 (696)
Autumn	23.4 (649)	23.3 (703)
Winter	24.9 (691)	25.0 (756)
Missing	*8.1 (246)*	*0 (0)*
25-hydroxyvitamin D level in mid-gestation (nmol/L)†	66.3 (19.4–125.4)	66.0 (19.4–124.8)
Missing	*8.2 (248)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers their children (n = 3,019). (continued)

	Observed	Imputed
Child characteristics		
Sex (%)		
Male	50.9 (1,514)	50.9 (1,514)
Female	49.1 (1,505)	49.1 (1,505)
Missing	*0 (0)*	*0 (0)*
Gestational age at birth (weeks)†	40.3 (36.0–42.4)	40.3 (36.0–42.4)
Missing	*0 (0)*	*0 (0)*
Birth weight (grams)*	3,513 (542)	3,513 (542)
Missing	*0 (0)*	*0 (0)*
Breastfeeding (%)		
Never	11.0 (283)	12.2 (369)
Non-exclusive for 4 months	61.8 (1,582)	60.6 (1,831)
Exclusive for 4 months	27.2 (696)	27.1 (819)
Missing	*15.2 (458)*	*0 (0)*
Vitamin D supplementation at age 2 months (%)		
No	29.0 (757)	30.1 (910)
Yes	71.0 (1,850)	69.9 (2,109)
Missing	*13.6 (412)*	*0 (0)*
Season of blood sampling at birth (%)		
Spring	24.1 (727)	24.1 (727)
Summer	26.4 (797)	26.4 (797)
Autumn	27.9 (842)	27.9 (842)
Winter	21.6 (653)	21.6 (653)
Missing	*0 (0)*	*0 (0)*
25-hydroxyvitamin D level at birth (nmol/L)†	39.6 (11.3–89.1)	40.2 (11.2–89.1)
Missing	*30.9 (932)*	*0 (0)*
Eczema last 6 months at age 6 months (%)		
No	84.9 (1,794)	70.8 (2,136)
Yes	15.1 (319)	29.2 (883)
Missing	*30.0 (906)*	*0 (0)*
Eczema last 6 months at age 1 year (%)		
No	81.8 (2,323)	79.8 (2,408)
Yes	18.2 (518)	20.2 (611)
Missing	*5.9 (178)*	*0 (0)*
Eczema last 12 months at age 2 years (%)		
No	87.2 (2,321)	85.3 (2,576)
Yes	12.8 (340)	14.7 (443)
Missing	*11.9 (358)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers their children (n = 3,019). (continued)

	Observed	Imputed
Eczema last 12 months at age 3 years (%)		
No	91.9 (2,277)	87.9 (2,655)
Yes	8.1 (201)	12.1 (364)
Missing	*17.9 (541)*	*0 (0)*
Eczema last 12 months at age 4 years (%)		
No	92.8 (2,298)	87.8 (2,650)
Yes	7.2 (177)	12.2 (369)
Missing	*18.0 (544)*	*0 (0)*
Eczema patterns (%)		
Never	61.9 (1,066)	49.6 (1,498)
Early	12.1 (210)	20.6 (622)
Late	12.9 (222)	15.4 (464)
Persistent	13.1 (225)	14.4 (435)
Missing	*42.9 (1,296)*	*0 (0)*

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed and imputed data.

Supplementary Table 2. Characteristics of mothers and children included and not included in the study.

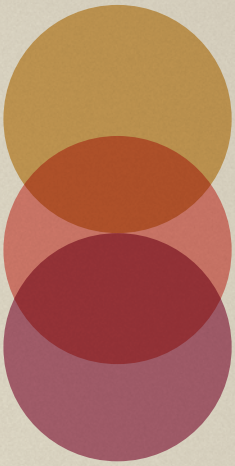
	Included n = 3,019	**Not included n = 105**	***P*-value for difference**
Maternal characteristics			
Age at enrollment (years)*	31.8 (4.1)	28.8 (5.9)	**< 0.001**
Missing	*0 (0)*	*0 (0)*	
Education (%)			**< 0.001**
Primary	2.3 (69)	18.4 (19)	
Secondary	33.7 (1,006)	47.6 (49)	
Higher	64.0 (1,909)	34.0 (35)	
Missing	*1.2 (35)*	*1.9 (2)*	
History of allergy, eczema or asthma (%)			0.76
No	65.3 (1,793)	63.8 (60)	
Yes	34.7 (951)	36.2 (34)	
Missing	*9.1 (275)*	*10.5 (11)*	
Parity (%)			**< 0.01**
0	59.8 (1,804)	46.7 (49)	
≥ 1	40.2 (1,209)	53.3 (56)	
Missing	*0.2 (6)*	*0 (0)*	
Pet keeping during pregnancy (%)			**< 0.01**
No	58.4 (1,578)	43.6 (41)	
Yes	41.6 (1,122)	56.4 (53)	
Missing	*10.6 (319)*	*10.5 (11)*	
Body mass index at enrollment (kg/m^2)†	23.3 (18.9–35.0)	23.5 (19.1–37.1)	0.16
Missing	*0.5 (16)*	*0 (0)*	
Smoking during pregnancy (%)			**< 0.001**
No	76.3 (2,113)	53.7 (51)	
Yes	23.7 (656)	46.3 (44)	
Missing	*8.3 (250)*	*9.5 (10)*	
Folate level in first trimester (nmol/L)†	19.7 (6.4–38.7)	13.6 (4.8–32.1)	**< 0.001**
Missing	*22.2 (671)*	*26 (24.8)*	
Psychiatric symptoms during pregnancy†	0.12 (0–0.77)	0.15 (0–1.74)	0.07
Missing	*31.4 (947)*	*32 (30.5)*	
Season of blood sampling in mid-gestation (%)			0.75
Spring	28.4 (787)	32.6 (29)	
Summer	23.3 (646)	24.7 (22)	
Autumn	23.4 (649)	21.3 (19)	
Winter	24.9 (691)	21.3 (19)	
Missing	*8.1 (246)*	*15.2 (16)*	

Supplementary Table 2. Characteristics of mothers and children included and not included in the study. (continued)

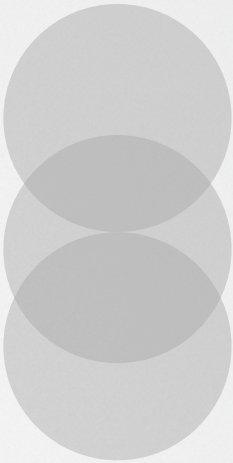
	Included n = 3,019	**Not included n = 105**	***P*-value for difference**
Child characteristics			
Sex (%)			0.65
Male	50.9 (1,514)	52.4 (55)	
Female	49.1 (1,505)	47.6 (50)	
Missing	*0 (0)*	*0 (0)*	
Gestational age at birth (weeks)†	40.3 (36.0–42.4)	40.0 (33.6–42.1)	**< 0.05**
Missing	*0 (0)*	*0 (0)*	
Birth weight (grams)*	3,513 (542)	3,378 (517)	**< 0.05**
Missing	*0 (0)*	*0 (0)*	
Breastfeeding (%)			**< 0.001**
Never	11.0 (283)	43.5 (20)	
Non-exclusive for 4 months	61.8 (1,582)	56.5 (26)	
Exclusive for 4 months	27.2 (696)	0 (0)	
Missing	*15.2 (458)*	*56.2 (59)*	
Vitamin D supplementation at age 2 months (%)			**< 0.001**
No	29.0 (757)	58.7 (27)	
Yes	71.0 (1,850)	41.3 (19)	
Missing	*13.6 (412)*	*56.2 (59)*	
Season of blood sampling at birth (%)			0.97
Spring	24.1 (727)	23.8 (25)	
Summer	26.4 (797)	26.7 (28)	
Autumn	27.9 (842)	29.5 (31)	
Winter	21.6 (653)	20.0 (21)	
Missing	*0 (0)*	*0 (0)*	

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed data. *P*-values for difference are calculated by independent samples T-test for continuous variables with a normal distribution, the Mann-Whitney U-test for continuous variables with a skewed distribution, and Pearson's Chi-square test for categorical variables. Bold values indicate statistical significance at the $\alpha = 0.05$ level.

CHAPTER 3



INFANT EXPOSURES AND CHILDHOOD ECZEMA, ALLERGIC SENSITIZATION OR ALLERGY



CHAPTER 3.1

ETHNIC ORIGIN AND ECZEMA IN PRESCHOOL-AGE CHILDREN

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ABSTRACT

Background The prevalence of childhood eczema varies considerably between ethnic groups. However, data from longitudinal studies remain scarce.

Methods We examined the associations of ethnic origin with the development of eczema from birth until age 4 years, and whether known environmental and genetic risk factors explain these associations. This study was performed in a multi-ethnic population-based prospective cohort among 5,082 children. Ethnic origin was based on the parents' country of birth. Data on physician-diagnosed eczema were obtained by annual questionnaires. Information on environmental risk factors was mostly obtained by questionnaires. Filaggrin mutations (2282del4, R2447X, R501X and S3247X) were genotyped for 3,096 children. We used generalized estimating equation models to examine the associations of ethnic origin with the longitudinal odds of eczema at ages 6 months and 1, 2, 3 and 4 years independently and overall.

Results Compared with Dutch children, Cape Verdean, Dutch Antillean, Surinamese-Creole, and Surinamese-Hindustani children had overall increased risks of eczema (odds ratio (95% confidence interval): 1.53 (1.15, 2.03), 1.60 (1.21, 2.12), 1.95 (1.56, 2.44), and 2.06 (1.65, 2.57), respectively). Effect estimates for the associations of Cape Verdean and Dutch Antillean origin with eczema became non-significant after adjustment for genetic risk factors or both environmental and genetic risk factors, respectively. Surinamese-Creole and Surinamese-Hindustani children remained to have increased risks of eczema.

Conclusions Cape Verdean, Dutch Antillean, Surinamese-Creole, and Surinamese-Hindustani children had increased risks of eczema in the first 4 years of life. Environmental and genetic risk factors partly weakened these associations.

INTRODUCTION

The prevalence of childhood eczema varies considerably between ethnic groups.[1] Cross-sectional studies from the USA and the UK showed that black children had an up to 2.2-fold increased risk of eczema, while Hispanic children had a 28% decreased risk, compared with white children.[2–4] Other European studies demonstrated that Surinamese and Dutch Antillean children more frequently, and Moroccan and Turkish children less frequently had eczema than Caucasian children.[5–7] Data from longitudinal studies remain scarce. We previously examined differences in the development of eczema between ethnic groups longitudinally in children until age 2 years.[8] We observed that Moroccan and Surinamese children more frequently had eczema in the first year of life, while Surinamese children more frequently and Turkish children less frequently had eczema in the second year of life, compared with Dutch children. Differences in the prevalence of eczema between ethnic groups must be explained by environmental and genetic factors. Known environmental risk factors for eczema include housing conditions, urban or rural living, dietary and feeding habits, air pollution, and microbial exposure.[9] 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.[10] Although 2282del4, R2447X, R501X, and S3247X are the most common *FLG* mutations in Caucasians[11], the prevalence of these mutations in other ethnic groups is less clear.

Therefore, we examined among 5,082 children participating in a large multi-ethnic population-based prospective cohort in Rotterdam, The Netherlands, the associations of ethnic origin with the development of eczema from birth until age 4 years, and whether any association was explained by known environmental and genetic risk factors.

METHODS

General design

This study was embedded in the Generation R Study, an ongoing population-based prospective cohort study from fetal life onwards.[12] The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, The Netherlands (MEC 217.595/2002/202). Written informed consent was obtained from parents or legal guardians of 7,295 children. For the current study, a total of 5,082 children were available for analysis of which 3,096 children had data on *FLG* genotype available (Supplementary Figure).

Ethnic origin

In accordance with Statistics Netherlands[13], the ethnic origin of the child was based on the parents' country of birth. This information was obtained by a questionnaire at enroll-

ment. Children of whom both parents were born in the Netherlands were classified as being of Dutch origin. A child was considered of non-Dutch origin if one of the parents was born abroad. If both parents were born abroad, the country of birth of the mother was decisive. Children of non-Dutch origin were classified into 'Cape Verdean', 'Dutch Antillean', 'Moroccan', 'Surinamese-Creole', 'Surinamese-Hindustani', and 'Turkish'.

Eczema

Information on physician-diagnosed eczema in the last 6 months/last year (no; yes) was obtained by parental questionnaires at ages 6 months and 1, 2, 3 and 4 years; response rates were 73%, 71%, 76%, 72% and 73%, respectively. Questions on eczema were adapted from the International Study of Asthma and Allergies in Childhood core questionnaires.[14]

Environmental risk factors

Information on maternal age, education (primary or secondary; higher), history of allergy, eczema or asthma (no; yes), parity (nulliparous; multiparous), pet keeping (no; yes) and body mass index (BMI) was obtained by a questionnaire, completed by the mother at enrollment. Information on maternal smoking (no; yes) was obtained by postal questionnaires sent during each trimester of pregnancy. Maternal psychiatric symptoms in the second trimester of pregnancy were assessed using the Global Severity Index of the Brief Symptom Inventory[15], denoting overall psychiatric symptoms. We obtained information on child's sex, gestational age at birth and birth weight from obstetric and midwife records at birth. Delivery reports and postal questionnaires completed by the mother when the child was 2, 6 and 12 months old provided data on ever breastfeeding (no; yes) or day care attendance (no; yes). The child's BMI was calculated using weight and height measured at age 10–13 months during a visit to the research center. Information on wheezing (no; yes) was obtained by annual questionnaires from birth until age 4 years.

Genetic risk factors

Four *FLG* mutations (2282del4, R2447X, R501X, and S3247X) were genotyped by modified Taqman allelic discrimination assays, using previously described primers.[16] Children without any mutant alleles were classified as wild-type. Because we observed merely two children who carried a homozygous *FLG* mutation, we created a combined *FLG* genotype, meaning that children were classified as having a *FLG* mutation if they were heterozygous or homozygous for any of the four mutations.

Statistical analysis

We examined differences in population characteristics between ethnic groups by one-way ANOVA, Kruskal-Wallis tests, and Pearson's Chi-square tests. We performed a non-response analysis by comparing children included in the analyses and those lost to follow-up using independent samples T-tests, Mann-Whitney U-tests, and Pearson's Chi-square tests. We used generalized estimating equation models to examine the associations of ethnic origin with the longitudinal odds of eczema at ages 6 months and 1, 2, 3 and 4 years independently and overall, taking into account correlations between repeated measurements of eczema within the same child. In addition to the crude model, we first adjusted for known environmental risk factors, including maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, BMI at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, breastfeeding, day care attendance, BMI at age 10–13 months, and wheezing (environmental model). Second, we adjusted for known genetic risk factors by means of the combined *FLG* genotype (genetic model). Third, we adjusted for both environmental and genetic risk factors (full model). Confounders were included in the models based on previous literature, if they were associated with both the determinant and the outcome, or if they changed the effect estimate with ≥ 10%. Missing covariates were ≤ 20%, except for maternal psychiatric symptoms (21.8%), and child's day care attendance (26.6%) and wheezing (36.0%) (Supplementary Table 1). To reduce potential bias from missing data and to increase precision, we performed a multiple imputation analysis of covariates and outcomes generating 25 independent datasets using the Markov chain Monte Carlo method, and calculated pooled estimates. We present results based on imputed analyses only. Measures of association are presented as odds ratios (OR) with their 95% confidence intervals (CI). Statistical analyses were performed using SPSS 21.0.0.1 for Windows (IBM Corp., Armonk, NY, USA) and SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

General

Of the participating children, 73.9% (n = 3,756) was of Dutch origin. Children of non-Dutch origin were Cape Verdean (2.5%; n = 125), Dutch Antillean (3.1%; n = 156), Moroccan (5.8%, n = 295), Surinamese-Creole (3.3%, n = 168), Surinamese-Hindustani (2.9%, n = 147) or Turkish (8.5%, n = 435) (Table 1). All maternal and child characteristics differed between the ethnic groups, except for maternal history of allergy, eczema or asthma. Overall, the prevalence of eczema declined from 17.4% at age 6 months to 8.9% at age 4 years. The highest prevalence of eczema was 16.5% at age 6 months for Dutch children, 22.2% at age 2 years for Cape Verdean children, 23.3% at age 2 years for Dutch Antillean children, 21.5% at age 6 months for Moroccan children, 27.4% at age 6 months

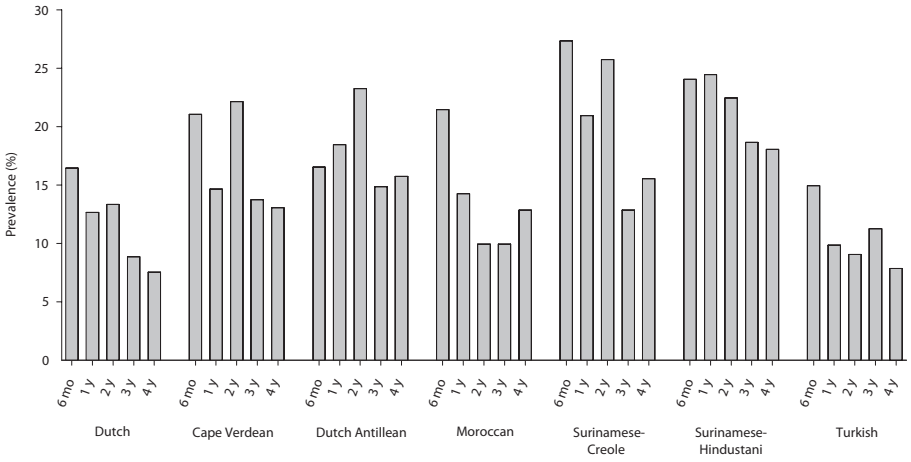
Table 1. Characteristics of mothers and their children.

	Dutch	Cape Verdean	Dutch Antillean	Moroccan	Surinamese-Creole	Surinamese-Hindustani	Turkish	*P*-value for difference
	n = 3,756	n = 125	n = 156	n = 295	n = 168	n = 147	n = 435	
Maternal characteristics								
Age at enrollment (years)*	31.7 (4.4)	29.8 (5.5)	27.5 (6.3)	28.8 (5.1)	30.0 (6.1)	28.7 (5.0)	28.2 (5.2)	**< 0.001**
Education, higher (%)	61.2 (2,298)	16.8 (21)	23.1 (36)	15.3 (45)	20.8 (35)	18.4 (27)	13.8 (60)	**< 0.001**
History of allergy, eczema or asthma, yes (%)	38.2 (1,434)	32.8 (41)	42.3 (66)	39.0 (115)	38.7 (65)	38.1 (56)	32.6 (142)	0.23
Parity, ≥ 1 (%)	41.2 (1,546)	57.6 (72)	37.8 (59)	58.6 (173)	49.4 (83)	40.8 (60)	58.2 (253)	**< 0.001**
Pet keeping during pregnancy, yes (%)	42.0 (1,576)	24.8 (31)	23.7 (37)	14.9 (44)	23.2 (39)	23.1 (34)	15.9 (69)	**< 0.001**
Body mass index at enrollment (kg/m^2)†	23.4 (18.8–35.2)	23.8 (17.8–35.3)	24.9 (17.8–38.2)	26.4 (19.5–37.9)	25.2 (18.8–41.5)	23.7 (18.3–42.0)	25.3 (19.3–38.7)	**< 0.001**
Smoking during pregnancy, yes (%)	23.2 (872)	25.6 (32)	29.5 (46)	6.8 (20)	36.9 (62)	19.0 (28)	37.2 (162)	**< 0.001**
Psychiatric symptoms during pregnancy†,‡	0.12 (0–0.98)	0.30 (0.02–1.95)	0.27 (0–1.47)	0.25 (0–2.22)	0.21 (0–1.48)	0.21 (0.01–1.54)	0.38 (0–2.14)	**< 0.001**
Child characteristics								
Sex, female (%)	49.4 (1,854)	47.2 (59)	64.1 (100)	50.5 (149)	45.2 (76)	49.0 (72)	49.7 (216)	**< 0.05**
Gestational age at birth (weeks)†	40.1 (36.0–42.3)	40.0 (36.0–42.2)	39.9 (34.1–42.2)	40.6 (36.1–42.5)	39.7 (33.5–42.1)	39.9 (35.5–41.9)	40.0 (35.4–42.4)	**< 0.001**
Birth weight (grams)*	3,501 (553)	3,286 (547)	3,187 (560)	3,505 (508)	3,220 (572)	3,083 (517)	3,388 (513)	**< 0.001**
Breastfed ever, yes (%)	89.6 (3,365)	92.8 (116)	92.3 (144)	95.6 (282)	91.7 (154)	98.6 (145)	98.4 (428)	**< 0.001**
Day care attendance, yes (%)	65.5 (2,460)	44.8 (56)	47.4 (74)	19.3 (57)	47.0 (79)	34.0 (50)	11.3 (50)	**< 0.001**
Body mass index at age 10–13 months (kg/m^2)†	17.3 (14.9–20.2)	17.8 (14.7–20.9)	17.2 (14.1–20.1)	17.7 (15.2–21.2)	17.3 (14.9–21.1)	16.6 (13.8–19.9)	17.5 (14.6–21.7)	**< 0.001**
Wheezing until age 4 years, yes (%)	44.1 (1,655)	48.8 (61)	53.2 (83)	40.7 (120)	45.2 (76)	48.3 (71)	55.9 (243)	**< 0.001**
Filaggrin mutation, ≥ 1 (%)§	10.0 (232)	4.2 (3)	4.3 (4)	0 (0)	0 (0)	1.1 (1)	1.2 (3)	**< 0.001**

Values are *means (SD),†medians (2.5–97.5th percentile) or percentages (absolute numbers) based on imputed data. *P*-values for difference were calculated by one-way ANOVA for continuous variables with a normal distribution, the Kruskal-Wallis test for continuous variables with a skewed distribution, and Pearson's Chi-square test for categorical variables. Bold values indicate statistical significance at the α = 0.05 level. ‡Scale comprises extremely low (0–0.20), low (0.21–0.52), below average (0.53–0.88), average (0.89–1.29), above average (1.30–1.86), high (1.87–2.63) and extremely high (2.64–4.00) levels of psychiatric symptoms.[37] §Missing data of filaggrin mutations (n = 1,986).

for Surinamese-Creole children, 24.5% at age 1 year of Surinamese-Hindustani children, and 15.0% at age 6 months for Turkish children (Figure 1). *FLG* mutations were present in 7.8% (n = 243) of all children. None of the Moroccan and Surinamese-Creole children carried a mutation. All other children of non-Dutch origin less frequently carried *FLG* mutations, compared with children of Dutch origin (*p*-value for difference < 0.05). Two children carried a homozygous *FLG* mutation (R501X and 2282del4). Compound heterozygosity was found in four different children. Differences between included children and their mothers and those lost to follow-up are listed in Supplementary Table 2.

Figure 1. Prevalence of eczema in children of different ethnic origin until age 4 years.

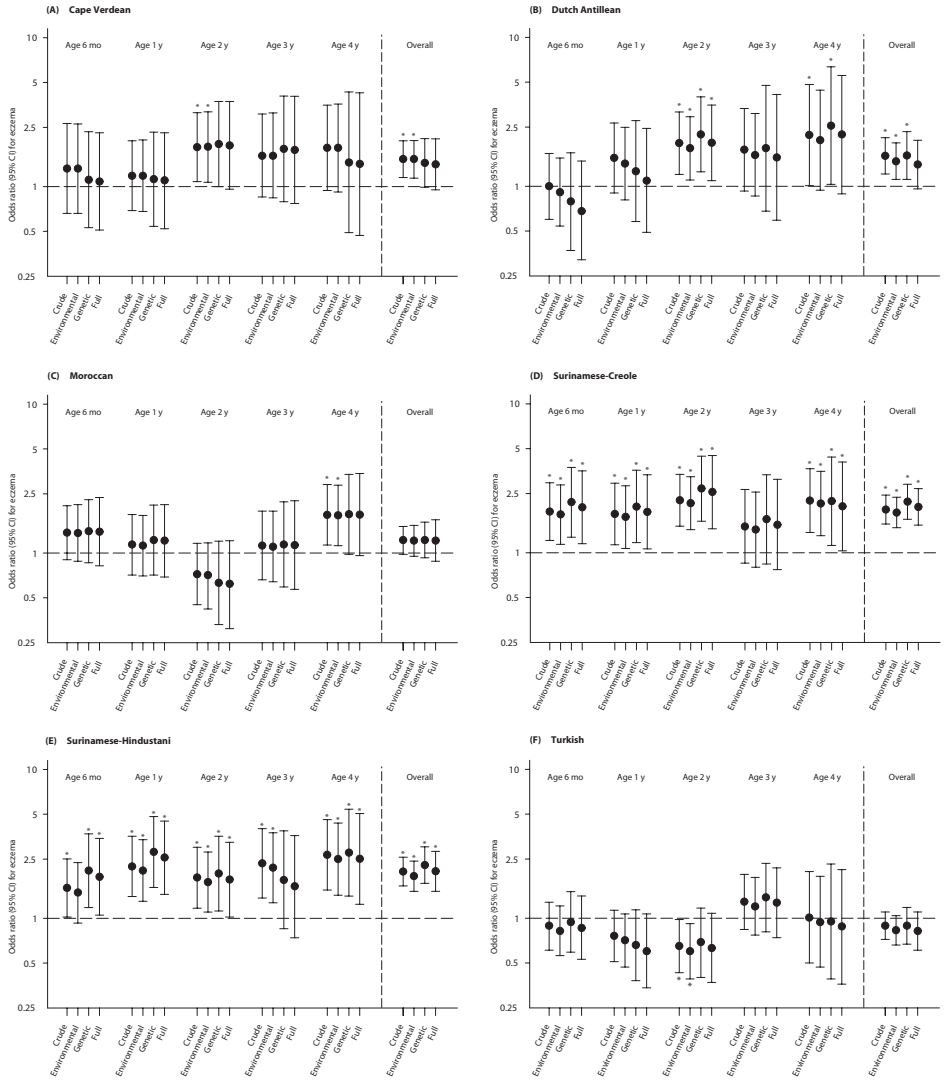


Mo = months; y = year(s).

Ethnic origin and eczema

Crude models showed that, compared with Dutch children, Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children had overall increased risks of eczema (OR (95% CI): 1.53 (1.15, 2.03), 1.60 (1.21, 2.12), 1.95 (1.56, 2.44) and 2.06 (1.65, 2.57), respectively) (Figure 2 and Supplementary Table 3), but Moroccan and Turkish children did not. When we adjusted for known environmental risk factors of mother and child separately, we observed that the maximum change in effect size for the associations of Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani origin occurred when maternal education (1.44 (1.08, 1.92), 1.52 (1.15, 2.01), 1.85 (1.48, 2.32) and 1.95 (1.56, 2.43), respectively) and psychiatric symptoms during pregnancy (1.44 (1.08, 1.92), 1.53 (1.15, 2.02), 1.88 (1.50, 2.35) and 1.97 (1.58, 2.46), respectively) were added to the crude models (Table 2). When we adjusted for all known environmental risk factors, we observed that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children remained to have increased risks of eczema. When

Figure 2. Associations of Cape Verdean (A), Dutch Antillean (B), Moroccan (C), Surinamese-Creole (D), Surinamese-Hindustani (E), and Turkish (F) origin with eczema in children until age 4 years.



Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Reference group is children of Dutch origin (dotted line). The crude model is unadjusted. The environmental model (all environmental risk factors) is adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index (BMI) at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, breastfeeding, day care attendance, BMI at age 10–13 months and wheezing. The genetic model (genetic risk factors) is adjusted for filaggrin genotype. The full model (all risk factors) is adjusted for both environmental and genetic risk factors. *P-value < 0.05. Mo = months; y = year(s).

Table 2. Effects of known environmental and genetic risk factors on the overall risk of eczema in children of different ethnic origin.

	Odds ratio (95% confidence interval) for eczema					
	Cape Verdean n = 125	**Dutch Antillean n = 156**	**Moroccan n = 295**	**Surinamese-Creole n = 168**	**Surinamese-Hindustani n = 147**	**Turkish n = 435**
Crude model	**1.53 (1.15, 2.03)**	**1.60 (1.21, 2.12)**	1.22 (0.98, 1.51)	**1.95 (1.56, 2.44)**	**2.06 (1.65, 2.57)**	0.89 (0.72, 1.10)
When additionally adjusted for:						
Environmental risk factors – mother						
Age at enrollment	**1.50 (1.13, 1.99)**	**1.53 (1.17, 2.01)**	1.18 (0.95, 1.47)	**1.92 (1.54, 2.39)**	**1.99 (1.60, 2.48)**	0.86 (0.69, 1.06)
Education	**1.44 (1.08, 1.92)**	**1.52 (1.15, 2.01)**	1.15 (0.92, 1.44)	**1.85 (1.48, 2.32)**	**1.95 (1.56, 2.43)**	0.84 (0.67, 1.04)
History of allergy, eczema or asthma	**1.56 (1.18, 2.06)**	**1.58 (1.19, 2.10)**	1.22 (0.98, 1.51)	**1.95 (1.56, 2.43)**	**2.06 (1.65, 2.58)**	0.91 (0.73, 1.13)
Parity	**1.57 (1.18, 2.09)**	**1.59 (1.20, 2.11)**	**1.25 (1.01, 1.56)**	**1.98 (1.58, 2.47)**	**2.06 (1.65, 2.57)**	0.91 (0.74, 1.13)
Pet keeping during pregnancy	**1.53 (1.15, 2.03)**	**1.60 (1.20, 2.12)**	1.22 (0.98, 1.50)	**1.95 (1.56, 2.43)**	**2.05 (1.65, 2.56)**	0.89 (0.71, 1.10)
Body mass index at enrollment	**1.53 (1.15, 2.03)**	**1.58 (1.20, 2.10)**	1.20 (0.96, 1.50)	**1.93 (1.54, 2.41)**	**2.05 (1.64, 2.56)**	0.88 (0.71, 1.09)
Smoking during pregnancy	**1.53 (1.15, 2.03)**	**1.59 (1.20, 2.11)**	1.24 (0.99, 1.54)	**1.93 (1.55, 2.42)**	**2.07 (1.66, 2.58)**	0.88 (0.71, 1.09)
Psychiatric symptoms during pregnancy	**1.44 (1.08, 1.92)**	**1.53 (1.15, 2.02)**	1.13 (0.91, 1.41)	**1.88 (1.50, 2.35)**	**1.97 (1.58, 2.46)**	0.80 (0.64, 1.00)
Environmental risk factors – child						
Sex	**1.52 (1.15, 2.02)**	**1.65 (1.24, 2.19)**	1.22 (0.99, 1.52)	**1.94 (1.55, 2.42)**	**2.06 (1.65, 2.57)**	0.89 (0.72, 1.10)
Gestational age at birth	**1.53 (1.15, 2.04)**	**1.61 (1.21, 2.13)**	1.22 (0.98, 1.51)	**1.96 (1.57, 2.45)**	**2.07 (1.66, 2.58)**	0.89 (0.72, 1.11)
Birth weight	**1.54 (1.16, 2.05)**	**1.61 (1.22, 2.14)**	1.22 (0.98, 1.51)	**1.97 (1.57, 2.46)**	**2.08 (1.67, 2.60)**	0.89 (0.72, 1.11)
Breastfed ever	**1.54 (1.16, 2.04)**	**1.61 (1.21, 2.13)**	1.23 (0.99, 1.53)	**1.96 (1.57, 2.44)**	**2.09 (1.67, 2.60)**	0.90 (0.73, 1.12)
Day care attendance	**1.54 (1.16, 2.04)**	**1.61 (1.21, 2.13)**	1.23 (0.98, 1.54)	**1.96 (1.57, 2.44)**	**2.07 (1.65, 2.61)**	0.90 (0.72, 1.13)
Body mass index at age 10–13 months	**1.56 (1.18, 2.06)**	**1.59 (1.20, 2.10)**	**1.24 (1.00, 1.54)**	**1.95 (1.56, 2.43)**	**2.00 (1.60, 2.50)**	0.90 (0.72, 1.11)
Wheezing until age 4 years	**1.50 (1.13, 2.00)**	**1.54 (1.16, 2.05)**	1.24 (1.00, 1.54)	**1.95 (1.56, 2.44)**	**2.03 (1.63, 2.53)**	0.84 (0.68, 1.05)
Environmental risk factors – all	**1.53 (1.14, 2.04)**	**1.47 (1.11, 1.96)**	1.21 (0.95, 1.53)	**1.86 (1.47, 2.36)**	**1.92 (1.52, 2.42)**	0.83 (0.66, 1.04)
Genetic risk factors	1.44 (0.99, 2.10)	**1.61 (1.11, 2.33)**	1.22 (0.93, 1.61)	**2.20 (1.68, 2.88)**	**2.28 (1.72, 3.02)**	0.89 (0.67, 1.18)
All risk factors	1.41 (0.95, 2.09)	1.40 (0.96, 2.04)	1.21 (0.88, 1.67)	**2.03 (1.53, 2.70)**	**2.07 (1.52, 2.82)**	0.82 (0.61, 1.10)

Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. Reference group is children of Dutch origin (n = 3,756). The crude model is unadjusted. The environmental model (all environmental risk factors) is adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index (BMI) at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, breastfeeding, day care attendance, BMI at age 10–13 months and wheezing. The genetic model (genetic risk factors) is adjusted for filaggrin genotype. The full model (all risk factors) is adjusted for both environmental and genetic risk factors.

we adjusted for genetic risk factors, we observed that only the effect estimate for the association of Cape Verdean origin with eczema became non-significant. After adjustment for both environmental and genetic risk factors, our results showed that only Surinamese-Creole and Surinamese-Hindustani children had increased risks of eczema, compared with Dutch children. Effect estimates did not materially change when wheezing was excluded from the models (data not shown).

DISCUSSION

We observed in a large multi-ethnic population-based prospective cohort that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children had overall increased risks of eczema in the first 4 years of life, compared with Dutch children. Only Surinamese-Creole and Surinamese-Hindustani children had increased risks of eczema after adjustment for both environmental and genetic risk factors.

Comparison of main findings with other studies

Previous birth cohort studies from the USA showed that black and Asian children had up to 2.58-fold increased risks of eczema[17, 18], compared with Caucasian children. In line with these studies, we observed that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children had increased risks of eczema, compared with Dutch children. European cross-sectional migrant studies demonstrated lower risks of eczema in Moroccan and Turkish than in Caucasian children.[5–7] We found no differences in risk of eczema between these children. This discrepancy might be explained by differences in the definition of ethnic origin, the method of eczema measurement, and the age at which eczema was measured. In our previous study, we observed that Moroccan and Surinamese children more frequently had eczema in the first year of life, compared with Dutch children.[8] In the second year of life, Surinamese children more frequently and Turkish children less frequently had eczema. In the current study, we examined associations of ethnic origin with eczema until age 4 years, took correlations between repeated measurements of eczema within the same child into account, and explored the role of known environmental and genetic risk factors. We observed that Dutch Antillean children had an increased risk of eczema at age 2 years, and Surinamese-Creole and Surinamese-Hindustani children at ages 6 months to 4 years. Moroccan and Turkish children had similar overall risks of eczema and similar risks of eczema per year, compared with Dutch children. Results for these children seemed inconsistent, and, therefore, need to be replicated before any conclusion can be drawn. In our cohort, the overall carrier frequency of *FLG* mutations was 7.8%, which is consistent with previously reported frequencies in European cohorts (range 6.6–13.5%).[19] Carrier frequencies of *FLG* mutations in children of non-Dutch origin was low. Furthermore, our results did

not materially change when *FLG* genotype was taken into account. This suggests that different mutations of the *FLG* gene and other genes might underlie the risk of the development of eczema in these children.[11]

Interpretation of results

Our results showed that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children had increased risks of eczema in the first 4 years of life, compared with Dutch children. Effect estimates for the association of Cape Verdean origin with eczema became non-significant after adjustment for genetic risk factors, and of Dutch Antillean origin with eczema after adjustment for both environmental and genetic risk factors. The largest change in effect size for the associations of Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani origin occurred when maternal education and psychiatric symptoms during pregnancy were added to the crude model separately. Mothers of Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children were lower educated and had higher psychiatric symptom scores during pregnancy, compared with mothers of Dutch children. However, precise quantification of attribution of each risk factor separately to the association of ethnic origin with eczema is difficult due to collinearity of environmental and genetic risk factors. Differences in the overall risk of eczema for children of different ethnic origin may result from residual, unmeasured variation in hygiene levels[20], staphylococcal colonization[21], sun exposure[22], vitamin D levels[23], and genetic factors.[11, 24] Also, functional and structural differences in skin barrier properties, such as transepidermal water loss[25], natural moisturizing factor[16], and stratum corneum lipid composition[26], between children of different ethnic origin might have influenced the results. Recently, it was observed that skin barrier characteristics in adults of African American, Caucasian, and East Asian descent differ.[27] East Asian skin seemed to have the most fragile skin barrier, but without dryness and scaliness due to high levels of stratum corneum lipids. African American skin had a stronger skin barrier, but dry skin due to low levels of stratum corneum lipids. A dry skin is one of the hallmarks of eczema. We demonstrated that children of Dutch and non-Dutch origin had different carrier frequencies of *FLG* mutations (10.0% vs. 1.4%). Recently, it was found in a European population that 2282del4, R2447X, R501X and S3247X mutations accounted for > 90% of the *FLG* mutation spectrum, while in an Asian population this spectrum contained 11 other *FLG* mutations.[11] Further studies are needed to examine the underlying genetic mechanisms of other *FLG* mutations for the association of ethnic origin with the development of eczema.

Strengths and limitations

The major strengths of this study are the use of a population-based prospective study design with a large number of children of different ethnic origin, detailed information

on eczema, and multiple known environmental and genetic risk factors. Our results could be applied to a general population of children of different ethnic origin. However, some methodological limitations should be considered. First, selection bias in longitudinal studies mainly arises due to subjects that are lost to follow-up, which was 10% in our study. Non-included subjects were more often of non-Dutch origin and non-affluent background, and were less healthy. Loss to follow-up might have led to bias if the associations of ethnic origin with eczema were different between those included and not included in the study. This we do not know. Nearly 74% of the participating children was of Dutch origin and the number of children in some of the non-Dutch ethnic groups was small. This may have underpowered our results. Second, the ethnic origin of the child was defined according to the Dutch standard classification.[13] This classification is objective, reproducible and easily applicable in epidemiological studies. However, some misclassification might have occurred since third-generation migrants were defined as being of Dutch origin but might still have a different skin structure. This may have reduced the contrast between children of Dutch origin and other ethnic groups, and underestimated our effect estimates. Third, information on eczema was obtained by parental questionnaires, consisting widely accepted and commonly used questions that reliably reflect the prevalence of eczema in young children at the population level.[14, 28] Furthermore, self-reported diagnosis of eczema in the past year based on a single question demonstrates sufficient validity for the epidemiological study of childhood eczema.[29] Nonetheless, we cannot rule out that parental reporting of eczema might be different among ethnic groups due to language difficulties and cultural differences, such as different attitudes toward health care utilization and different reporting of symptoms.[30] We had no additional information on eczema from medical records or physical examinations. Fourth, we did not have data on other important environmental factors and possible intermediating factors, such as housing conditions, air pollution, and microbial exposure.[9] In addition, environmental risk factors such as maternal BMI and history of allergy, eczema or asthma and child's birth weight, BMI and wheezing have a genetic component of their own.[31–33] These genetic components may also partly explain associations of environmental risk factors with eczema. Therefore, changing environmental factors might not be sufficient to lower the risk of childhood eczema. Fifth, we only genotyped four *FLG* mutations. Despite other known loci associated with eczema[34], *FLG* mutations are known to be most prevalent in Caucasian populations and associations of these mutations with eczema in children have been replicated in many studies.[10] Furthermore, the *FLG* gene is the only gene of which its function in relation with eczema has been studied. An *in vitro* study showed that knockout of *FLG* gene expression extensively alters keratinocyte differentiation and integrity and function of the stratum corneum.[35] In non-Caucasian populations, specifically those born in the host country as in our study, the prevalence of other mutations and their role in the associa-

tions of ethnic origin with the risk of childhood eczema is less clear[36], and remains to be studied. Children without any mutant alleles were classified as wild-type, although their carrier status of other non-genotyped *FLG* mutations was unknown. Further studies are needed to explore the role of other mutations, which might be more prevalent in children of non-Dutch origin, in the association of ethnic origin with the development of childhood eczema. Last, we considered possible collider bias resulting from adjustment for child's wheezing. This seems unlikely because effect estimates did not materially change when wheezing was excluded from the models.

In conclusion, we observed that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children had increased risks of eczema in the first 4 years of life. Only the associations of Surinamese-Creole and Surinamese-Hindustani origin with eczema remained present when environmental and genetic risk factors were taken into account. Further studies are needed to explore the possible underlying mechanisms and the clinical relevance of our findings.

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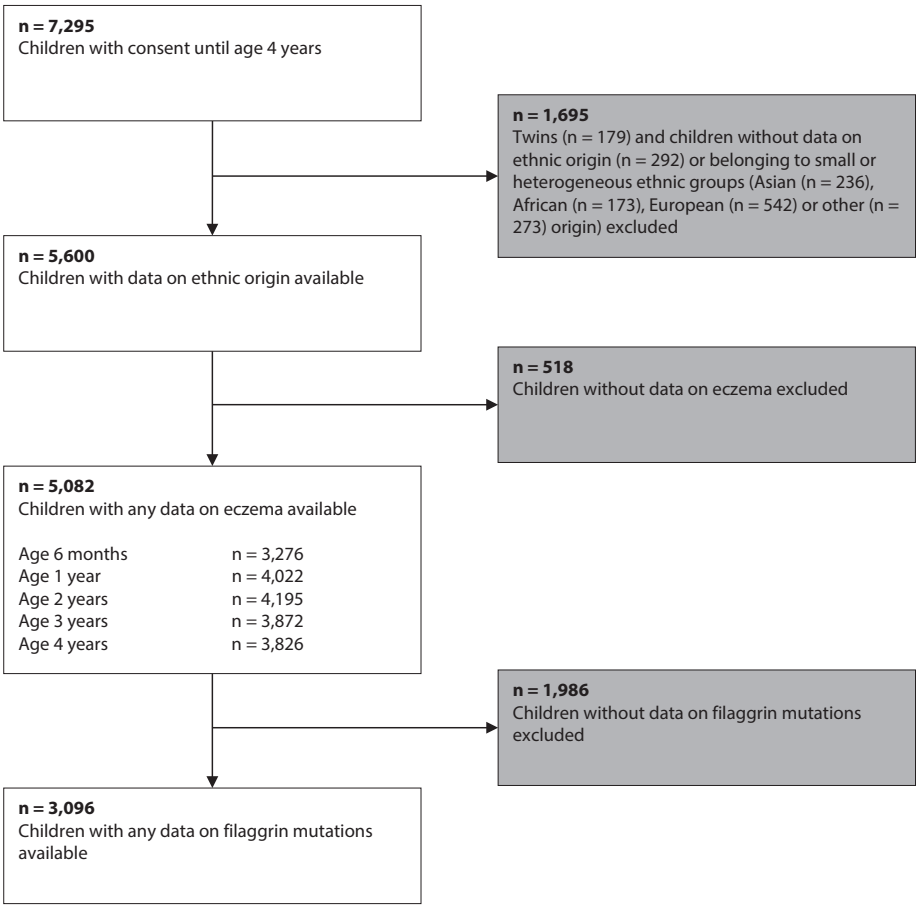
SUPPLEMENTARY MATERIAL

Supplementary Figure. Flowchart of participants.



Supplementary Table 1. Characteristics of mothers and their children (n = 5,082).

	Observed	Imputed
Maternal characteristics		
Age at enrollment (years)*	30.9 (4.9)	30.9 (4.9)
Missing	*0 (0)*	*0 (0)*
Education (%)		
Primary or secondary	49.3 (2,406)	50.4 (2,559)
Higher	50.7 (2,479)	49.6 (2,523)
Missing	*3.9 (197)*	*0 (0)*
History of allergy, eczema or asthma (%)		
No	61.4 (2,623)	62.2 (3,163)
Yes	38.6 (1,652)	37.8 (1,919)
Missing	*15.9 (807)*	*0 (0)*
Parity (%)		
0	56.8 (2,809)	55.8 (2,837)
≥ 1	43.2 (2,136)	44.2 (2,245)
Missing	*2.7 (137)*	*0 (0)*
Pet keeping during pregnancy (%)		
No	65.3 (2,653)	64.0 (3,253)
Yes	34.7 (1,409)	36.0 (1,829)
Missing	*20.1 (1,020)*	*0 (0)*
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.6)	23.8 (18.8–35.9)
Missing	*9.4 (480)*	*0 (0)*
Smoking during pregnancy (%)		
No	76.0 (3,502)	76.0 (3,860)
Yes	24.0 (1,105)	24.0 (1,222)
Missing	*9.4 (475)*	*0 (0)*
Psychiatric symptoms during pregnancy†	0.13 (0–1.31)	0.15 (0–1.38)
Missing	*21.8 (1,108)*	*0 (0)*
Child characteristics		
Sex (%)		
Male	50.3 (2,556)	50.3 (2,556)
Female	49.7 (2,526)	49.7 (2,526)
Missing	*0 (0)*	*0 (0)*
Gestational age at birth (weeks)†	40.1 (36.0–42.3)	40.1 (36.0–42.3)
Missing	*0.3 (14)*	*0 (0)*
Birth weight (grams)*	3,455 (556)	3,455 (556)
Missing	*0.1 (3)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers and their children (n = 5,082). (continued)

	Observed	Imputed
Breastfed ever (%)		
No	8.6 (416)	8.8 (449)
Yes	91.4 (4,431)	91.2 (4,633)
Missing	*4.6 (235)*	*0 (0)*
Day care attendance until age 1 year (%)		
No	40.6 (1,513)	44.4 (2,256)
Yes	59.4 (2,214)	55.6 (2,826)
Missing	*26.6 (1,355)*	*0 (0)*
Body mass index at age 10–13 months (kg/m^2)†	17.4 (14.8–20.4)	17.4 (14.9–20.4)
Missing	*20.2 (1,027)*	*0 (0)*
Wheezing until age 4 years (%)		
No	51.8 (1,685)	54.5 (2,771)
Yes	48.2 (1,566)	45.5 (2,311)
Missing	*36.0 (1,831)*	*0 (0)*
Ethnic origin (%)		
Dutch	73.9 (3,756)	73.9 (3,756)
Cape Verdean	2.5 (125)	2.5 (125)
Dutch Antillean	3.1 (156)	3.1 (156)
Moroccan	5.8 (295)	5.8 (295)
Surinamese-Creole	3.3 (168)	3.3 (168)
Surinamese-Hindustani	2.9 (147)	2.9 (147)
Turkish	8.5 (435)	8.5 (435)
Missing	*0 (0)*	*0 (0)*
Eczema last 6 months at age 6 months (%)		
No	83.8 (2,746)	82.6 (4,198)
Yes	16.2 (530)	17.4 (884)
Missing	*35.5 (1,806)*	*0 (0)*
Eczema last 6 months at age 1 year (%)		
No	87.4 (3,515)	86.6 (4,402)
Yes	12.6 (507)	13.4 (680)
Missing	*20.9 (1,060)*	*0 (0)*
Eczema last 12 months at age 2 years (%)		
No	86.5 (3,598)	86.0 (4,369)
Yes	13.5 (561)	14.0 (713)
Missing	*18.2 (923)*	*0 (0)*

Supplementary Table 1. Characteristics of mothers and their children (n = 5,082). (continued)

	Observed	Imputed
Eczema last 12 months at age 3 years (%)		
No	91.1 (3,527)	90.1 (4,578)
Yes	8.9 (345)	9.9 (504)
Missing	*23.8 (1,210)*	*0 (0)*
Eczema last 12 months at age 4 years (%)		
No	92.2 (3,527)	91.1 (4,631)
Yes	7.8 (299)	8.9 (451)
Missing	*24.7 (1,256)*	*0 (0)*

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed and imputed data.

Supplementary Table 2. Characteristics of mothers and children included and not included in the study.

	Included n = 5,082	Not included n = 518	*P*-value for difference
Maternal characteristics			
Age at enrollment (years)*	30.9 (4.9)	27.6 (5.7)	**< 0.001**
Missing	*0 (0)*	*0.02 (1)*	
Education (%)			**< 0.001**
Primary or secondary	49.3 (2,406)	84.7 (388)	
Higher	50.7 (2,479)	15.3 (70)	
Missing	*3.9 (197)*	*11.6 (60)*	
History of allergy, eczema or asthma (%)			0.36
No	61.4 (2,623)	59.0 (236)	
Yes	38.6 (1,652)	41.0 (164)	
Missing	*15.9 (807)*	*22.8 (118)*	
Parity (%)			**< 0.001**
0	56.8 (2,809)	43.9 (223)	
≥ 1	43.2 (2,136)	56.1 (285)	
Missing	*2.7 (137)*	*1.9 (10)*	
Pet keeping during pregnancy (%)			**< 0.001**
No	65.3 (2,653)	74.5 (306)	
Yes	34.7 (1,409)	25.5 (105)	
Missing	*20.1 (1,020)*	*20.6 (107)*	
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.6)	24.4 (18.6–37.4)	**< 0.001**
Missing	*9.4 (480)*	*4.6 (24)*	
Smoking during pregnancy (%)			**< 0.001**
No	76.0 (3,502)	64.7 (292)	
Yes	24.0 (1,105)	35.3 (159)	
Missing	*9.4 (475)*	*12.9 (67)*	

Supplementary Table 2. Characteristics of mothers and children included and not included in the study. (continued)

	Included n = 5,082	Not included n = 518	*P*-value for difference
Psychiatric symptoms during pregnancy†	0.13 (0–1.31)	0.33 (0.02–2.07)	**< 0.001**
Missing	*21.8 (1,108)*	*42.9 (222)*	
Child characteristics			
Sex (%)			**< 0.05**
Male	50.3 (2,556)	56.1 (290)	
Female	49.7 (2,526)	43.9 (227)	
Missing	*0 (0)*	*0.2 (1)*	
Gestational age at birth (weeks)†	40.1 (36.0–42.3)	40.0 (35.5–42.3)	**< 0.05**
Missing	*0.3 (14)*	*0.4 (2)*	
Birth weight (grams)*	3,455 (556)	3,334 (546)	**< 0.001**
Missing	*0.1 (3)*	*0.4 (2)*	
Breastfed ever (%)			0.68
No	8.6 (416)	9.2 (36)	
Yes	91.4 (4,431)	90.8 (356)	
Missing	*4.6 (235)*	*24.3 (126)*	
Day care attendance until age 1 year (%)			**< 0.05**
No	40.6 (1,513)	84.6 (11)	
Yes	59.4 (2,214)	15.4 (2)	
Missing	*26.6 (1,355)*	*97.5 (505)*	
Body mass index at age 10–13 months (kg/m^2)†	17.4 (14.8–20.4)	17.5 (14.8–20.8)	0.19
Missing	*20.2 (1,027)*	39.8 (206)	
Wheezing until age 4 years (%)			0.07
No	51.8 (1,685)	0 (0)	
Yes	48.2 (1,566)	100.0 (3)	
Missing	*36.0 (1,831)*	*99.4 (515)*	
Ethnic origin (%)			**< 0.001**
Dutch	73.9 (3,756)	34.9 (181)	
Cape Verdean	2.5 (125)	12.2 (63)	
Dutch Antillean	3.1 (156)	8.1 (42)	
Moroccan	5.8 (295)	22.4 (116)	
Surinamese-Creole	3.3 (168)	7.0 (36)	
Surinamese-Hindustani	2.9 (147)	5.2 (27)	
Turkish	8.5 (435)	10.2 (53)	
Missing	*0 (0)*	*0 (0)*	

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed data. *P*-values for difference are calculated by independent samples T-test for continuous variables with a normal distribution, the Mann-Whitney U-test for continuous variables with a skewed distribution, and Pearson's Chi-square test for categorical variables. Bold values indicate statistical significance at the $\alpha = 0.05$ level.

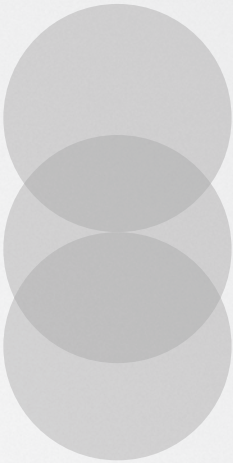
Supplementary Table 3. Associations of ethnic origin with eczema per year and overall in children until age 4 years.

	Odds ratio (95% confidence interval) for eczema					
	6 months	**1 year**	**2 years**	**3 years**	**4 years**	**Overall**
Dutch (n = 3,756)	Reference	Reference	Reference	Reference	Reference	Reference
Cape Verdean						
Crude model (n = 125)	1.32 (0.66, 2.65)	1.18 (0.69, 2.03)	**1.84 (1.08, 3.14)**	1.61 (0.85, 3.07)	1.82 (0.94, 3.52)	**1.53 (1.15, 2.03)**
Environmental model (n = 125)	1.32 (0.66, 2.63)	1.18 (0.68, 2.06)	**1.85 (1.07, 3.18)**	1.61 (0.84, 3.12)	1.82 (0.92, 3.58)	**1.53 (1.14, 2.04)**
Genetic model (n = 72)	1.11 (0.53, 2.34)	1.12 (0.54, 2.32)	1.93 (1.00, 3.73)	1.79 (0.79, 4.05)	1.45 (0.49, 4.32)	1.44 (0.99, 2.10)
Full model (n = 72)	1.08 (0.51, 2.30)	1.10 (0.52, 2.30)	1.89 (0.96, 3.73)	1.76 (0.77, 4.04)	1.42 (0.47, 4.27)	1.41 (0.95, 2.09)
Dutch Antillean						
Crude model (n = 156)	1.00 (0.60, 1.66)	1.55 (0.90, 2.66)	**1.95 (1.20, 3.16)**	1.76 (0.93, 3.33)	**2.21 (1.01, 4.82)**	**1.60 (1.21, 2.12)**
Environmental model (n = 156)	0.91 (0.54, 1.55)	1.42 (0.81, 2.49)	**1.80 (1.10, 2.93)**	1.62 (0.86, 3.08)	2.04 (0.94, 4.43)	**1.47 (1.11, 1.96)**
Genetic model (n = 93)	0.79 (0.37, 1.68)	1.26 (0.58, 2.76)	**2.23 (1.25, 3.99)**	1.80 (0.68, 4.76)	**2.55 (1.03, 6.35)**	**1.61 (1.11, 2.33)**
Full model (n = 93)	0.68 (0.32, 1.48)	1.09 (0.49, 2.45)	**1.96 (1.09, 3.51)**	1.56 (0.59, 4.14)	2.23 (0.89, 5.56)	1.40 (0.96, 2.04)
Moroccan						
Crude model (n = 295)	1.37 (0.90, 2.08)	1.14 (0.71, 1.81)	0.72 (0.45, 1.16)	1.12 (0.66, 1.91)	**1.80 (1.13, 2.88)**	1.22 (0.98, 1.51)
Environmental model (n = 295)	1.36 (0.88, 2.11)	1.12 (0.70, 1.79)	0.71 (0.42, 1.17)	1.10 (0.64, 1.91)	**1.79 (1.12, 2.86)**	1.21 (0.95, 1.53)
Genetic model (n = 172)	1.40 (0.86, 2.28)	1.22 (0.71, 2.10)	0.63 (0.33, 1.20)	1.14 (0.59, 2.21)	1.82 (0.98, 3.37)	1.22 (0.93, 1.61)
Full model (n = 172)	1.39 (0.82, 2.36)	1.21 (0.69, 2.11)	0.62 (0.31, 1.21)	1.13 (0.57, 2.24)	1.81 (0.96, 3.42)	1.21 (0.88, 1.67)
Surinamese-Creole						
Crude model (n = 168)	**1.89 (1.21, 2.95)**	**1.82 (1.13, 2.93)**	**2.25 (1.51, 3.36)**	1.50 (0.85, 2.66)	**2.24 (1.37, 3.67)**	**1.95 (1.56, 2.44)**
Environmental model (n = 168)	**1.81 (1.14, 2.86)**	**1.74 (1.07, 2.82)**	**2.15 (1.43, 3.24)**	1.43 (0.80, 2.56)	**2.14 (1.30, 3.53)**	**1.86 (1.47, 2.36)**
Genetic model (n = 99)	**2.19 (1.27, 3.75)**	**2.04 (1.17, 3.58)**	**2.70 (1.63, 4.45)**	1.68 (0.84, 3.34)	**2.22 (1.12, 4.40)**	**2.20 (1.68, 2.88)**
Full model (n = 99)	**2.02 (1.15, 3.55)**	**1.88 (1.06, 3.34)**	**2.50 (1.50, 4.18)**	1.54 (0.77, 3.11)	**2.05 (1.03, 4.07)**	**2.03 (1.53, 2.70)**

Supplementary Table 3. Associations of ethnic origin with eczema per year and overall in children until age 4 years. (continued)

	Odds ratio (95% confidence interval) for eczema					
	6 months	**1 year**	**2 years**	**3 years**	**4 years**	**Overall**
Surinamese-Hindustani						
Crude model (n = 435)	**1.60 (1.02, 2.51)**	**2.23 (1.40, 3.56)**	**1.88 (1.17, 3.00)**	**2.34 (1.37, 4.00)**	**2.67 (1.55, 4.61)**	**2.06 (1.65, 2.57)**
Environmental model (n = 435)	1.49 (0.93, 2.37)	**2.09 (1.30, 3.37)**	**1.75 (1.10, 2.79)**	**2.19 (1.27, 3.76)**	**2.50 (1.43, 4.36)**	**1.92 (1.52, 2.42)**
Genetic model (n = 88)	**2.09 (1.18, 3.69)**	**2.79 (1.61, 4.83)**	**2.00 (1.12, 3.56)**	1.81 (0.85, 3.87)	**2.76 (1.41, 5.40)**	**2.28 (1.72, 3.02)**
Full model (n = 88)	**1.90 (1.05, 3.44)**	**2.56 (1.45, 4.50)**	**1.82 (1.02, 3.24)**	1.64 (0.74, 3.59)	**2.51 (1.24, 5.06)**	**2.07 (1.52, 2.82)**
Turkish						
Crude model (n = 435)	0.89 (0.61, 1.28)	0.76 (0.51, 1.13)	**0.65 (0.43, 0.98)**	1.29 (0.84, 1.97)	1.01 (0.50, 2.06)	0.89 (0.72, 1.10)
Environmental model (n = 435)	0.82 (0.56, 1.21)	0.71 (0.47, 1.07)	**0.60 (0.39, 0.92)**	1.20 (0.77, 1.88)	0.94 (0.47, 1.91)	0.83 (0.66, 1.04)
Genetic model (n = 250)	0.94 (0.59, 1.51)	0.66 (0.38, 1.14)	0.69 (0.40, 1.17)	1.38 (0.81, 2.34)	0.95 (0.39, 2.31)	0.89 (0.67, 1.18)
Full model (n = 250)	0.86 (0.53, 1.41)	0.60 (0.34, 1.07)	0.63 (0.37, 1.08)	1.27 (0.74, 2.18)	0.88 (0.36, 2.12)	0.82 (0.61, 1.10)

Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Bold values indicate statistical significance at the $\alpha = 0.05$ level. The crude model is unadjusted. The environmental model (all environmental risk factors) is adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index (BMI) at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, breastfeeding, day care attendance, BMI at age 10–13 months and wheezing. The genetic model (genetic risk factors) is adjusted for filaggrin genotype. The full model (all risk factors) is adjusted for both environmental and genetic risk factors. Missing data of filaggrin mutations in the genetic and full model (n = 1,986).



CHAPTER 3.2

DURATION AND EXCLUSIVENESS OF BREASTFEEDING AND ECZEMA, ALLERGIC SENSITIZATION AND ALLERGY IN SCHOOL-AGE CHILDREN

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ABSTRACT

Background Breastfeeding may have immune modulatory effects that influence the development of childhood allergic sensitization and atopic diseases. We aimed to examine the associations of breastfeeding with childhood allergic sensitization, inhalant or food allergy and eczema, and whether any association was affected by disease-related modification of the exposure or modified by maternal history of allergy, eczema or asthma.

Methods This study among 5,828 children was performed in a population-based prospective cohort from fetal life onwards. We collected information on duration (< 2 months, 2–4 months, 4–6 months and ≥ 6 months) and exclusiveness (non-exclusive vs. exclusive for 4 months) of breastfeeding in infancy by postal questionnaires. At age 10 years, inhalant and food allergic sensitization were measured by skin prick tests, and physician-diagnosed inhalant and food allergy by a postal questionnaire. Data on parental-reported eczema were available from birth until age 10 years.

Results We observed no association of breastfeeding with any allergic sensitization, physician-diagnosed allergy, or combination of these outcomes. Shorter breastfeeding duration was associated with an overall increased risk of eczema (*p*-value for trend < 0.05). Non-exclusively breastfed children had an overall increased risk of eczema (adjusted odds ratio (95% confidence interval): 1.11 (1.01, 1.23)), compared with children exclusively breastfed for 4 months. Risk period-specific sensitivity analyses, additional adjustment for ointment use for eczema at age 2 months, and cross-lagged modeling showed no consistent results for disease-related modification of the exposure. Results were not modified by maternal history of allergy, eczema or asthma (*p*-value for interaction > 0.05).

Conclusions Shorter duration or non-exclusiveness of breastfeeding is associated with a weak overall increased risk of eczema but not allergic sensitization or physician-diagnosed allergy at age 10 years.

INTRODUCTION

Breastfeeding may affect the development of childhood allergic sensitization and atopic diseases such as allergy and eczema.[1] Underlying biological mechanisms are not fully clear but might include various components of human milk that modulate the child's immune system and alter the balance between pro-inflammatory and anti-inflammatory signals.[1, 2] Also, human milk oligosaccharides are suggested to influence the development of atopic diseases by modulating gut microbiota diversity.[2, 3] Previous birth cohort studies showed that breastfeeding was not consistently associated with allergic sensitization measured by skin prick tests.[4–8] A recent meta-analysis of observational studies showed that longer duration and exclusiveness of breastfeeding were associated with an up to 26% decreased risk of allergic rhinitis and eczema in preschool-age children, but not with food allergy.[9] Furthermore, results from a cluster-randomized controlled trial among 13,889 children showed that children of mothers who were promoted to breastfeed longer and more exclusively did not have reduced prevalences of inhalant allergic sensitization, allergic rhinitis and eczema until age 6.5 years, compared with children of mothers who were not promoted.[10] However, the effects of breastfeeding on allergic sensitization and atopic diseases at older age are less known.[4, 6] Also, the effects of disease-related modification of the exposure, meaning that early symptoms of allergy or eczema in the child may encourage a mother to alter breastfeeding habits[11], and the modifying effects of maternal history of allergy, eczema or asthma on the associations of breastfeeding with atopic diseases in school-age children are less clear.[5, 6, 8]

Therefore, we aimed to examine the associations of duration and exclusiveness of breastfeeding with allergic sensitization, inhalant or food allergy at age 10 years, and eczema from birth until age 10 years among 5,828 children and their mothers participating in a population-based prospective cohort study. Additionally, we examined whether any association was affected by altered maternal breastfeeding habits due to early symptoms of allergy or eczema in the child or modified by maternal history of allergy, eczema or asthma.

METHODS

General design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards.[12] The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, The Netherlands (MEC-2012–165). Written informed consent was obtained from both parents or legal representatives. For the current study, twins (n = 208), and children without data on breastfeeding (n = 2,111), and allergic sensitization, allergy and eczema (n = 297) were excluded, leaving a total of 5,828 children for the analyses (Supplementary Figure 1).

Breastfeeding duration and exclusiveness

Detailed information on breastfeeding initiation and continuation was obtained from parental postal questionnaires at ages 2, 6 and 12 months. Mothers were asked whether they ever breastfed their child (no; yes) and at what age (in months) of the child they stopped breastfeeding. Of mothers that started breastfeeding, duration of breastfeeding was categorized into four groups: '< 2 months', '2–4 months', '4–6 months', and '≥ 6 months'.[13] Exclusiveness of breastfeeding was defined using information on the introduction of milk or solids, and categorized into 'non-exclusive breastfeeding for 4 months' and 'exclusive breastfeeding for 4 months'. Analyses that focused on breastfeeding duration and exclusiveness were performed among children that were ever breastfed.

Allergic sensitization, allergy and eczema

Children visited the research center at a median age of 9.7 years (2.5–97.5th percentile: 9.3–10.6). Inhalant and food allergic sensitization (no; yes) to house dust mite, 5-grass mixture, birch, cat and dog (ALK-Abelló B.V., Almere, The Netherlands), and hazelnut, cashew nut, peanut and peach were measured by skin prick tests using the scanned area method.[14] We used a positive control (histamine dihydrochloride 10 mg/mL) in duplicate and a negative control (sodium chloride 9 mg/mL). Skin responses were considered positive if the area of the wheal was ≥ 40% of the histamine response (i.e., histamine equivalent prick index area ≥ 0.40).[14] The scanned area method does not require adjustment for the negative control. However, children without a negative control or with any skin response to the negative control were omitted from the analyses. Questions adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires provided information on physician-diagnosed inhalant ("Was your child ever diagnosed with an allergy to pollen (hay fever)/house dust mite/cat/dog?") (no; yes) and food ("Was your child ever diagnosed with an allergy to cashew nut/peanut?") (no; yes) allergy at age 10 years.[15] We further combined allergic sensitization and physician-diagnosed allergy into groups of 'no allergic sensitization and no allergy', 'any allergic sensitization, but no allergy', 'no allergic sensitization, but any allergy', and 'any allergic sensitization and any allergy'. Physician-diagnosed eczema was parental-reported at ages 6 months and 1, 2, 3, 4 and 10 years ("Was your child diagnosed with eczema in the last 6 months/last year?") (no; yes).

Covariates

Information on maternal education (primary or secondary; higher), history of allergy, eczema or asthma (no; yes), parity (nulliparous; multiparous), pet keeping (no; yes) and body mass index (BMI) was obtained by postal questionnaires completed by the mother at enrollment. Information on maternal smoking was obtained by questionnaires multiple times during pregnancy and combined (no; yes). We assessed maternal psychiatric

symptoms in the second trimester of pregnancy using the Global Severity Index of the Brief Symptom Inventory[16], denoting overall psychiatric symptoms. Information on child's sex, gestational age at birth and birth weight was obtained from obstetric and midwife records at birth. We based ethnic origin (European; non-European) of the child on the country of birth of the parents.[17] We obtained information on ointment use for eczema (no; yes) and day care attendance (no; yes) by questionnaires at ages 2 months and 1 year, respectively.

Statistical analysis

We compared characteristics of children included and not included using independent samples T-tests, Mann-Whitney U-tests, and Pearson's Chi-square tests. We used logistic regression or multinomial logistic regression models to examine the associations of duration and exclusiveness of breastfeeding with the risk of allergic sensitization and physician-diagnosed allergy or combined allergic sensitization and allergy groups, respectively, at age 10 years. We used generalized estimating equation models to examine the associations of duration and exclusiveness of breastfeeding with the longitudinal odds of eczema at ages 6 months and 1, 2, 3, 4 and 10 years independently and overall, taking into account correlations between repeated measurements of eczema within the same child. Confounders were included in the models based on literature, if they were associated with both the determinant and the outcome, or if they changed the effect estimates with ≥ 10% in bivariate analyses. Analyses with inhalant or food allergic sensitization or allergy as the outcomes were mutually adjusted for each other. Tests for trends were performed by including the categorized breastfeeding duration as a continuous variable in the models. We performed risk period-specific sensitivity analyses by excluding children who developed eczema during the period of breastfeeding until age 6 months (n = 962) and additional adjustment for ointment use for eczema at age 2 months to account for possible altered maternal breastfeeding habits due to early eczema in the child. We applied an adjusted cross-lagged model to examine bidirectional associations of breastfeeding with eczema.[18, 19] More detailed information on cross-lagged modeling is provided in the Supplementary Methods. For these analyses, ointment use for eczema at age 2 months was used as a proxy for the presence of eczema at age 2 months. The modifying effect of maternal history of allergy, eczema or asthma and the time-varying effect of age at eczema measurement were tested by adding them as product terms with the breastfeeding variables in the models. Missing data of covariates and eczema were multiple-imputed to reduce potential bias and improve efficiency (Supplementary Table 1). We assumed that missing data were missing at random.[20] The best indicator for the presence or absence of eczema is an eczema measurement at a different age. Therefore, at least one eczema measurement was available in our population for analysis to predict other eczema measurements. Because we lacked repeated

measurements on allergic sensitization and physician-diagnosed allergy, we did not impute missing data of these outcomes. The size or direction of the effect estimates did not materially differ between analyses with imputed data and complete cases only (data not shown). Therefore, we present results based on imputed analyses only. Measures of association are presented as adjusted odds ratios (aOR) with their 95% confidence intervals (CI). Statistical analyses were performed using SPSS 21.0.0.1 (IBM Corp., Armonk, NY, USA), SAS 9.3 (SAS Institute Inc., Cary, NC, USA), and Mplus 7.11 for Windows (Muthén & Muthén, Los Angeles, CA, USA).

RESULTS

General

Maternal and child characteristics are presented in Table 1 and Supplementary Table 2. Of the children, 92.1% (n = 5,365) was ever breastfed, 25.6% (n = 1,182) was breastfed for < 2 months, 21.2% (n = 980) for 2–4 months, 11.8% (n = 547) for 4–6 months, 31.4% (n = 1,449) for ≥ 6 months, and 25.2% (n = 1,201) exclusively breastfed for 4 months. Inhalant or food allergic sensitization was present in 32.1% (n = 1,085) and 6.8% (n = 230) of the children at age 10 years, respectively. Physician-diagnosed inhalant or food allergy was present in 12.1% (n = 467) and 2.3% (n = 87) of the children at age 10 years, respectively. The prevalence of eczema declined from 16.5% (n = 962) at age 6 months to 7.9% (n = 460) at age 10 years. Participants without follow-up data had younger, lower educated mothers who had higher parity and BMI at enrollment, smoked more during pregnancy, and had more psychiatric symptoms during pregnancy. Children were more often of non-European origin and attended day care more often than those included in the study (Supplementary Table 3).

Table 1. Characteristics of mothers and their children.

	n = 5,828
Maternal characteristics	
Education, higher (%)	51.8 (3,017)
History of eczema, allergy or asthma, yes (%)	38.2 (2,228)
Parity, ≥ 1 (%)	43.8 (2,553)
Pet keeping during pregnancy, yes (%)	35.1 (2,047)
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.7)
Smoking during pregnancy, yes (%)	23.5 (1,372)
Psychiatric symptoms during pregnancy†	0.15 (0–1.40)

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

	n = 5,828
Child characteristics	
Sex, female (%)	50.1 (2,919)
Gestational age at birth (weeks)†	40.1 (36.0–42.3)
Birth weight (grams)*	3,455 (548)
Ethnic origin, European (%)	68.8 (4,012)
Ointment use for eczema at age 2 months, yes (%)	8.0 (464)
Day care attendance until age 1 year, yes (%)	55.9 (3,259)
Breastfed ever, yes (%)	92.1 (5,365)
Breastfeeding duration (%)	
Never	10.0 (463)
< 2 months	25.6 (1,182)
2–4 months	21.2 (980)
4–6 months	11.8 (547)
≥ 6 months	31.4 (1,449)
Breastfeeding exclusiveness (%)	
Never	9.7 (463)
Non-exclusive for 4 months	65.0 (3,095)
Exclusive for 4 months	25.2 (1,201)
Allergic sensitization at age 10 years, yes (%)	
Inhalant	32.1 (1,085)
Food	6.8 (230)
Physician-diagnosed allergy at age 10 years, yes (%)	
Inhalant	12.1 (467)
Food	2.3 (87)
Allergic sensitization and allergy combined at age 10 years (%)	
No allergic sensitization and no allergy	66.3 (1,868)
Any allergic sensitization, but no allergy	22.6 (636)
No allergic sensitization, but any allergy	1.3 (36)
Any allergic sensitization and any allergy	9.9 (279)
Eczema, yes (%)	
Age 6 months	16.5 (962)
Age 1 year	13.2 (768)
Age 2 years	14.2 (825)
Age 3 years	10.1 (586)
Age 4 years	8.7 (508)
Age 10 years	7.9 (460)

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on imputed data. Data on breastfeeding duration and exclusiveness, allergic sensitizations and physician-diagnosed allergies are not imputed.

Breastfeeding and childhood atopic diseases

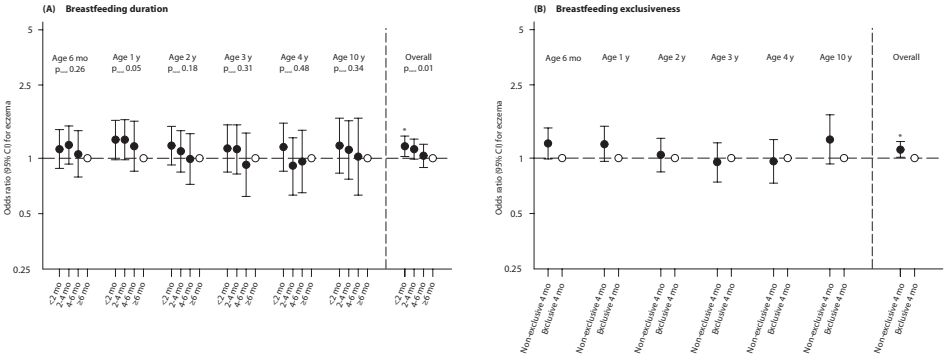
Never breastfeeding, duration and exclusiveness of breastfeeding were not associated with allergic sensitization, physician-diagnosed allergy or combined allergic sensitization and allergy groups (Table 2 and Supplementary Table 4). Never breastfeeding was not associated with eczema per year or overall, compared with ever breastfeeding (Supplementary Table 5). A shorter breastfeeding duration was associated with a higher overall risk of eczema (*p*-value for trend < 0.05) (Figure A and Supplementary Table 5). Specifically, children breastfed < 2 months had an overall increased risk of eczema (aOR (95% CI): 1.16 (1.02, 1.32)), compared with children breastfed ≥ 6 months. Children non-exclusively breastfed for 4 months had an overall increased risk of eczema (1.11 (1.01, 1.23), compared with children exclusively breastfed for 4 months. (Figure B and Supplementary Table 5). Sensitivity analyses excluding children who developed eczema during the period of breastfeeding until age 6 months showed that effect estimates for the associations of breastfeeding < 2 months and non-exclusive breastfeeding for 4 months with eczema overall attenuated to non-significant (1.10 (0.92, 1.32) and 0.99 (0.86, 1.14), respectively). Effect estimates did not materially change when we

Table 2. Associations of duration and exclusiveness of breastfeeding with allergic sensitizations and physician-diagnosed allergies in children at age 10 years.

	Odds ratio (95% confidence interval) for allergic sensitization		**Odds ratio (95% confidence interval) for physician-diagnosed allergy**	
	Inhalant n = 3,381	**Food n = 3,369**	**Inhalant n = 3,771**	**Food n = 3,846**
Breastfeeding				
Never (n = 463)	0.83 (0.60, 1.14)	0.83 (0.42, 1.66)	1.11 (0.73, 1.67)	0.83 (0.30, 2.29)
Ever (n = 5,365)	Reference	Reference	Reference	Reference
Breastfeeding duration				
< 2 months (n = 1,182)	1.02 (0.80, 1.30)	1.05 (0.62, 1.76)	1.28 (0.93, 1.77)	1.54 (0.71, 3.32)
2–4 months (n = 980)	1.14 (0.89, 1.46)	1.26 (0.75, 2.12)	0.94 (0.67, 1.33)	1.53 (0.69, 3.39)
4–6 months (n = 547)	1.22 (0.92, 1.63)	0.57 (0.29, 1.13)	0.97 (0.64, 1.46)	1.08 (0.39, 3.01)
≥ 6 months (n = 1,449)	Reference	Reference	Reference	Reference
P-value for trend	0.79	0.53	0.20	0.22
Breastfeeding exclusiveness				
Non-exclusive for 4 months (n = 3,095)	1.05 (0.86, 1.28)	1.38 (0.88, 2.14)	1.02 (0.78, 1.35)	0.76 (0.41, 1.40)
Exclusive for 4 months (n = 1,201)	Reference	Reference	Reference	Reference

Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Models are adjusted for maternal education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin and day care attendance, and mutually for inhalant and food allergic sensitization or allergy.

Figure. Associations of duration (A) and exclusiveness (B) of breastfeeding with eczema in children until age 10 years.



Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Reference group is children who were (A) breastfed ≥ 6 months or (B) exclusively breastfed for 4 months. Models are adjusted for maternal education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin and day care attendance. *P-value < 0.05. Mo = months; y = year(s).

additionally adjusted for ointment use for eczema at age 2 months (data not shown). Cross-lagged modeling showed that effect estimates for the associations of eczema or ointment use for eczema with breastfeeding, which were considered as associations in the opposite direction, were non-significant (Supplementary Figure 2). We observed no modifying effect of maternal history of allergy, eczema or asthma, and no time-varying effect of age at eczema measurement on the associations of breastfeeding with child's allergic sensitization, physician-diagnosed allergy, combined allergic sensitization and allergy groups or eczema (p-values for interaction > 0.05).

DISCUSSION

In this large prospective population-based study, we observed that shorter duration or non-exclusiveness of breastfeeding was associated with a weak overall increased risk of eczema, but not allergic sensitization or physician-diagnosed allergy at age 10 years.

Comparison of main findings with other studies

Previous birth cohort studies showed conflicting results on the association of breastfeeding with the risk of allergic inhalant or food sensitization.[4–8, 21] Recently, observational studies that examined the associations of breastfeeding with allergic rhinitis, food allergy and eczema were meta-analyzed.[9] Longer duration of breastfeeding was associated with a 21% decreased risk of allergic rhinitis, and exclusive breastfeeding for 3–4 months with a 26% decreased risk of eczema in children until age 5 and 2 years, re-

spectively, but not at older ages and effect estimates generally had high heterogeneity.[9] Our results suggest that shorter duration or non-exclusiveness of breastfeeding is associated with an overall increased risk of eczema until age 10 years. In line with the results of the meta-analysis, we found no association of breastfeeding with food allergy, and no modifying effect of maternal history of allergy, eczema or asthma on the associations of breastfeeding with allergic sensitization or atopic diseases. Differences in results might be explained by recall bias of feeding history, different study populations (general population vs. full-term born children only vs. high-risk children), definitions of breastfeeding (never vs. ever, duration, degree of exclusiveness), types of inhalant or food allergens that were measured, age at which measurements were performed (preschool-age vs. school-age), and measurement of and adjustment for potential confounders. Further studies are needed to examine whether early introduction of allergenic foods might prevent the development of food allergy.[22–24]

Interpretation of results

Our results showed that duration or exclusiveness of breastfeeding was not associated with allergic sensitization or physician-diagnosed allergy at age 10 years. Children breastfed during a shorter period or non-exclusively had an up to 1.16-fold overall increased risk of eczema until age 10 years. For exclusiveness of breastfeeding, aORs translate to an estimated number of 17–122 children that would need to be breastfed exclusively for 4 months to prevent one case of eczema at age 6 months to 10 years.[25] Our findings partly support current breastfeeding guidelines of the European Academy of Allergy and Clinical Immunology and the Dutch Youth Health Centre.[26, 27] We observed associations of duration or exclusiveness of breastfeeding with eczema overall but not per year. This might be explained by increased statistical power when using eczema overall rather than a chance finding. Differences in observed associations of breastfeeding with eczema and allergic sensitizations or physician-diagnosed allergies might be due to differences in timing of these outcome measurements. Eczema was assessed longitudinally, while allergic sensitizations and physician-diagnosed allergies were measured at one time point only. Future studies focusing on associations of breastfeeding with potential shifts in allergic sensitization and allergy patterns using skin prick tests and measurements of physician-diagnosed allergy longitudinally from early childhood onwards are needed.[28] Underlying biological mechanisms for the associations of breastfeeding with allergic sensitization, allergy and eczema might involve complex interactions of secretory immunoglobulin A, cytokines, chemokines, polyamines, sensitizing and tolerance promoting allergens, and eosinophil-derived granular proteins in human milk, which modulate the infant's developing immune system.[1] N-3 polyunsaturated fatty acids in breast milk are suggested to play a mediating role in the development of allergic sensitization, allergy and eczema, but evidence on this topic is rather conflicting.[29] Also, human milk

oligosaccharides with prebiotic properties are suggested to influence the development of atopic diseases by modulating gut microbiota diversity.[3] The impact of human milk on the diversity of the infant's gut microbiota in relation to allergic sensitization and atopic diseases requires further investigation in population-based cohorts.

Risk period-specific sensitivity analyses, additional adjustment for ointment use for eczema at age 2 months and cross-lagged modeling were used to examine potential disease-related modification of the exposure.[11, 18, 30] Failure to account for disease-related modification of the exposure could erroneously suggest that shorter or non-exclusive breastfeeding leads to eczema, while it could have been that the development of early-onset eczema encouraged mothers to extend breastfeeding. For risk period-specific sensitivity analyses, the effect estimates attenuated to non-significant, but this might have mainly been due to power issues and less likely due to differences in etiology, severity and further development of atopic diseases between children with early and later onset of eczema.[31, 32] The size and direction of the effect estimates did not materially change. Therefore, we consider our results most probably not affected by disease-related modification of the exposure.

We found no modifying effect of maternal history of allergy, eczema or asthma on the associations of breastfeeding with allergic sensitization or atopic diseases. Genetic studies are needed to fully explore the role of maternal history of atopic diseases.

Strengths and limitations

Strengths of this study are the use of a population-based prospective study design from fetal life onwards with a large number of participants, detailed information on breastfeeding status, allergic sensitization, allergy and eczema, and longitudinal measurements of eczema over time. However, some methodological limitations should be considered. First, selection bias in longitudinal studies mainly arises due to participants that are lost to follow-up. Characteristics of non-included subjects differed from those included in the study. This might have led to bias if the associations of breastfeeding with allergic sensitization, allergy and eczema were different between those included and not included in the study. Response rates of questionnaires ranged from 71% to 82%. The main reason for this was that not all children received each questionnaire due to logistical constraints and delayed implementation of some questionnaires after children reached the target age for those questionnaires. Second, we used an advanced software tool to calculate the histamine equivalent prick index. The scanned area method corrects for interobserver variability and ethnic differences in skin response to histamine and is recommended in research settings.[14] The histamine equivalent prick index area cut-off value was based on skin response to food allergens but assumed similar for inhalant allergens. We evaluated inhalant and food allergens that we considered most relevant to children of age 10 years at the population level. Other allergens, such as milk

and egg, were not taken into account because of low sensitization rates at this age.[33] This may have resulted in non-differential misclassification and, subsequently, a dilution of the effect. It might be of interest to further explore the effects of breastfeeding on allergic sensitization to specific inhalant and food allergens with low sensitization in high-risk populations. Third, we used parental questionnaires to obtain data on child's allergy and eczema, which may have led to non-differential misclassification of these outcomes, specifically food allergy. This most probably led to an underestimation of the effect estimates. Also, breastfeeding mothers are likely to be more health-conscious and might, therefore, be more likely to have allergic symptoms or skin rashes in their children presented for medical review, thus increasing the probability of diagnosing allergy or eczema. This may have resulted in differential misclassification and, subsequently, also an underestimation of the true effect. To reduce potential underestimation of the effect estimates, we opted for a self-reported physician's diagnosis of eczema to maximize the number of children without eczema correctly classified (i.e., specificity) at the expense of misclassifying children with stable disease not requiring medical attention as non-cases (i.e., sensitivity).[34] We used widely accepted, validated questions adapted from the ISAAC core questionnaire that reliably reflect the prevalence of eczema in young children at the population level.[15, 35] Self-reported diagnosis of eczema in the last year based on a single question seems sufficiently valid for studying current childhood eczema in epidemiological studies when compared with a physician's diagnosis.[36] Fourth, we aimed to reduce potential bias due to missing data by using multiple imputation methods assuming that missing data were missing at random. However, it remains difficult to quantify the effect of missing data that were missing not at random, partly due to computational limitations and current statistical software availability. Fifth, as in any observational study, residual confounding due to insufficiently or unmeasured confounders might be an issue.

In conclusion, we observed that shorter duration or non-exclusiveness of breastfeeding was associated with a weak overall increased risk of eczema, but not allergic sensitization or physician-diagnosed allergy at age 10 years. Results seemed not affected by disease-related modification of the exposure, and not modified by maternal atopic disease history. Despite its small protective effect, breastfeeding is encouraged because of its nutritional, immunological and psychosocial benefits. Further studies using detailed information on human milk components are needed to explore the specific underlying pathophysiological mechanisms.

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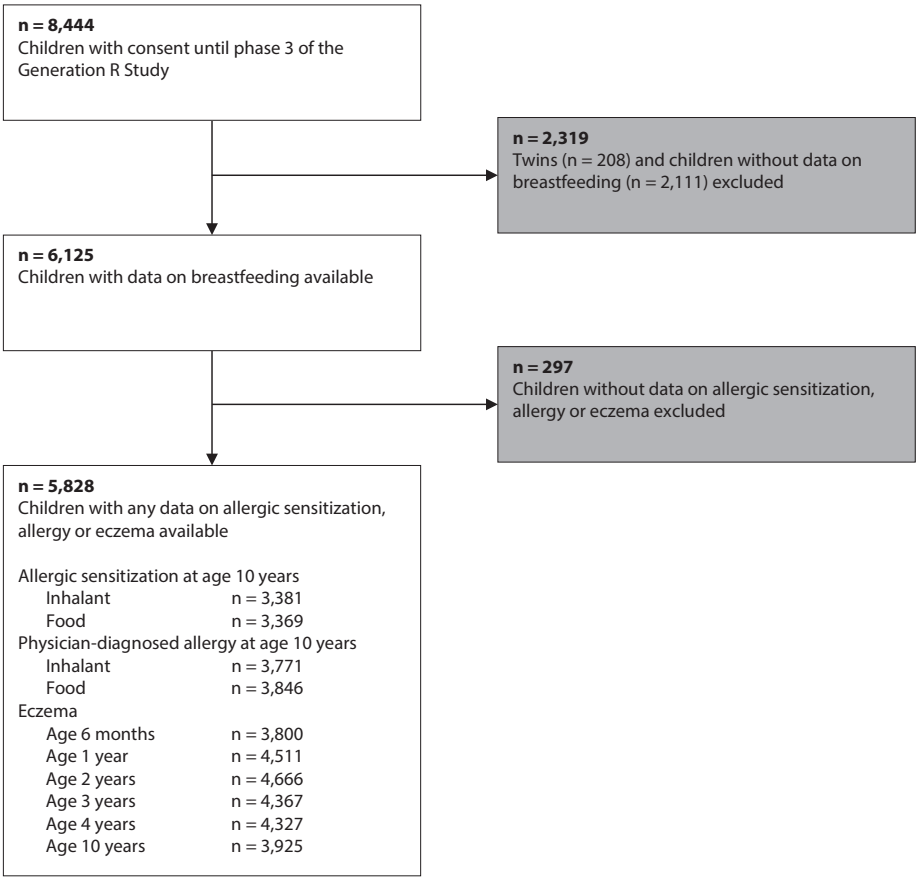
SUPPLEMENTARY MATERIAL

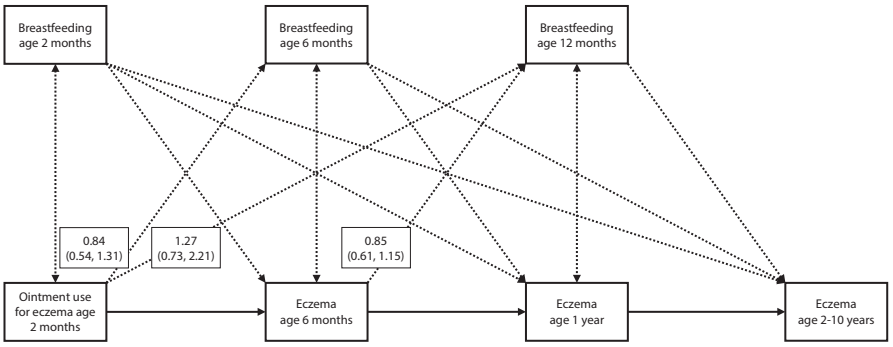
Supplementary Methods

With a cross-lagged model, bidirectional associations of breastfeeding (i.e., exposure) with childhood eczema (i.e., outcome) can be studied within the same model. In this model, we used logistic regression models to study associations of breastfeeding at age 2 months with ointment use for eczema at age 2 months and eczema at ages 6 months to 10 years. Similarly, we studied associations of breastfeeding at age 6 months with eczema at ages 6 months to 10 years, and of breastfeeding at age 12 months with eczema at ages 1 to 10 years. In addition, we studied the associations of ointment use for eczema at age 2 months with breastfeeding at ages 6 and 12 months, and of eczema at age 6 months with breastfeeding at age 12 months. Furthermore, the cross-lagged model takes associations of eczema measurements over time into account.

Supplementary Figure 1. Flowchart of participants.



Supplementary Figure 2. All hypothesized directions of the associations of breastfeeding with eczema using cross-lagged modeling.



Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data, using cross-lagged modeling which takes all potential directions of associations into account. Reference group is children who were not breastfed, did not use ointment for eczema or did not have eczema. Models are adjusted for maternal education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin and day care attendance. Arrows indicate the direction of the associations and whether they are significant (bold) or non-significant (dashed). Only odds ratios (95% confidence interval) for associations of eczema with breastfeeding, the opposite direction of the hypothesis, are presented.

Supplementary Table 1. Details of the multiple imputation model.

Software used: SPSS 21.0.0.1 for Windows (IBM Corp., Armonk, NY, USA)

Imputation method used: fully conditional specification

Model type for scale variables used: predictive mean matching

Number of imputed datasets created: 25

Maximum number of iterations: 20

Imputed variables*:

Outcomes: eczema at ages 6 months and 1, 2, 3, 4 and 10 years

Covariates: maternal education, history of allergy, eczema or asthma, parity, pet keeping during pregnancy, body mass index at enrollment, smoking during pregnancy, and psychiatric symptoms during pregnancy; child's sex, gestational age at birth, birth weight, ethnic origin, day care attendance until age 1 year, ever asthma at age 10 years, and ointment use for eczema at age 2 months

Additional indicator variables:

Outcomes: ever eczema at age 10 years; histamine equivalent intracutaneous coefficient value for house dust mite, birch, 5-grass mixture, dog, cat, cashew, peanut and peach

Covariates: paternal education, history of allergy, eczema or asthma, body mass index at enrollment, and smoking during pregnancy; household income

Treatment of binary or categorical variables: logistic

Statistical interactions included in imputation models: none

*Also included in the multiple imputation model as indicator variables.

Supplementary Table 2. Characteristics of mothers and their children (n = 5,828).

	Observed	Imputed
Maternal characteristics		
Education (%)		
Primary or secondary	46.7 (2,558)	48.2 (2,811)
Higher	53.3 (2,919)	51.8 (3,017)
Missing	*6.0 (351)*	*0 (0)*
History of allergy, eczema or asthma (%)		
No	60.9 (2,922)	61.8 (3600)
Yes	39.1 (1,873)	38.2 (2228)
Missing	*17.1 (1,033)*	*0 (0)*
Parity (%)		
0	57.2 (3,264)	56.2 (3,275)
≥ 1	42.8 (2,445)	43.8 (2,553)
Missing	*2.0 (119)*	*0 (0)*
Pet keeping during pregnancy (%)		
No	66.4 (3,064)	64.9 (3,781)
Yes	33.6 (1,553)	35.1 (2,047)
Missing	*20.8 (1,211)*	*0 (0)*
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.7)	23.7 (18.8–35.7)
Missing	*9.2 (537)*	*0 (0)*
Smoking during pregnancy (%)		
No	76.7 (3,986)	76.5 (4,456)
Yes	23.3 (1,213)	23.5 (1,372)
Missing	*10.8 (629)*	*0 (0)*
Psychiatric symptoms during pregnancy†	0.13 (0–1.31)	0.15 (0–1.40)
Missing	*22.4 (1,303)*	*0 (0)*
Child characteristics		
Sex (%)		
Male	49.9 (2,909)	49.9 (2,909)
Female	50.1 (2,919)	50.1 (2,919)
Missing	*0 (0)*	*0 (0)*
Gestational age at birth (weeks)†	40.1 (36.0–42.3)	40.1 (36.0–42.3)
Missing	*0.1 (8)*	*0 (0)*
Birth weight (grams)*	3,455 (548)	3,455 (548)
Missing	*0.1 (3)*	*0 (0)*
Ethnic origin (%)		
European	69.9 (4,006)	68.8 (4,012)
Non-European	30.1 (1,726)	31.2 (1,816)
Missing	*1.6 (96)*	*0 (0)*

Supplementary Table 2. Characteristics of mothers and their children (n = 5,828). (continued)

	Observed	Imputed
Ointment use for eczema at age 2 months (%)		
No	92.8 (3,548)	92.0 (5,364)
Yes	7.2 (274)	8.0 (464)
Missing	*34.4 (2,006)*	*0 (0)*
Day care attendance until age 1 year (%)		
No	40.5 (1,714)	44.1 (2,569)
Yes	59.5 (2,518)	55.9 (3,259)
Missing	*27.4 (1,596)*	*0 (0)*
Breastfed ever (%)		
No	7.9 (463)	7.9 (463)
Yes	92.1 (5,365)	92.1 (5,365)
Missing	*0 (0)*	*0 (0)*
Breastfeeding duration (%)		
Never	10.0 (463)	10.0 (463)
< 2 months	25.6 (1,182)	25.6 (1,182)
2–4 months	21.2 (980)	21.2 (980)
4–6 months	11.8 (547)	11.8 (547)
≥ 6 months	31.4 (1,449)	31.4 (1,449)
Missing	*20.7 (1,207)*	*20.7 (1,207)*
Breastfeeding exclusiveness (%)		
Never	9.7 (463)	9.7 (463)
Non-exclusive for 4 months	65.0 (3,095)	65.0 (3,095)
Exclusive for 4 months	25.2 (1,201)	25.2 (1,201)
Missing	*18.3 (1,069)*	*18.3 (1,069)*
Allergic sensitization at age 10 years – inhalant (%)		
No	67.9 (2,296)	67.9 (2,296)
Yes	32.1 (1,085)	32.1 (1,085)
Missing	*42.0 (2,447)*	*42.0 (2,447)*
Allergic sensitization at age 10 years – food (%)		
No	93.2 (3,139)	93.2 (3,139)
Yes	6.8 (230)	6.8 (230)
Missing	*42.2 (2,459)*	*42.2 (2,459)*
Physician-diagnosed allergy at age 10 years – inhalant (%)		
No	87.9 (3,379)	87.9 (3,379)
Yes	12.1 (467)	12.1 (467)
Missing	*34.0 (1,982)*	*34.0 (1,982)*

Supplementary Table 2. Characteristics of mothers and their children (n = 5,828). (continued)

	Observed	Imputed
Physician-diagnosed allergy at age 10 years – food (%)		
No	97.7 (3,684)	97.7 (3,684)
Yes	2.3 (87)	2.3 (87)
Missing	*35.3 (2,057)*	*35.3 (2,057)*
Allergic sensitization and allergy combined at age 10 years (%)		
No allergic sensitization and no allergy	66.3 (1,868)	66.3 (1,868)
Any allergic sensitization, but no allergy	22.6 (636)	22.6 (636)
No allergic sensitization, but any allergy	1.3 (36)	1.3 (36)
Any allergic sensitization and any allergy	9.9 (279)	9.9 (279)
Missing	*51.6 (3,009)*	*51.6 (3,009)*
Eczema last 6 months at age 6 months (%)		
No	83.9 (3,188)	83.5 (4,866)
Yes	16.1 (612)	16.5 (962)
Missing	*34.8 (2,028)*	*0 (0)*
Eczema last 6 months at age 1 year (%)		
No	87.2 (3,933)	86.8 (5,060)
Yes	12.8 (578)	13.2 (768)
Missing	*22.6 (1,317)*	*0 (0)*
Eczema last 12 months at age 2 years (%)		
No	86.5 (4,034)	85.8 (5003)
Yes	13.5 (632)	14.2 (825)
Missing	*19.9 (1,162)*	*0 (0)*
Eczema last 12 months at age 3 years (%)		
No	90.8 (3,966)	89.9 (5,242)
Yes	9.2 (401)	10.1 (586)
Missing	*25.1 (1,461)*	*0 (0)*
Eczema last 12 months at age 4 years (%)		
No	92.2 (3,991)	91.3 (5,320)
Yes	7.8 (336)	8.7 (508)
Missing	*25.8 (1,501)*	*0 (0)*
Eczema last 12 months at age 10 years (%)		
No	93.3 (3,663)	92.1 (5,368)
Yes	6.7 (262)	7.9 (460)
Missing	*32.7 (1,903)*	*0 (0)*

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed and imputed data. Data on breastfeeding duration and exclusiveness, allergic sensitizations and physician-diagnosed allergies are not imputed.

Supplementary Table 3. Characteristics of mothers and children included and not included in the study.

	Included n = 5,828	Not included n = 297	*P*-value for difference
Maternal characteristics			
Education (%)			**< 0.001**
Primary or secondary	46.7 (2,558)	86.0 (190)	
Higher	53.3 (2,919)	14.0 (31)	
Missing	*6.0 (351)*	*25.6 (76)*	
History of allergy, eczema or asthma (%)			0.69
No	60.9 (2,922)	62.4 (121)	
Yes	39.1 (1,873)	37.6 (73)	
Missing	*17.1 (1,033)*	*34.7 (103)*	
Parity (%)			**< 0.001**
0	57.2 (3,264)	45.9 (135)	
≥ 1	42.8 (2,445)	54.1 (159)	
Missing	*2.0 (119)*	*1.0 (3)*	
Pet keeping during pregnancy (%)			0.19
No	66.4 (3,064)	70.8 (143)	
Yes	33.6 (1,553)	29.2 (59)	
Missing	*20.8 (1,211)*	*32.0 (95)*	
Body mass index at enrollment (kg/m^2)†	23.7 (18.8–35.7)	24.5 (17.9–37.4)	**< 0.05**
Missing	*9.2 (537)*	*7.4 (22)*	
Smoking during pregnancy (%)			**< 0.001**
No	76.7 (3,986)	64.9 (146)	
Yes	23.3 (1,213)	35.1 (79)	
Missing	*10.8 (629)*	*24.2 (72)*	
Psychiatric symptoms during pregnancy†	0.13 (0–1.31)	0.27 (0–1.48)	**< 0.001**
Missing	*22.4 (1,303)*	*47.8 (142)*	
Child characteristics			
Sex (%)			0.12
Male	49.9 (2,909)	54.5 (162)	
Female	50.1 (2,919)	45.5 (135)	
Missing	*0 (0)*	*0 (0)*	
Gestational age at birth (weeks)†	40.1 (36.0–42.3)	39.9 (36.2–42.3)	0.05
Missing	*0.1 (8)*	*0.3 (1)*	
Birth weight (grams)*	3,455 (548)	3,393 (494)	0.06
Missing	*0.1 (3)*	*0 (0)*	

Supplementary Table 3. Characteristics of mothers and children included and not included in the study. (continued)

	Included n = 5,828	**Not included n = 297**	***P*-value for difference**
Ethnic origin (%)			**< 0.001**
European	69.9 (4,006)	36.9 (90)	
Non-European	30.1 (1,726)	63.1 (154)	
Missing	*1.6 (96)*	*17.8 (53)*	
Day care attendance until age 1 year (%)			**< 0.05**
No	40.5 (1,714)	85.7 (6)	
Yes	59.5 (2,518)	14.3 (1)	
Missing	*27.4 (1,596)*	*97.6 (290)*	
Breastfed ever (%)			**< 0.05**
No	7.9 (463)	11.8 (35)	
Yes	92.1 (5,365)	88.2 (262)	
Missing	*0 (0)*	*0 (0)*	
Breastfeeding duration (%)			**< 0.001**
Never	10.0 (463)	35.0 (35)	
< 2 months	25.6 (1,182)	52.0 (52)	
2–4 months	21.2 (980)	11.0 (11)	
4–6 months	11.8 (547)	1.0 (1)	
≥ 6 months	31.4 (1,449)	1.0 (1)	
Missing	*20.7 (1,207)*	*66.3 (197)*	
Breastfeeding exclusiveness (%)			**< 0.001**
Never	9.7 (463)	27.3 (35)	
Non-exclusive for 4 months	65.0 (3,095)	71.9 (92)	
Exclusive for 4 months	25.2 (1,201)	0.8 (1)	
Missing	*18.3 (1,069)*	*56.9 (169)*	

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed data. *P*-values for difference are calculated by independent samples T-test for continuous variables with a normal distribution, the Mann-Whitney U-test for continuous variables with a skewed distribution, and Pearson's Chi-square test for categorical variables. Bold values indicate statistical significance at the $\alpha = 0.05$ level.

Supplementary Table 4. Associations of duration and exclusiveness of breastfeeding with combined allergic sensitization and physician-diagnosed allergy groups in children at age 10 years.

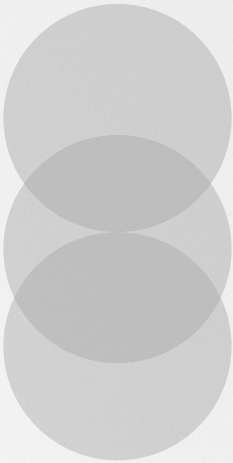
	Odds ratio (95% confidence interval) for any allergic sensitization and any physician-diagnosed allergy combined		
	Any allergic sensitization, but no allergy n = 636	**No allergic sensitization, but any allergy n = 36**	**Any allergic sensitization and any allergy n = 279**
Breastfeeding			
Never (n = 463)	0.90 (0.63, 1.29)	0.32 (0.04, 2.43)	0.94 (0.56, 1.57)
Ever (n = 5,365)	Reference	Reference	Reference
Breastfeeding duration			
< 2 months (n = 1,182)	0.97 (0.73, 1.28)	1.71 (0.58, 5.01)	1.22 (0.83, 1.78)
2–4 months (n = 980)	1.16 (0.88, 1.52)	2.30 (0.80, 6.59)	0.96 (0.64, 1.45)
4–6 months (n = 547)	1.04 (0.74, 1.45)	1.49 (0.36, 6.14)	1.13 (0.72, 1.79)
≥ 6 months (n = 1,449)	Reference	Reference	Reference
P-value for trend	0.93	0.26	0.43
Breastfeeding exclusiveness			
Non-exclusive for 4 months (n = 3,095)	1.08 (0.86, 1.36)	1.60 (0.58, 4.69)	1.04 (0.76, 1.42)
Exclusive for 4 months (n = 1,201)	Reference	Reference	Reference

Values are odds ratios (95% confidence interval) from multinomial logistic regression models based on imputed data. Reference group is children without any allergic sensitization or physician-diagnosed allergy (n = 1,868). Models are adjusted for maternal education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin and day care attendance.

Supplementary Table 5. Associations of duration and exclusiveness of breastfeeding with eczema per year and overall in children until age 10 years.

	Odds ratio (95% confidence interval) for eczema						
	6 months	**1 year**	**2 years**	**3 years**	**4 years**	**10 years**	**Overall**
Breastfeeding							
Never (n = 463)	0.99 (0.73, 1.33)	1.04 (0.77, 1.33)	1.17 (0.86, 1.58)	1.17 (0.83, 1.64)	1.20 (0.84, 1.71)	1.15 (0.76, 1.74)	1.10 (0.97, 1.26)
Ever (n = 5,365)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Breastfeeding duration							
< 2 months (n = 1,182)	1.12 (0.88, 1.43)	1.26 (0.98, 1.61)	1.17 (0.92, 1.49)	1.13 (0.84, 1.52)	1.15 (0.85, 1.55)	1.17 (0.83, 1.65)	**1.16 (1.02, 1.32)**
2–4 months (n = 980)	1.18 (0.93, 1.50)	1.26 (0.98, 1.62)	1.09 (0.84, 1.41)	1.12 (0.82, 1.52)	0.91 (0.63, 1.29)	1.11 (0.77, 1.60)	1.12 (0.99, 1.27)
4–6 months (n = 547)	1.05 (0.79, 1.41)	1.16 (0.85, 1.59)	0.99 (0.72, 1.36)	0.92 (0.62, 1.37)	0.96 (0.65, 1.42)	1.02 (0.63, 1.65)	1.03 (0.89, 1.19)
≥ 6 months (n = 1,449)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
P-value for trend	0.26	0.05	0.18	0.31	0.48	0.34	**< 0.05**
Breastfeeding exclusiveness							
Non-exclusive for 4 months (n = 3,095)	1.20 (0.99, 1.46)	1.19 (0.96, 1.49)	1.04 (0.84, 1.28)	0.95 (0.74, 1.21)	0.96 (0.73, 1.26)	1.26 (0.93, 1.72)	**1.11 (1.01, 1.23)**
Exclusive for 4 months (n = 1,201)	Reference	Reference	Reference	Reference	Reference	Reference	Reference

Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Bold values indicate statistical significance at the α = 0.05 level. Models are adjusted for maternal education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin and day care attendance.



CHAPTER 3.3

ALLERGENIC FOOD INTRODUCTION AND ECZEMA, ALLERGIC SENSITIZATION AND ALLERGY IN SCHOOL-AGE CHILDREN

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ABSTRACT

Background The role of timing and diversity of allergenic food introduction in the development of childhood allergic sensitization and atopic diseases is controversial.

Objective To examine whether timing and diversity of allergenic food introduction are associated with allergic sensitization, allergy and eczema in children until age 10 years.

Methods This study among 5,202 children was performed in a population-based prospective cohort. Timing (age ≤ 6 months vs. > 6 months) and diversity (0, 1, 2 and ≥ 3 foods) of allergenic food (cow's milk, hen's egg, peanut, tree nuts, soy and gluten) introduction were assessed by questionnaires at ages 6 and 12 months. At age 10 years, inhalant and food allergic sensitization were measured by skin prick tests, and physician-diagnosed inhalant and food allergy by questionnaire. Data on parental-reported physician-diagnosed eczema were obtained from birth until age 10 years.

Results Timing of allergenic food introduction was not associated with allergic sensitization or physician-diagnosed allergy. Children introduced to gluten at age ≤ 6 months had a decreased risk of eczema (adjusted odds ratio (95% confidence interval): 0.84 (0.72, 0.99)), compared with children introduced to gluten at age > 6 months. Diversity of allergenic food introduction was not associated with allergic sensitization, physician-diagnosed food allergy or eczema. Children introduced to ≥ 3 allergenic foods at age ≤ 6 months had a decreased risk of physician-diagnosed inhalant allergy (0.64 (0.42, 0.98)), compared with children not introduced to any allergenic food at age ≤ 6 months.

Conclusions Neither timing nor diversity of allergenic food introduction was consistently associated with childhood allergic sensitization, allergy or eczema.

INTRODUCTION

The role of timing and diversity of allergenic food introduction in the development of childhood allergic sensitization and atopic diseases, such as allergy and eczema, is controversial. Currently, the World Health Organization and the American Academy of Pediatrics recommend not to introduce any complementary foods until age 6 months[1, 2], while the European Academy of Allergy and Clinical Immunology and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition recommend not to avoid or delay the introduction of potentially allergenic foods beyond age 4 months, irrespective of atopic heredity.[3, 4]

We previously demonstrated that the introduction of allergenic foods, such as cow's milk and peanut, at age ≤ 6 months was not associated with eczema until age 4 years.[5] Recently, the LEAP and LEAP-On trials showed that peanut introduction in high-risk children aged 4–11 months was associated with a decreased frequency of peanut allergy that persisted after 12 months of peanut avoidance.[6, 7] This resulted in addendum guidelines for the prevention of peanut allergy in high-risk children, recommending introduction of peanut as early as age 4–6 months in children with severe eczema, egg allergy or both.[8] Another trial did not show an effect of early introduction of six common allergenic foods on the frequency of food allergies between age 1 and 3 years among a selected group of exclusively breast-fed children from the general population.[9] However, less is known about the effects of the timing of introduction of common allergenic foods in early life on allergic sensitization, allergy and eczema in an unselected group of school-age children.[10] Previous birth cohort studies have examined the association of diversity of solid food introduction, by means of the number of solid foods introduced, with allergic sensitization and diseases,[11-15] but none of these studies focused specifically on allergenic foods.

Because animal studies suggest that acquiring immune tolerance is an active process and that exposure to dietary factors during a critical early window at age 4–6 months may be essential to this process[16], we hypothesized that timing and diversity of allergenic food introduction might induce immune tolerance and, subsequently, influence the risk of developing allergic sensitization and atopic diseases in childhood. Therefore, we aimed to examine among 5,202 children participating in a population-based prospective cohort study whether timing and diversity of introduction of allergenic foods (cow's milk, hen's egg, peanut, tree nuts, soy and gluten) were associated with the development of allergic sensitization, inhalant or food allergy and eczema until age 10 years.

METHODS

General design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards.[17] The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, The Netherlands (MEC-2012–165). Written informed consent was obtained from both parents or legal representatives. A total of 5,202 children were included for the current analyses (Supplementary Figure).

Allergenic food introduction

We collected data on the introduction of cow's milk, hen's egg, peanut, tree nuts, soy and gluten by parental questionnaires at ages 6 and 12 months ("How often do you give your child cow's milk/hen's egg/peanut/tree nuts/soy/gluten at present?" and "How old was your child when you first gave him or her cow's milk/hen's egg/peanut/tree nuts/soy/gluten?"). Data from the questionnaires were combined and categorized into 'introduction at age ≤ 6 months' and 'introduction at age > 6 months'.[5] Reported introductions of food products were cross-checked with a short food-frequency questionnaire also completed by the mother when the child was 6 and 12 months old. This questionnaire consisted of food products frequently consumed by children around these ages according to a Dutch food consumption survey.[18] For example, if parents indicated that they had never introduced peanut in the child's diet at age 12 months, but at age 6 months parents reported that the child had consumed peanut butter more than once, then the introduction of peanut was considered to be at age ≤ 6 months. Additionally, the introduction of cow's milk and soy was cross-checked with the type of bottle feeding (regular, soy-based or based on fully or partly hydrolyzed whey protein), and the introduction of gluten was cross-checked with the consumption of specific brands of bread, biscuits and porridge (gluten-containing or gluten-free brands) at ages 6 and 12 months. To assess diversity of allergenic food introduction at age ≤ 6 months, we categorized the number of allergenic foods introduced at this age into '0', '1', '2' and '≥ 3'.

Allergic sensitization, allergy and eczema

Children visited the research center at a median age of 9.7 years (2.5–97.5th percentile: 9.3–10.5). Inhalant and food allergic sensitization to house dust mite, 5-grass mixture, birch, cat and dog (ALK-Abelló B.V., Almere, The Netherlands), and hazelnut, cashew nut, peanut and peach were measured by skin prick tests using the scanned area method.[19] Questions adapted from the International Study of Asthma and Allergies in Childhood core questionnaires provided information on physician-diagnosed inhalant ("Was your child ever diagnosed with an allergy to pollen (hay fever)/house dust mite/cat/dog?") (no; yes) and food ("Was your child ever diagnosed with an allergy to cashew nut/peanut?") (no; yes) allergy at age 10 years.[20] We further combined allergic sensitization

and physician-diagnosed allergy into groups of 'no allergic sensitization and no allergy', 'any allergic sensitization, but no allergy', 'no allergic sensitization, but any allergy', and 'any allergic sensitization and any allergy'. Physician-diagnosed eczema was parental-reported at ages 6 months and 1, 2, 3, 4 and 10 years ("Was your child diagnosed with eczema in the last 6 months/last year?") (no; yes).

Covariates

Information on maternal age, education (primary or secondary; higher), history of allergy, eczema or asthma (no; yes), parity (nulliparous; multiparous), pet keeping (no; yes) and body mass index (BMI) was obtained by questionnaires completed by the mother at enrollment. Information on maternal smoking (no; yes) was obtained by postal questionnaires multiple times during pregnancy. We assessed maternal psychiatric symptoms in the second trimester of pregnancy using the Global Severity Index of the Brief Symptom Inventory.[21] Information on child's sex, gestational age at birth and birth weight was obtained from obstetric and midwife records at birth. We based ethnic origin (European; non-European) of the child on the country of birth of the parents.[22] Delivery reports and postal questionnaires completed by the mother when the child was 2, 6 and 12 months old provided data on ever breastfeeding (no; yes) and breastfeeding duration (never; < 6 months; ≥ 6 months). We obtained information on ointment use for eczema (no; yes), physician-diagnosed cow's milk allergy (no; yes), day care attendance (no; yes) and antibiotic use (no; yes) by questionnaires at ages 2, 6 and 12 months. BMI was calculated from the child's weight and height measured at age 10–13 months during a visit to the research center.

Statistical analysis

We used logistic regression or multinomial logistic regression models to examine the associations of timing and diversity of allergenic food introduction with the risk of allergic sensitization and physician-diagnosed allergy or combined allergic sensitization and allergy groups, respectively, at age 10 years. We used generalized estimating equation models to examine the associations of timing and diversity of allergenic food introduction with the longitudinal odds of eczema at ages 6 months and 1, 2, 3, 4 and 10 years independently and overall, taking into account correlations between repeated measurements of eczema within the same child.[23] We used an unstructured correlation matrix, allowing a distinct correlation between every pair of measurements within a child. First, we adjusted for potential confounders, including maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, BMI at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin, breastfeeding, day care attendance and antibiotic use. We considered this the main model. Second, child's BMI at age 10–13 months was considered as an intermediate and additionally adjusted

for in the model. Confounders were included in the models based on literature, if they were associated with both the determinant and the outcome, or if they changed the effect estimates with ≥ 10%. Analyses with inhalant or food allergic sensitization or allergy as the outcomes were mutually adjusted for each other. Tests for trends were performed by including diversity of allergenic food introduction as a continuous variable in the models. Because the associations evaluated in the current study are complementary and share a single underlying biological hypothesis, we did not apply multiple testing correction.[24] We performed additional analyses to assess the robustness of our results, accounting for disease-related modification of the exposure and effect modification. First, we performed risk period-specific sensitivity analyses by excluding children who developed eczema until age 6 months (n = 928). Second, we additionally adjusted our main model for ointment use for eczema at age 2 months. Third, we tested the modifying effects of maternal history of allergy, eczema or asthma, and child's breastfeeding duration and history of cow's milk allergy until age 1 year, and the time-varying effect of age at eczema measurement by adding them as product terms with the allergenic food variables in the models. Missing data of covariates, allergenic foods and eczema were multiple-imputed to reduce potential bias associated with missing data (Supplementary Table 1). The best indicator for the presence or absence of eczema is an eczema measurement at a different age. Therefore, at least one eczema measurement was available in our population for analysis to predict other eczema measurements. Because we lacked repeated measurements on allergic sensitization and physician-diagnosed allergy, we did not impute missing data of these outcomes. The size or direction of the effect estimates did not materially differ between analyses with imputed data and complete cases only (data not shown). Therefore, we present pooled results based on imputed analyses only. Measures of association are presented as adjusted odds ratios (aOR) with their 95% confidence intervals (CI). Statistical analyses were performed using SPSS 21.0.0.1 for Windows (IBM Corp., Armonk, NY, USA) and SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

General

The majority of children were introduced to at least 1 allergenic food (81.8%; n = 4,215) at age ≤ 6 months, most commonly cow's milk (74.0%; n = 3,847) and gluten (55.8%; n = 2,904) (Table 1 and Supplementary Table 2). Inhalant or food allergic sensitization was present in 31.5% (n = 949) and 6.7% (n = 202) of the children at age 10 years, respectively. Physician-diagnosed inhalant or food allergy was present in 12.0% (n = 435) and 2.3% (n = 81) of the children at age 10 years, respectively. The prevalence of eczema declined from 17.8% (n = 928) at age 6 months to 8.6% (n = 448) at age 10 years. Mothers without follow-up data were younger, lower educated, had higher parity, more often kept pets

Table 1. Characteristics of mothers and their children.

	n = 5,202
Maternal characteristics	
Age at enrollment (years)*	31.1 (4.8)
Education, higher (%)	53.5 (2,784)
History of eczema, allergy or asthma, yes (%)	37.9 (1,972)
Parity, ≥ 1 (%)	41.5 (2,158)
Pet keeping during pregnancy, yes (%)	34.9 (1,814)
Body mass index at enrollment (kg/m^2)†	23.6 (18.8–35.6)
Smoking during pregnancy, yes (%)	23.0 (1,195)
Psychiatric symptoms during pregnancy‡	0.13 (0–1.33)
Child characteristics	
Sex, female (%)	50.4 (2,623)
Gestational age at birth (weeks)†	40.1 (36.0–42.3)
Birth weight (grams)*	3,454 (549)
Ethnic origin, European (%)	72.5 (3,770)
Breastfeeding (%)	
Ever, yes	91.6 (4,763)
Duration, ≥ 6 months	34.9 (1,814)
Ointment use for eczema at age 2 months, yes (%)	7.6 (393)
Cow's milk allergy until age 1 year, yes (%)	5.9 (305)
Day care attendance until age 1 year, yes (%)	57.5 (2,992)
Antibiotic use until age 1 year, yes (%)	22.0 (1,143)
Body mass index at age 10–13 months (kg/m^2)†	17.3 (14.9–20.3)
Timing of allergenic food introduction, ≤ 6 months (%)	
Cow's milk	74.0 (3,847)
Hen's egg	14.2 (741)
Peanut	5.8 (303)
Tree nuts	4.5 (236)
Soy	20.3 (1,055)
Gluten	55.8 (2,904)
Diversity of allergenic foods introduced at age ≤ 6 months (%)	
No allergenic foods introduced	18.2 (945)
1 allergenic food introduced	33.7 (1,754)
2 allergenic foods introduced	29.1 (1,516)
≥ 3 allergenic foods introduced	19.0 (987)
Allergic sensitization at age 10 years, yes (%)	
Inhalant	31.5 (949)
Food	6.7 (202)

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

	n = 5,202
Physician-diagnosed allergy at age 10 years, yes (%)	
Inhalant	12.0 (435)
Food	2.3 (81)
Allergic sensitization and allergy combined at age 10 years (%)	
No allergic sensitization and no allergy	67.1 (1,759)
Any allergic sensitization, but no allergy	21.9 (574)
No allergic sensitization, but any allergy	1.2 (31)
Any allergic sensitization and any allergy	9.8 (258)
Eczema, yes (%)	
Age 6 months	17.8 (928)
Age 1 year	13.3 (690)
Age 2 years	13.7 (712)
Age 3 years	10.0 (519)
Age 4 years	8.5 (440)
Age 10 years	8.6 (448)

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on imputed data. Data on allergic sensitizations and physician-diagnosed allergies are not imputed.

during pregnancy, had a higher BMI at enrollment, and smoked more and had more psychiatric symptoms during pregnancy. Their children were more often males, born younger, had a lower birth weight, and were more often of non-European origin and less often breastfed and exposed to gluten at age ≤ 6 months (Supplementary Table 3).

Allergenic food introduction

Timing of allergenic food introduction was not associated with allergic sensitization, physician-diagnosed allergy or combined allergic sensitization and allergy groups (Table 2 and Supplementary Table 4). Children introduced to gluten at age ≤ 6 months had a decreased risk of eczema until age 10 years (aOR (95% CI): 0.84 (0.72, 0.99)), compared with children introduced to gluten at age > 6 months (Figure 1 and Supplementary Table 5). Timing of introduction of other allergenic foods was not associated with eczema at specific ages or overall. Diversity of allergenic food introduction was not associated with allergic sensitization, physician-diagnosed food allergy or combined allergic sensitization and allergy groups (Table 2 and Supplementary Table 4). Children introduced to ≥ 3 allergenic foods at age ≤ 6 months had a decreased risk of physician-diagnosed inhalant, but not food allergy at age 10 years (0.64 (0.42, 0.98)), compared with children not introduced to any allergenic food at age ≤ 6 months (Table 2). We did not observe a significant trend for a lower risk of physician-diagnosed inhalant allergy

Table 2. Associations of timing and diversity of allergenic food introduction with allergic sensitizations and physician-diagnosed allergies in children at age 10 years.

	Odds ratio (95% confidence interval) for allergic sensitization		Odds ratio (95% confidence interval) for physician-diagnosed allergy	
	Inhalant n = 3,017	**Food n = 3,006**	**Inhalant n = 3,617**	**Food n = 3,546**
Allergenic food introduced at age ≤ 6 months*				
Cow's milk (n = 3,847)	0.99 (0.81, 1.22)	0.76 (0.50, 1.17)	0.83 (0.65, 1.08)	1.44 (0.76, 2.73)
Hen's egg (n = 741)	0.99 (0.76, 1.31)	1.06 (0.61, 1.85)	0.74 (0.48, 1.15)	0.83 (0.32, 2.13)
Peanut (n = 303)	0.74 (0.46, 1.20)	1.68 (0.69, 4.10)	0.59 (0.26, 1.37)	2.55 (0.67, 9.67)
Tree nuts (n = 236)	1.07 (0.55, 2.09)	0.63 (0.09, 4.34)	0.84 (0.26, 2.71)	2.43 (0.47, 12.45)
Soy (n = 1,055)	1.05 (0.85, 1.30)	0.91 (0.57, 1.45)	0.97 (0.72, 1.32)	1.39 (0.72, 2.68)
Gluten (n = 2,904)	0.99 (0.83, 1.19)	0.71 (0.48, 1.06)	0.80 (0.63, 1.02)	0.65 (0.36, 1.18)
Diversity of allergenic foods introduced at age ≤ 6 months†				
1 allergenic food introduced (n = 1,754)	0.97 (0.75, 1.25)	0.90 (0.53, 1.53)	0.86 (0.63, 1.19)	1.56 (0.73, 3.32)
2 allergenic foods introduced (n = 1,516)	0.94 (0.72, 1.22)	0.76 (0.44, 1.32)	0.85 (0.61, 1.18)	0.77 (0.33, 1.82)
≥ 3 allergenic foods introduced (n = 987)	1.01 (0.75, 1.36)	0.69 (0.36, 1.31)	**0.64 (0.42, 0.98)**	1.56 (0.63, 3.86)
P-value for trend	0.96	0.18	0.05	0.92

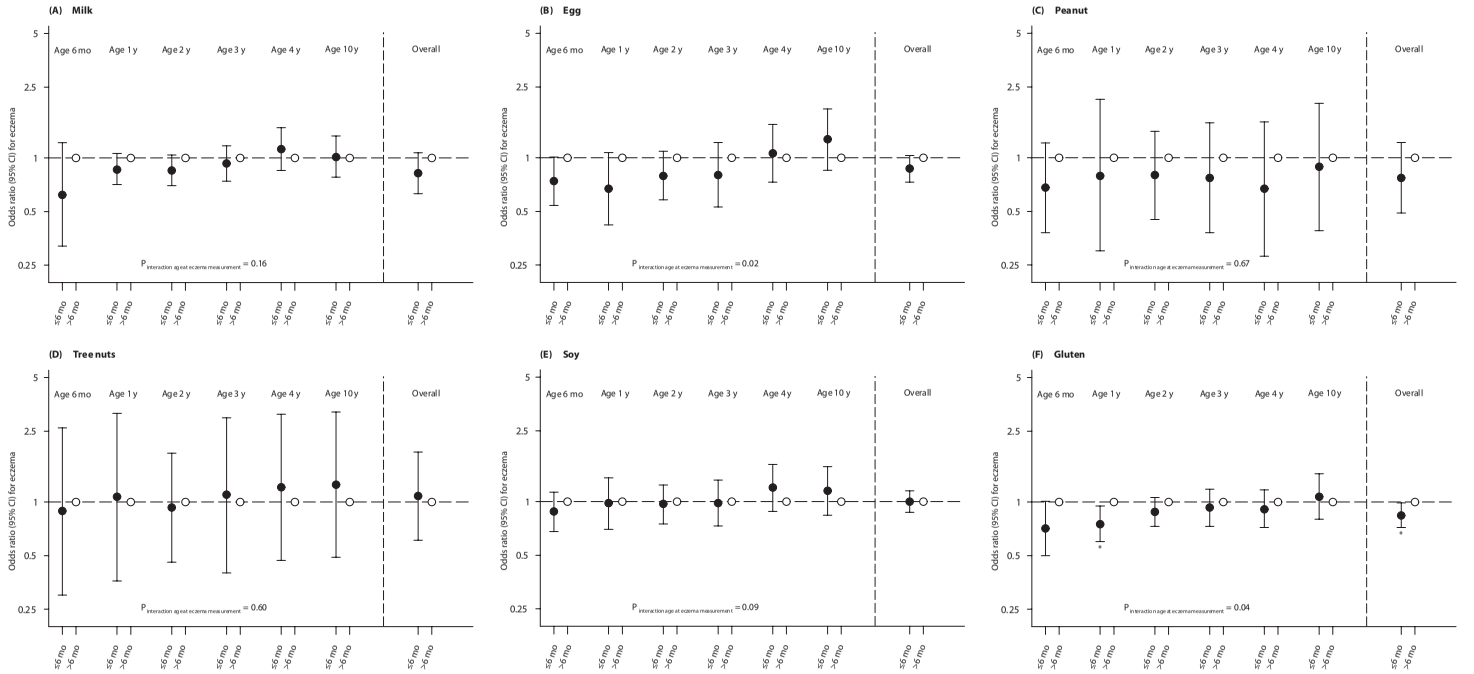
Values are odds ratios (95% confidence interval) from logistic regression models based on imputed data. Bold values indicate statistical significance at the α = 0.05 level. Reference group is children with *allergenic food introduction at age > 6 months or †no allergenic foods introduced at age ≤ 6 months. Models are adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin, breastfeeding, day care attendance and antibiotic use, and mutually for inhalant and food allergic sensitization or allergy.

when introduced to a higher number of allergenic foods at age ≤ 6 months (*p*-value = 0.05). Diversity of allergenic food introduction was not consistently associated with eczema (Figure 2 and Supplementary Table 5). Additional adjustment for child's BMI at age 10–13 month did not materially affect the size and the direction of the effect estimates (data not shown).

Additional analyses

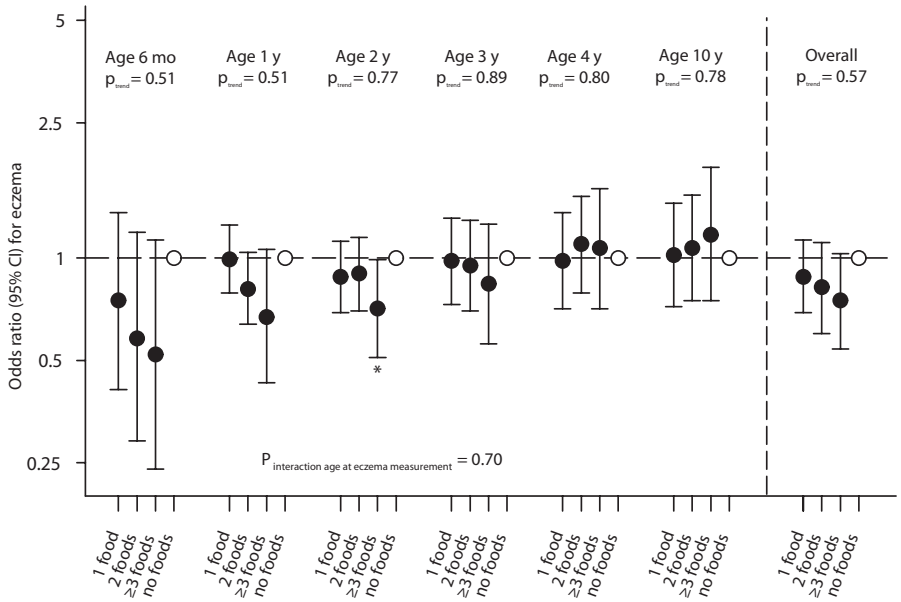
Risk period-specific sensitivity analyses showed that effect estimates for the associations of early gluten introduction with eczema until age 10 years, and of introduction of ≥ 3 allergenic foods with physician-diagnosed inhalant allergy attenuated to non-significant (0.95 (0.81, 1.10) and 0.65 (0.39, 1.08), respectively). Effect estimates did not materially change in size or direction when we additionally adjusted our main analyses for ointment use for eczema at age 2 months (data not shown). Results were similar among

Figure 1. Associations of timing of milk (A), egg (B), peanut (C), tree nuts (D), soy (E) and gluten (F) introduction with eczema in children until age 10 years.



Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Reference group is children with allergenic food introduction at age > 6 months. Models are adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin, breastfeeding, day care attendance and antibiotic use. *P-value < 0.05. Mo = months; y = year(s).

Figure 2. Associations of diversity of allergenic food introduction with eczema in children until age 10 years.



Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Reference group is children with no allergenic foods introduced at age ≤ 6 months. Models are adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin, breastfeeding, day care attendance and antibiotic use. *P-value < 0.05. Mo = months; y = year(s).

children with and without a maternal history of allergy, eczema or asthma or a history of cow's milk allergy until age 1 year, and we observed no modifying effect of child's breastfeeding duration on any of the examined associations (p-values for interaction > 0.05). For egg and gluten introduction, a time-varying effect of age at eczema measurement was observed with stronger associations of egg and gluten introduction with eczema in early than in later life (p-values for interaction < 0.05) (Figure 1).

DISCUSSION

In this large prospective population-based study, we observed no consistent association of timing and diversity of allergenic food introduction with allergic sensitization or atopic diseases in children until age 10 years. However, children introduced to gluten at age ≤ 6 months had a decreased risk of eczema until age 10 years, and children introduced to

≥ 3 allergenic foods at age ≤ 6 months had a decreased risk of physician-diagnosed inhalant allergy at age 10 years.

Comparison of main findings with other studies

For the allergenic foods evaluated in the current study, a recent meta-analysis showed no association of early allergenic food introduction with inhalant or food allergic sensitization, measured by skin prick tests or antigen-specific immunoglobulin E (IgE), and allergic rhinitis.[25] However, introduction of hen's egg at age 4–6 months was associated with a 44% decreased risk of egg allergy at age 1 year, and introduction of peanut at age 4–11 months was associated with a 71% decreased risk of peanut allergy at age 3–5 years. There was no consistent evidence for an association of early cow's milk, tree nuts, soy or gluten introduction with food allergies. Previous birth cohort studies showed inconsistent results on the association of diversity of solid food introduction, by means of the number of solid foods introduced, with the risk of childhood allergic sensitization and atopic diseases.[11-15] However, literature focusing specifically on diversity of allergenic food introduction is lacking. Differences between results from the meta-analysis and the current study might be explained by differences in study population (general population vs. high-risk children; different age groups) and study aim (observational vs. tolerance induction for specific allergenic foods). Other possible explanations may include differences in recall bias, selection of allergenic foods, definitions of diversity of food introduction (solid vs. specific allergenic foods; number of foods per group), outcome methods (antigen-specific IgE vs. skin prick tests; physician-diagnosed vs. parental-reported), child's age at time of outcome measurement, and measurement of and adjustment for potential confounders.

Interpretation of results

Dietary factors may serve as substrates for the production of microbial metabolites that regulate immune activity and immune tolerance mechanisms.[26] Therefore, we hypothesized that early allergenic food introduction might influence immune tolerance and, subsequently, the development of childhood allergic sensitization and atopic diseases. However, we found no consistent association of the timing of introduction of allergenic foods with childhood allergic sensitization or atopic diseases. We did find an inverse association of early gluten introduction with eczema, which might be explained by the fact that when gluten are introduced to older children, the amounts tend to be greater than in younger children.[27] We can speculate that a higher gluten load may have resulted in T-cell activation rather than immune tolerance.[27] We observed an association of early gluten introduction with eczema overall but not consistently per year. This might be explained by increased statistical power when using eczema overall rather than a chance finding. Differences in observed associations of allergenic food introduction

with eczema and allergic sensitizations or physician-diagnosed allergies might be due to differences in timing of these outcome measurements. Eczema was assessed longitudinally, while allergic sensitizations and physician-diagnosed allergies were measured at one time point only.

It is suggested that exposure to a variety of food products during the first year of life, especially beyond age 6 months, may be important for the development of immune tolerance.[13, 14] Again, we observed no consistent association of diversity of allergenic food introduction with allergic sensitization or atopic diseases. We did observe a suggestive trend of a decreased risk of physician-diagnosed inhalant allergy with a higher (i.e., per 1-food group increase) allergenic food diversity. A recent birth cohort study showed that introduction of a higher number of solid foods was associated with a decreased expression of Cε germline transcript, a marker for antibody isotype switching of B-cells to IgE-producing cells.[14] The inhibition of isotype switching to IgE is one of the mechanisms that might be involved in the inhibition of atopic diseases by regulatory T-cells.[28] We did not find an association of diversity of allergenic food introduction with food allergic sensitization and physician-diagnosed food allergy, which could partly be explained by the low prevalence of food allergic sensitization (6.7%) and physician-diagnosed food allergy (2.3%). Therefore, these results should be interpreted with caution.

Our findings might be explained by disease-related modification of the exposure, meaning that early symptoms of allergy or eczema in the child may encourage parents to alter feeding practices. Among children with early allergy-related symptoms and among those with a parental history of allergy, eczema or asthma, introduction of complementary foods, especially allergenic foods, tends to be delayed.[14] We tried to assess the effects of such bias by performing risk period-specific sensitivity analyses and tests for interaction with a maternal history of allergy, eczema or asthma or a history of cow's milk allergy until age 1 year. We showed that to some degree disease-related modification of the exposure was present in our study, particularly for the association of early gluten introduction with eczema until age 10 years. Therefore, caution is warranted in interpreting our results, which require further studies for replication and exploration of underlying pathophysiological mechanisms.

Strengths and limitations

The strengths of this study are the use of a population-based prospective study design from fetal life onwards with a large number of participants and detailed information on allergic sensitization, allergy and eczema. Also, we adjusted for multiple social, behavioral and environmental factors. However, some methodological limitations should be considered. First, characteristics of non-included subjects differed from those included in the study. Although this may affect the generalizability of our results, it is unlikely that these differences affected the observed associations. Second, our data did not allow us

to evaluate the effects of allergenic food introduction during the specific window of immunological opportunity to induce immune tolerance at age 4–6 months. Instead, we assessed the associations of allergenic food introduction at age ≤ 6 months, which may have underestimated our results. Also, our data did not allow us to evaluate the effects of continued breastfeeding during the period of allergenic food introduction, which may be important for promoting immune tolerance.[16] However, we did not observe any modifying effect of breastfeeding duration. It has recently been suggested that the prevention of food allergy by means of early introduction of multiple allergenic foods is dose-dependent[9], but we lacked data on the precise amounts of allergenic foods introduced. Third, we cannot rule out that our results may be affected by disease-related modification of the exposure. Randomized controlled trials are required to completely exclude this potential bias.[14] Fourth, for measuring allergic sensitization, we selected a panel of common inhalant and food allergens relevant to children of age 10 years. Other allergens, such as milk and egg, were not considered because of low sensitization rates at this age.[29] The scanned area method is recommended in research settings and corrects for interobserver variability and ethnic differences in skin response to histamine.[19] Fifth, we did not perform double-blind, placebo-controlled food challenges or physical examinations to establish a diagnosis of food allergy or eczema, respectively. This may have led to non-differential misclassification of these outcomes and most probably an underestimation of the observed effects. Instead, we used parental questionnaires with widely accepted and commonly used questions that reliably reflect the prevalence of eczema in young children at the population level.[20, 30] Finally, as in any observational study, residual confounding due to insufficiently or unmeasured confounders might still be present.

In conclusion, we observed no consistent association of timing and diversity of allergenic food introduction with childhood allergic sensitization, physician-diagnosed allergy or eczema. Children introduced to gluten and those introduced to ≥ 3 allergenic foods at early age had a decreased risk of eczema or physician-diagnosed inhalant allergy, respectively. However, these results do not provide strong evidence to change current feeding guidelines. Further studies are needed to replicate our findings and to explore the specific underlying pathophysiological mechanisms.

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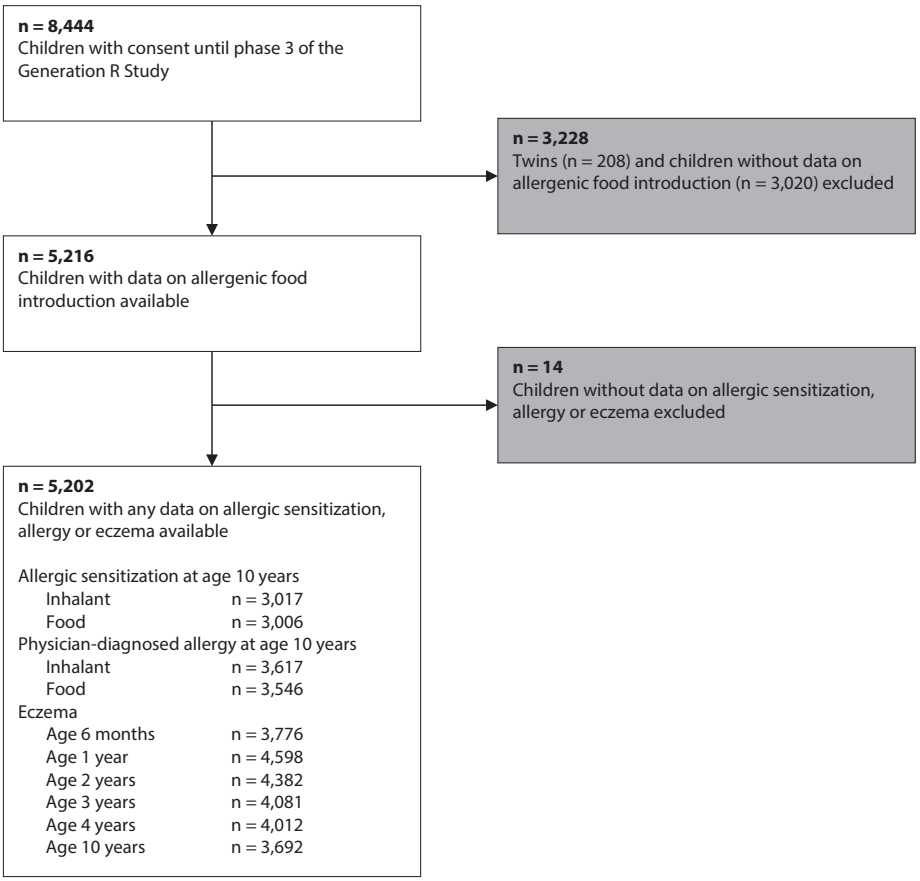
SUPPLEMENTARY MATERIAL

Supplementary Figure. Flowchart of participants.



Supplementary Table 1. Details of the multiple imputation model.

Software used: SPSS 21.0.0.1 for Windows (IBM Corp., Armonk, NY, USA)	
Imputation method used: fully conditional specification	
Model type for scale variables used: predictive mean matching	
Number of imputed datasets created: 25	
Maximum number of iterations: 20	
Imputed variables*:	
Outcomes:	eczema at ages 6 months and 1, 2, 3, 4 and 10 years
Determinants:	introduction of milk, egg, peanut, tree nuts, soy and gluten
Covariates:	maternal age, education, history of allergy, eczema or asthma, parity, pet keeping during pregnancy, body mass index at enrollment, smoking during pregnancy, and psychiatric symptoms during pregnancy; child's sex, gestational age at birth, birth weight, ethnic origin, breastfeeding ever, day care attendance until age 1 year, antibiotic use until age 1 year, body mass index at age 10–13 months, cow's milk allergy at age 1 year, and ointment use for eczema at age 2 months
Additional indicator variables:	
Outcomes:	ever eczema at age 10 years; histamine equivalent intracutaneous coefficient value for house dust mite, birch, 5-grass mixture, dog, cat, cashew, peanut and peach; physician-diagnosed allergy to pollen, house dust mite, cat, dog, peanut and cashew nut
Determinants:	introduction of fruits or vegetables
Covariates:	paternal age, education, history of allergy, eczema or asthma, body mass index at enrollment, smoking during pregnancy, and psychiatric symptoms during pregnancy; child's exclusiveness of breastfeeding; household income
Treatment of binary or categorical variables: logistic	
Statistical interactions included in imputation models: none	

*Also included in the multiple imputation model as indicator variables.

Supplementary Table 2. Characteristics of mothers and their children (n = 5,202).

	Observed	Imputed
Maternal characteristics		
Age at enrollment (years)*	31.1 (4.8)	31.1 (4.8)
Missing	*0 (0)*	*0 (0)*
Education (%)		
Primary or secondary	44.8 (2,229)	46.5 (2,418)
Higher	55.2 (2,751)	53.5 (2,784)
Missing	*4.3 (222)*	*0 (0)*
History of allergy, eczema or asthma (%)		
No	60.9 (2,658)	62.1 (3,230)
Yes	39.1 (1,705)	37.9 (1,972)
Missing	*16.1 (839)*	*0 (0)*
Parity (%)		
0	58.7 (2,984)	58.5 (3,044)
≥ 1	41.3 (2,102)	41.5 (2,158)
Missing	*2.2 (116)*	*0 (0)*
Pet keeping during pregnancy (%)		
No	65.4 (2,774)	65.1 (3,388)
Yes	34.6 (1,467)	34.9 (1,814)
Missing	*18.5 (961)*	*0 (0)*
Body mass index at enrollment (kg/m^2)†	23.6 (18.8–35.6)	23.6 (18.8–35.6)
Missing	*7.5 (391)*	*0 (0)*
Smoking during pregnancy (%)		
No	77.2 (3,633)	77.0 (4,007)
Yes	22.8 (1,076)	23.0 (1,195)
Missing	*9.5 (493)*	*0 (0)*
Psychiatric symptoms during pregnancy†	0.13 (0–1.29)	0.13 (0–1.33)
Missing	*19.0 (990)*	*0 (0)*
Child characteristics		
Sex (%)		
Male	49.6 (2,579)	49.6 (2,579)
Female	50.4 (2,623)	50.4 (2,623)
Missing	*0 (0)*	*0 (0)*
Gestational age at birth (weeks)†	40.1 (36.0–42.3)	40.1 (36.0–42.3)
Missing	*0.2 (9)*	*0 (0)*
Birth weight (grams)*	3,454 (550)	3,454 (549)
Missing	*0.1 (5)*	*0 (0)*

Supplementary Table 2. Characteristics of mothers and their children (n = 5,202). (continued)

	Observed	Imputed
Ethnic origin (%)		
European	72.8 (3,750)	72.5 (3,770)
Non-European	27.2 (1,400)	27.5 (1,432)
Missing	*1.0 (52)*	*0 (0)*
Breastfed ever (%)		
No	7.8 (399)	8.4 (439)
Yes	92.2 (4,709)	91.6 (4,763)
Missing	*1.8 (94)*	*0 (0)*
Breastfeeding duration (%)		
Never	9.0 (399)	8.4 (439)
< 6 months	58.3 (2,574)	56.7 (2,949)
≥ 6 months	32.7 (1,443)	34.9 (1,814)
Missing	*15.1 (786)*	*0 (0)*
Ointment use for eczema at age 2 months (%)		
No	93.0 (3,336)	92.4 (4,809)
Yes	7.0 (252)	7.6 (393)
Missing	*31.0 (1,614)*	*0 (0)*
Cow's milk allergy until age 1 year (%)		
No	94.2 (4,856)	94.1 (4,897)
Yes	5.8 (301)	5.9 (305)
Missing	*0.9 (45)*	*0 (0)*
Day care attendance until age 1 year (%)		
No	41.0 (1,767)	42.5 (2,210)
Yes	59.0 (2,541)	57.5 (2,992)
Missing	*17.2 (894)*	*0 (0)*
Antibiotic use until age 1 year (%)		
No	77.8 (3,032)	78.0 (4,059)
Yes	22.2 (864)	22.0 (1,143)
Missing	*25.1 (1,306)*	*0 (0)*
Body mass index at age 10–13 months (kg/m^2)[†]	17.3 (14.9–20.3)	17.3 (14.9–20.3)
Missing	*18.3 (954)*	*0 (0)*
Introduction of cow's milk (%)		
≤ 6 months	73.9 (3,830)	74.0 (3,847)
> 6 months	26.1 (1,351)	26.0 (1,355)
Missing	*0.4 (21)*	*0 (0)*

Supplementary Table 2. Characteristics of mothers and their children (n = 5,202). (continued)

	Observed	Imputed
Introduction of hen's egg (%)		
≤ 6 months	11.5 (551)	14.2 (741)
> 6 months	88.5 (4,244)	85.8 (4,461)
Missing	*7.8 (407)*	*0 (0)*
Introduction of peanut (%)		
≤ 6 months	2.9 (141)	5.8 (303)
> 6 months	97.1 (4,647)	94.2 (4,899)
Missing	*8.0 (414)*	*0 (0)*
Introduction of tree nuts (%)		
≤ 6 months	0.5 (26)	4.5 (236)
> 6 months	99.5 (4,717)	95.5 (4,966)
Missing	*8.8 (459)*	*0 (0)*
Introduction of soy (%)		
≤ 6 months	18.7 (930)	20.3 (1,055)
> 6 months	81.3 (4,046)	79.7 (4,147)
Missing	*4.3 (226)*	*0 (0)*
Introduction of gluten (%)		
≤ 6 months	43.4 (2,196)	44.2 (2,298)
> 6 months	56.6 (2,861)	55.8 (2,904)
Missing	*2.8 (145)*	*0 (0)*
Diversity of allergenic foods introduced at age ≤ 6 months (%)		
No allergenic foods introduced	19.7 (916)	18.2 (945)
1 allergenic food introduced	36.6 (1,705)	33.7 (1,754)
2 allergenic foods introduced	31.0 (1,442)	29.1 (1,516)
≥ 3 allergenic foods introduced	12.7 (594)	19.0 (987)
Missing	*10.5 (545)*	*0 (0)*
Allergic sensitization at age 10 years – inhalant (%)		
No	68.5 (2,068)	68.5 (2,068)
Yes	31.5 (949)	31.5 (949)
Missing	*42.0 (2,185)*	*42.0 (2,185)*
Allergic sensitization at age 10 years – food (%)		
No	93.3 (2,804)	93.3 (2,804)
Yes	6.7 (202)	6.7 (202)
Missing	*42.2 (2,196)*	*42.2 (2,196)*
Physician-diagnosed allergy at age 10 years – inhalant (%)		
No	88.0 (3,182)	88.0 (3,182)
Yes	12.0 (435)	12.0 (435)
Missing	*30.5 (1,585)*	*30.5 (1,585)*

Supplementary Table 2. Characteristics of mothers and their children (n = 5,202). (continued)

	Observed	Imputed
Physician-diagnosed allergy at age 10 years – food (%)		
No	97.7 (3,465)	97.7 (3,465)
Yes	2.3 (81)	2.3 (81)
Missing	*31.8 (1,656)*	*31.8 (1,656)*
Allergic sensitization and allergy combined at age 10 years (%)		
No allergic sensitization and no allergy	67.1 (1,759)	67.1 (1,759)
Any allergic sensitization, but no allergy	21.9 (574)	21.9 (574)
No allergic sensitization, but any allergy	1.2 (31)	1.2 (31)
Any allergic sensitization and any allergy	9.8 (258)	9.8 (258)
Missing	*49.6 (2,580)*	*49.6 (2,580)*
Eczema last 6 months at age 6 months (%)		
No	83.8 (3,166)	83.2 (4,374)
Yes	16.2 (610)	17.8 (928)
Missing	*27.4 (1,426)*	*0 (0)*
Eczema last 6 months at age 1 year (%)		
No	87.2 (4,009)	86.7 (4,512)
Yes	12.8 (589)	13.3 (690)
Missing	*11.6 (604)*	*0 (0)*
Eczema last 12 months at age 2 years (%)		
No	86.7 (3,800)	86.3 (4,490)
Yes	13.3 (582)	13.7 (712)
Missing	*15.8 (820)*	*0 (0)*
Eczema last 12 months at age 3 years (%)		
No	90.7 (3,701)	90.0 (4,683)
Yes	9.3 (380)	10.0 (519)
Missing	*21.5 (1,121)*	*0 (0)*
Eczema last 12 months at age 4 years (%)		
No	92.2 (3,700)	91.6 (4,762)
Yes	7.8 (312)	8.5 (440)
Missing	*22.9 (1,190)*	*0 (0)*
Eczema last 12 months at age 10 years (%)		
No	93.4 (3,447)	90.1 (4,754)
Yes	6.6 (245)	8.6 (448)
Missing	*29.0 (1,510)*	*0 (0)*

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed and imputed data. Data on allergic sensitizations and physician-diagnosed allergies are not imputed.

Supplementary Table 3. Characteristics of mothers and children included and not included in the study.

	Included n = 5,202	Not included n = 3,242	*P*-value for difference
Maternal characteristics			
Age at enrollment (years)*	31.1 (4.8)	28.8 (5.7)	**< 0.001**
Missing	*0 (0)*	*0.1 (2)*	
Education (%)			**< 0.001**
Primary or secondary	44.8 (2,229)	75.0 (1,942)	
Higher	55.2 (2,751)	25.0 (649)	
Missing	*4.3 (222)*	*20.1 (651)*	
History of allergy, eczema or asthma (%)			0.68
No	60.9 (2,658)	61.4 (1,401)	
Yes	39.1 (1,705)	38.6 (879)	
Missing	*16.1 (839)*	*29.7 (962)*	
Parity (%)			**< 0.001**
0	58.7 (2,984)	50.0 (1,533)	
≥ 1	41.3 (2,102)	50.0 (1,535)	
Missing	*2.2 (116)*	*5.4 (174)*	
Pet keeping during pregnancy (%)			**< 0.01**
No	65.4 (2,774)	69.4 (1,550)	
Yes	34.6 (1,467)	30.6 (683)	
Missing	*18.5 (961)*	*31.1 (1,009)*	
Body mass index at enrollment (kg/m^2)†	23.6 (18.8–35.6)	24.4 (18.6–37.3)	**< 0.001**
Missing	*7.5 (391)*	*14.5 (469)*	
Smoking during pregnancy (%)			**< 0.001**
No	77.2 (3,633)	68.1 (1,735)	
Yes	22.8 (1,076)	31.9 (811)	
Missing	*9.5 (493)*	*21.5 (696)*	
Psychiatric symptoms during pregnancy†	0.13 (0–1.29)	0.23 (0–1.64)	**< 0.001**
Missing	*19.0 (990)*	*45.5 (1,474)*	
Child characteristics			
Sex (%)			**< 0.05**
Male	49.6 (2,579)	51.9 (1,683)	
Female	50.4 (2,623)	48.1 (1,557)	
Missing	*0 (0)*	*0.1 (2)*	
Gestational age at birth (weeks)†	40.1 (36.0–42.3)	39.9 (34.1–42.2)	**< 0.001**
Missing	*0.2 (9)*	*1.9 (62)*	
Birth weight (grams)*	3,454 (550)	3,310 (608)	**< 0.001**
Missing	*0.1 (5)*	*0.8 (27)*	

Supplementary Table 3. Characteristics of mothers and children included and not included in the study. (continued)

	Included n = 5,202	**Not included n = 3,242**	***P*-value for difference**
Ethnic origin (%)			**< 0.001**
European	72.8 (3,750)	47.9 (1,404)	
Non-European	27.2 (1,400)	52.1 (1,529)	
Missing	*1.0 (52)*	*9.5 (309)*	
Breastfed ever (%)			**< 0.05**
No	7.8 (399)	10.1 (118)	
Yes	92.2 (4,709)	89.9 (1,050)	
Missing	*1.8 (94)*	*64.0 (2,074)*	
Day care attendance until age 1 year (%)			0.52
No	41.0 (1,767)	44.0 (51)	
Yes	59.0 (2,541)	56.0 (65)	
Missing	*17.2 (894)*	*96.4 (3,126)*	
Antibiotic use until age 1 year (%)			0.26
No	77.8 (3,032)	73.6 (92)	
Yes	22.2 (864)	26.4 (33)	
Missing	*25.1 (1,306)*	*96.1 (3,117)*	
Body mass index at age 10–13 months (kg/m^2)†	17.3 (14.9–20.3)	17.4 (14.7–20.6)	0.29
Missing	*18.3 (954)*	*63.5 (2,060)*	
Introduction of cow's milk (%)			0.32
≤ 6 months	73.9 (3,830)	70.2 (99)	
> 6 months	26.1 (1,351)	29.8 (42)	
Missing	*0.4 (21)*	*95.7 (3,101)*	
Introduction of hen's egg (%)			0.09
≤ 6 months	11.5 (551)	6.6 (8)	
> 6 months	88.5 (4,244)	93.4 (114)	
Missing	*7.8 (407)*	*96.2 (3,120)*	
Introduction of peanut (%)			0.83
≤ 6 months	2.9 (141)	3.3 (4)	
> 6 months	97.1 (4,647)	96.7 (118)	
Missing	*8.0 (414)*	*96.2 (3,120)*	
Introduction of tree nuts (%)			0.70
≤ 6 months	0.5 (26)	0.8 (1)	
> 6 months	99.5 (4,717)	99.2 (122)	
Missing	*8.8 (459)*	*96.2 (3,119)*	

Supplementary Table 3. Characteristics of mothers and children included and not included in the study. (continued)

	Included n = 5,202	**Not included n = 3,242**	***P*-value for difference**
Introduction of soy (%)			0.41
≤ 6 months	18.7 (930)	21.5 (29)	
> 6 months	81.3 (4,046)	78.5 (106)	
Missing	*4.3 (226)*	*95.8 (3,107)*	
Introduction of gluten (%)			**< 0.05**
≤ 6 months	43.4 (2,196)	32.6 (42)	
> 6 months	56.6 (2,861)	67.4 (87)	
Missing	*2.8 (145)*	*96.0 (3,113)*	
Diversity of allergenic foods introduced at age ≤ 6 months (%)			0.09
No allergenic foods introduced	19.7 (916)	28.3 (34)	
1 allergenic food introduced	36.6 (1,705)	37.5 (45)	
2 allergenic foods introduced	31.0 (1,442)	22.5 (27)	
≥ 3 allergenic foods introduced	12.7 (594)	11.7 (14)	
Missing	*10.5 (545)*	*96.3 (3,122)*	

Values are *means (SD), †medians (2.5–97.5th percentile) or percentages (absolute numbers) based on observed data. *P*-values for difference are calculated by independent samples T-test for continuous variables with a normal distribution, the Mann-Whitney U-test for continuous variables with a skewed distribution, and Pearson's Chi-square test for categorical variables. Bold values indicate statistical significance at the $\alpha = 0.05$ level.

Supplementary Table 4. Associations of timing and diversity of allergenic food introduction with combined allergic sensitization and physician-diagnosed allergy groups in children at age 10 years.

	Odds ratio (95% confidence interval) for any allergic sensitization and any physician-diagnosed allergy combined		
	Any allergic sensitization, but no allergy n = 574	**No allergic sensitization, but any allergy n = 31**	**Any allergic sensitization and any allergy n = 258**
Allergenic food introduced at age ≤ 6 months*			
Cow's milk (n = 3,847)	0.89 (0.71, 1.12)	0.55 (0.24, 1.24)	0.84 (0.61, 1.14)
Hen's egg (n = 741)	1.12 (0.82, 1.52)	0.48 (0.11, 2.17)	0.86 (0.52, 1.43)
Peanut (n = 303)	0.67 (0.36, 1.25)	N.A.	0.83 (0.39, 1.78)
Tree nuts (n = 236)	1.04 (0.46, 2.33)	N.A.	1.08 (0.26, 4.49)
Soy (n = 1,055)	1.00 (0.77, 1.28)	1.24 (0.50, 3.05)	1.15 (0.82, 1.60)
Gluten (n = 2,904)	0.93 (0.76, 1.14)	0.45 (0.20, 1.02)	0.95 (0.72, 1.27)
Diversity of allergenic foods introduced at age ≤ 6 months†			
1 allergenic food introduced (n = 1,754)	0.84 (0.63, 1.12)	0.54 (0.20, 1.47)	0.88 (0.59, 1.32)
2 allergenic foods introduced (n = 1,516)	0.82 (0.61, 1.10)	0.48 (0.17, 1.37)	0.89 (0.59, 1.35)
≥ 3 allergenic foods introduced (n = 987)	0.88 (0.62, 1.23)	0.30 (0.08, 1.12)	0.88 (0.54, 1.43)
P-value for trend	0.27	0.55	0.63

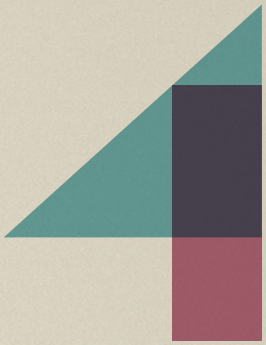
Values are odds ratios (95% confidence interval) from multinomial logistic regression models based on imputed data. Reference group is children without any allergic sensitization or physician-diagnosed allergy (n = 1,759), and with *allergenic food introduction at age > 6 months or †no allergenic foods introduced at age ≤ 6 months. Models are adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin, breastfeeding, day care attendance and antibiotic use. N.A. = not available due to low number of children.

Supplementary Table 5. Associations of timing and diversity of allergenic food introduction with eczema per year and overall in children until age 10.

	Odds ratio (95% confidence interval) for eczema						
	6 months	**1 year**	**2 years**	**3 years**	**4 years**	**10 years**	**Overall**
Allergenic food introduced at age ≤ 6 months*							
Cow's milk (n = 3,847)	0.62 (0.32, 1.22)	0.86 (0.71, 1.06)	0.85 (0.70, 1.04)	0.93 (0.74, 1.17)	1.12 (0.85, 1.48)	1.01 (0.78, 1.33)	0.82 (0.63, 1.07)
Hen's egg (n = 741)	0.74 (0.54, 1.01)	0.67 (0.42, 1.07)	0.79 (0.58, 1.09)	0.80 (0.53, 1.22)	1.06 (0.73, 1.54)	1.27 (0.85, 1.88)	0.87 (0.73, 1.03)
Peanut (n = 303)	0.68 (0.38, 1.21)	0.79 (0.30, 2.13)	0.80 (0.45, 1.41)	0.77 (0.38, 1.57)	0.67 (0.28, 1.59)	0.89 (0.39, 2.02)	0.77 (0.49, 1.22)
Tree nuts (n = 236)	0.89 (0.30, 2.61)	1.07 (0.36, 3.15)	0.93 (0.46, 1.88)	1.10 (0.40, 2.97)	1.21 (0.47, 3.11)	1.25 (0.49, 3.20)	1.08 (0.61, 1.91)
Soy (n = 1,055)	0.88 (0.68, 1.13)	0.98 (0.70, 1.36)	0.97 (0.75, 1.24)	0.98 (0.73, 1.32)	1.20 (0.88, 1.62)	1.15 (0.84, 1.57)	1.00 (0.87, 1.15)
Gluten (n = 2,904)	0.71 (0.50, 1.01)	**0.75 (0.60, 0.95)**	0.88 (0.73, 1.06)	0.93 (0.73, 1.18)	0.91 (0.72, 1.17)	1.07 (0.80, 1.44)	**0.84 (0.72, 0.99)**
Diversity of allergenic foods introduced at age ≤ 6 months†							
1 allergenic food introduced (n = 1,754)	0.75 (0.41, 1.36)	0.99 (0.79, 1.25)	0.88 (0.69, 1.12)	0.98 (0.73, 1.31)	0.98 (0.71, 1.36)	1.02 (0.72, 1.45)	0.88 (0.69, 1.13)
2 allergenic foods introduced (n = 1,516)	0.58 (0.29, 1.19)	0.81 (0.64, 1.04)	0.90 (0.70, 1.15)	0.95 (0.70, 1.29)	1.10 (0.79, 1.52)	1.07 (0.75, 1.53)	0.82 (0.60, 1.11)
≥ 3 allergenic foods introduced (n = 987)	0.52 (0.24, 1.13)	0.67 (0.43, 1.06)	**0.71 (0.51, 0.99)**	0.84 (0.56, 1.26)	1.07 (0.71, 1.60)	1.17 (0.75, 1.85)	0.75 (0.54, 1.03)
P-value for trend	0.52	0.51	0.77	0.89	0.80	0.78	0.57

Values are odds ratios (95% confidence interval) from generalized estimating equation models based on imputed data. Bold values indicate statistical significance at the α = 0.05 level. Reference group is children with *allergenic food introduction at age > 6 months or †no allergenic foods introduced at age ≤ 6 months. Models are adjusted for maternal age at enrollment, education, history of allergy, eczema or asthma, parity, pet keeping, body mass index at enrollment, smoking, psychiatric symptoms, and child's sex, gestational age, birth weight, ethnic origin, breastfeeding, day care attendance and antibiotic use.

CHAPTER 4



GENERAL DISCUSSION

INTRODUCTION

Eczema is a chronic inflammatory skin disease that inflicts important health and economic effects at the population level.[1] Eczema may occur solely or coincide with allergic sensitization and symptoms of allergy as part of an atopic constitution.[2] It is suggested that eczema might have at least part of its origin in fetal life and infancy.[3,4] This phenomenon of early-life exposures that modulate the susceptibility to chronic diseases in later life is commonly referred to as 'developmental programming' or the 'Developmental Origins of Health and Disease (DOHaD)' hypothesis.[5] Adverse exposures may result in specific adaptations with short-term developmental and survival benefits, but may eventually lead to disease.

The aim of this thesis was to identify fetal and infant exposures that affect the development of eczema, allergic sensitization or allergy in childhood. In previous chapters, the main findings, merits and limitations of the studies presented in this thesis have already been discussed in detail. This chapter provides a general overview of the main findings, highlights methodological considerations in epidemiological studies, discusses the clinical implications of the main findings and suggests directions for future research.

MAIN FINDINGS

Previous epidemiological research has identified important early-life risk and protective factors associated with the development of eczema, allergic sensitization or allergy, but studies have focused primarily on preschool-age and high-risk children of Caucasian origin.[6,7] Data from multi-ethnic longitudinal studies relating early-life environmental and genetic exposures with eczema, allergic sensitization and allergy in older children from the general population remain scarce. The main findings of the studies presented in this thesis are summarized in the Table.

Fetal exposures

In the studies presented in this thesis, we examined the associations of two important fetal exposures with childhood eczema, allergic sensitization or allergy.

First, maternal psychiatrics symptoms during pregnancy is suggested to increase the risk of childhood eczema or allergy via developmental adaptations of the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic adrenomedullary system and immune responses in the offspring.[8] We observed that maternal overall and anxiety symptoms during pregnancy were associated with an up to 1.21-fold increased risk of eczema until age 10 years. Furthermore, maternal overall psychiatric, depressive and anxiety symptoms during pregnancy were associated with an up to 1.96-fold increased risk of physician-diagnosed inhalant allergy at age 10 years. These associations were independent of maternal psychiatric symptoms after delivery, paternal psychiatric symptoms during or

after delivery, and a large set of potential confounding factors such as maternal education, ethnic origin and history of allergy, eczema or asthma. We observed no association of maternal psychiatric symptoms during pregnancy with allergic sensitization or food allergy. Our findings suggest a possible intrauterine programming effect of maternal psychiatric symptoms that increases the child's risk of developing atopic diseases.

Second, vitamin D is suggested to influence immune regulation, epidermal barrier function and bacterial defense.[9, 10] However, the role of vitamin D in the development of childhood eczema remains controversial as previous cohort studies, that varied greatly in sample size, method and timing of vitamin D measurement, definition of eczema, and confounder adjustment reported conflicting results.[9] We observed among 3,019 preschool-age children of Dutch origin that 25-hydroxyvitamin D levels in mid-gestation and at birth were not associated with the risk of childhood eczema. Other environmental factors, such as dietary and feeding habits and microbial exposure, or genetic factors might have a more important role in the onset of childhood eczema.

In summary, we identified adverse effects of maternal psychiatric symptoms on eczema and physician-diagnosed inhalant allergy in school-age children, but no effect of vitamin D levels in mid-gestation and at birth on eczema in preschool-age children. Future studies are needed to develop intervention strategies focused on reducing maternal psychiatric symptoms during pregnancy to prevent the occurrence of eczema and allergy.

Infant exposures

In the studies presented in this thesis, we examined the associations of three important common infant exposures with childhood eczema, allergic sensitization or allergy.

First, the prevalence of eczema varies considerably among children of different ethnic groups.[11] Also, it was recently observed that differences in structural and functional epidermal barrier characteristics exist between adults of African American, Caucasian and East Asian descent.[12] In our multi-ethnic population-based prospective cohort, we observed that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children had an up to 2.06-fold increased risk of eczema until age 4 years. The maximum change in effect size for these associations occurred when we adjusted separately for maternal education and overall psychiatric symptoms during pregnancy. Surinamese-Creole and Surinamese-Hindustani children remained to have increased risks of eczema after adjustment for environmental and genetic risk factors. However, precise quantification of attribution of each of these factors separately to the association of ethnic origin with the risk of eczema is difficult due to collinearity.

Second, breastfeeding might have immune modulatory effects that influence the development of childhood eczema, allergic sensitization and allergy via complex interactions of human milk components such as immunoglobulin A, cytokines, chemokines, long-chain polyunsaturated fatty acids, polyamines, and sensitizing and tolerance promoting al-

lergens.[13] Recently, a meta-analysis of observational studies showed that longer duration and exclusiveness of breastfeeding were associated with an up to 26% decreased risk of eczema and allergic rhinitis in preschool-age children.[14] Using more detailed information on breastfeeding status, we showed that shorter duration or non-exclusiveness of breastfeeding is associated with an up to 1.16-fold increased risk of eczema until age 10 years. However, we observed no association of breastfeeding with allergic sensitization or physician-diagnosed allergy. Our results seemed neither affected by disease-related modification of the exposure nor modified by maternal history of eczema, allergy or asthma. Despite the small observed protective effect, breastfeeding should be encouraged because of its nutritional, immunological and psychosocial benefits.

Third, the role of timing and diversity of allergenic food introduction in the development of childhood eczema, allergic sensitization and allergy has been a much debated topic in the past decades. An accumulating body of evidence suggests that a critical window of immunological opportunity exists at age 4–6 months to expose children to food proteins and induce immune tolerance.[15, 16] However, we showed that neither timing nor diversity of allergenic food introduction, including the introduction of cow's milk, hen's egg, peanut, tree nuts, soy and gluten, at age ≤ 6 months is consistently associated with eczema, allergic sensitization or allergy in children until age 10 years. Children introduced to gluten at age ≤ 6 months had a 16% decreased risk of eczema, and children introduced to ≥ 3 allergenic foods at age ≤ 6 months had a 36% decreased risk of physician-diagnosed inhalant allergy. To some extent, disease-related modification of the exposure was present within our study, particularly for the association of early gluten introduction with eczema until age 10 years. Overall, our results do not provide strong evidence to adapt current international feeding guidelines.

In summary, we observed that in our multi-ethnic cohort the risk of developing eczema at preschool-age varies among children of different ethnic origin. Furthermore, we identified adverse effects of shorter and non-exclusive breastfeeding, but no consistent protective effect of timing or higher diversity of allergenic food introduction at early age on eczema in school-age children. Further research is warranted to replicate our findings and to explore the underlying biological mechanisms.

METHODOLOGICAL CONSIDERATIONS

The research presented in this thesis was embedded in the Generation R Study, a large multi-ethnic population-based prospective cohort study from fetal life onwards in Rotterdam, The Netherlands.[17] Specific methodological considerations for individual studies have been discussed in the respective chapters of this thesis. In the following paragraphs, general methodological issues with regard to internal and external validity are discussed.

Selection bias

Selection bias may occur if the association of the exposure with the outcome of interest is different between study participants and those who are eligible but do not participate in the study. The overall response rate of participating children eligible at birth was 61%.[17] It is unlikely that this non-response at baseline is at random. Participating children were less often of non-Dutch origin. Parents of participating children had a higher household income and higher educational attainment[18], which is in line with data from other large prospective cohort studies.[19] Also, parents of participating children had less depressive symptoms than expected from the population figures in Rotterdam.[20] Altogether, the non-response at baseline suggests a selection toward a relatively more affluent and healthier study population, which might have resulted in lower prevalence rates of diseases and, subsequently, reduced statistical power. However, several studies have shown that in cohort studies associations are not markedly influenced by selective non-participation at baseline.[21, 22] Therefore, we consider it less likely that our results are biased by selective non-response at baseline.

Selection bias may also occur if the association of the exposure with the outcome of interest is different between those included in the analyses and those lost to follow-up. Of all live born children enrolled in the Generation R Study (n = 9,749), 75.8% (n = 7,393) participated in the follow-up studies at age 10 years[17], of whom 76.4% (n = 5,645) had information available on eczema, allergic sensitization or physician-diagnosed allergy. Mothers who did not answer questionnaires related to specific research questions or did not visit the research center for skin prick testing of their child were younger, lower educated and had lower folate levels during pregnancy, and their children were more often of non-Dutch origin and less often breastfed. This selective loss to follow-up toward a more affluent and healthier population might have biased the observed effect estimates, but quantifying this bias is difficult. We performed multiple imputation analyses to minimize the risk of selection bias due to missing values of covariates.[23, 24]

Information bias

Information bias refers to a systematic error due to misclassification of participant data. Misclassification is differential (i.e., non-random) if it is different for participants with and without the exposure or outcome of interest. Misclassification is non-differential (i.e., random) if it is unrelated to the occurrence or presence of the exposure or outcome. Differential misclassification may result in an over- or underestimation of the true effect, while non-differential misclassification usually leads to underestimated effect estimates.[25]

Participant information on exposures of interest to the studies described in this thesis, including maternal psychiatric symptoms during pregnancy, maternal and fetal vitamin D levels in mid-gestation and at birth, respectively, child's ethnic origin, breastfeeding

and allergenic food introduction, were collected longitudinally and before assessment of most outcomes. Both researchers and participants were unaware of specific research questions, which limits the probability of differential misclassification. Although the classification of the child's ethnic origin is objective, reproducible and easily applicable in epidemiological studies, some non-differential misclassification might have occurred as third-generation migrants were defined as being of Dutch origin but might still have a different skin structure. This might have reduced the contrast between children of Dutch origin and other ethnic groups and, as a result, diluted our effect estimates. Also, non-differential misclassification of maternal psychiatric symptoms during pregnancy or breastfeeding or complementary feeding habits might have occurred due to socially desirable responding. It is difficult to speculate on the direction of the bias introduced by this random measurement error.

Childhood eczema, allergic sensitization and allergy were the outcomes examined in this thesis. For eczema, we relied on data derived from parental questionnaires, consisting widely accepted and commonly used questions that reliably reflect the prevalence of eczema in children at the population level.[26] A parental-reported physician diagnosis of eczema in the past year based on a single question has demonstrated sufficient validity for the epidemiological study of childhood eczema.[27] For allergy, we were not able to use an accepted and validated questionnaire but used questions adapted from the International Study of Asthma and Allergies in Childhood core questionnaires instead.[28] Parental-reported disease may give rise to non-differential misclassification, which most probably resulted in an underestimation of the true effects. Also, mothers with psychiatric symptoms or breastfeeding mothers may be more anxious or aware of their child's health and, therefore, be more likely to have skin rashes or allergic symptoms in their child presented for medical review, thus increasing the probability of diagnosing eczema or allergy. This might have resulted in differential misclassification and, subsequently, an overestimation of the harmful effect of maternal psychiatric symptoms during pregnancy and an underestimation of the protective effect of breastfeeding. Allergic sensitization was measured by skin prick tests using the scanned area method.[29] Because this method is objective, computerized and corrects for interobserver variability and ethnic differences in skin response to histamine, it is unlikely that differential or non-differential misclassification occurred.

Confounding

A confounder is an extraneous factor associated with both the exposure and the outcome, and is not an intermediary step in the causal pathway. If not controlled for, confounding may cause biased effect estimates and lead to erroneous conclusions. In this thesis, we used two approaches to account for confounding.

First, we adjusted our analyses for multiple potential confounders. We selected covariates based on previous literature and further examined their potential confounding effect using statistical models. In most studies presented in this thesis, adjustment for potential confounders only moderately affected the effect estimates, which suggests that the observed associations of the exposures with the outcomes are possibly true associations. Although we adjusted for many potential confounders in our studies, we cannot rule out that specific results were affected by residual confounding due to insufficiently or unmeasured confounders, such as housing conditions, air pollution, vitamin or supplement intake, and microbial exposure.[6] Residual confounding might have resulted in an overestimation of the effect estimates.

Second, we used information on both maternal and paternal psychiatric symptoms to disentangle potential intrauterine and confounding mechanisms.[30, 31] Stronger effect estimates for the association of maternal than paternal psychiatric symptoms during pregnancy with childhood eczema or allergy suggest underlying intrauterine mechanisms, while similar effect estimates indicate that these associations may result from residual confounding of unmeasured factors. We observed that children of mothers with overall psychiatric, depressive or anxiety symptoms during pregnancy had an increased risk of physician-diagnosed inhalant allergy, and children of mothers with overall psychiatric or anxiety symptoms during pregnancy an increased risk of eczema. These results were independent of maternal psychiatric symptoms after delivery, and of paternal psychiatric symptoms during pregnancy and after delivery, suggesting a possible intrauterine programming effect of maternal psychiatric symptoms that increases the child's risk of developing atopic diseases.

External validity

External validity is the extent to which results of a study can be generalized to other populations. The Generation R Study is conducted in the general population of Rotterdam, The Netherlands. The largest ethnic groups were formed by children of Dutch, Surinamese, Turkish and Moroccan origin, but the distribution of ethnic groups differed moderately from that of the study area.[18] In our cohort, children were more often of Dutch origin, and in particular children of Moroccan origin were underrepresented. Both household income and educational attainment suggest a selection toward a population with a higher socioeconomic status. As discussed previously, parents of participating children had less depressive symptoms than expected from the population figures in Rotterdam.[20] This selection toward a more affluent and healthier population is similar in our follow-up assessments until age 10 years and may affect the generalizability of our findings. Therefore, caution is advised when extrapolating the results to other, less ethnically diverse, less affluent or less healthy populations.

CAUSALITY

We described associations, but not causal relationships, of fetal and infant exposures with childhood eczema, allergic sensitization and allergy because of the observational design of the Generation R Study. The Bradford Hill criteria provide a widely recognized basis for inferring causality in epidemiological studies.[32] These criteria include the strength, specificity, consistency and temporality of an association. Evidence for a causal relationship is further strengthened if a biological gradient or dose-response effect, a plausible mechanism between cause and effect, coherence with current knowledge on the biology of the disease, analogy with comparable exposures or experimental evidence is present.

When we apply the Bradford Hill criteria to our studies, we observed small to moderate overall effects estimates for the associations of fetal and infant exposures with childhood eczema, our main outcome of interest. Effect estimates were adjusted for a large number of confounders, and in general consistency with results reported by previous studies. A clear temporal relationship, meaning that the disease must occur after the exposure, is present in our study on the associations of maternal psychiatric symptoms during pregnancy with childhood eczema. However, ensuring temporality in our studies on breastfeeding and complementary feeding habits is more difficult. Early signs and symptoms of eczema or allergy in the child might have encouraged parents to alter breastfeeding or complementary feeding habits, because they might have been aware of a possible association of breastfeeding or complementary feeding habits with childhood eczema or allergy.[33] Similarly, breastfeeding or complementary feeding habits may be influenced by a family history of eczema, allergy or asthma. Mothers with a history of eczema, allergy or asthma may be more motivated or advised to prolong breastfeeding or avoid delayed introduction of allergenic foods and might have been more aware of symptoms of eczema or allergy in their children. Failure to account for this disease-related modification of the exposure may erroneously suggest that, for example, shorter duration of breastfeeding or delayed allergenic food introduction leads to eczema or allergy, while it might have been that the early onset of eczema or allergy encouraged mothers to extend breastfeeding or expedite complementary feeding. Therefore, we performed risk period-specific sensitivity analyses, additional adjustments, tests for interaction or cross-lagged modeling.[34, 35] For analyses on breastfeeding, the size and direction of the effect estimates did not materially change and, therefore, we consider our results most probably not affected by disease-related modification of the exposure. For analyses on timing or diversity of allergenic food introduction, to some extent disease-related modification of the exposure existed within our study. More specifically, for the association of early gluten introduction with eczema until age 10 years, we observed that the effect estimate attenuated toward the null and was no longer significant. Furthermore, we observed a dose-response effect for maternal psychiatric symptoms during preg-

nancy and breastfeeding duration on the risk of developing childhood eczema. For all observed associations of fetal and infant exposures with childhood eczema, plausible underlying biological mechanisms and coherence with animal studies or laboratory findings are available. We observed that gluten was the only allergenic food associated with a decreased risk of eczema when introduced at age < 6 months. However, studies that assessed analogue factors for the allergenic foods investigated in our study may strengthen the evidence for a causal relationship. For example, early introduction of fish has been associated with a decreased risk of childhood eczema.[36] Theoretically, a causal effect of fetal or infant exposures on childhood eczema, allergic sensitization or allergy can be proven using a randomized controlled trial (RCT). However, randomized exposure to factors with known adverse or beneficial health effects, such as maternal stress during pregnancy and breastfeeding, respectively, is unethical. Instead, experimental interventions that promote breastfeeding or early introduction of allergenic foods in conformity with current guidelines may provide additional insight into the causality of these exposures on childhood eczema or allergy. This is illustrated by the PROBIT, LEAP, LEAP-On and EAT trials. The PROBIT trial showed that children of mothers who were intensively promoted to breastfeed according to guidelines of the World Health Organization (WHO) had a decreased risk of eczema during the first year of life.[37] The LEAP and LEAP-On trials showed that peanut introduction in high-risk children aged 4–11 months was associated with a decreased frequency of peanut allergy that persisted after 1 year of peanut avoidance[38, 39], which resulted in recent addendum guidelines for the prevention of peanut allergy in high-risk children.[40] However, the EAT trial did not show an effect of early introduction of common allergenic foods on the frequency of food allergies at age 1–3 years among a selected group of exclusively breast-fed children from the general population.[41] In summary, our epidemiological studies provide moderate to good evidence for causal relationships of fetal and infant exposures with childhood eczema based on the Bradford Hill criteria and the results from previous animal and experimental studies.

CLINICAL IMPLICATIONS

We identified several fetal and infant exposures associated with an increased risk of childhood eczema or allergy, supporting the hypothesis that atopic diseases might partly have their origin in fetal life and infancy.[3, 4] Our results might have clinical implications, including the development of lifestyle interventions for the primary prevention of atopic diseases already from fetal life onwards, and the identification of high-risk children.

Specifically, our observation that children of mothers with psychiatric symptoms during pregnancy have increased risks of eczema and physician-diagnosed inhalant allergy

could be encouraging to develop public preventive intervention strategies aimed at stress reduction or elimination.[42] Strategies such as physical relaxation and meditation, stress assessment and education, and counseling and social support have shown promising results on reducing the risk of preterm birth, low birth weight and intrauterine growth retardation.[43] Pregnancy is an important period where women are likely to be more motivated to make beneficial lifestyle changes.

Eczema is more difficult to diagnose in children with skin of colour because erythema may be less noticeable.[44] Therefore, our observation that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children have an increased risk of eczema, compared with Dutch children, should raise clinicians' awareness of the disease among these ethnic groups.

Currently, the European Academy of Allergy and Clinical Immunology, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition and the Dutch Youth Health Centre recommend exclusive breastfeeding for 4–6 months and not to avoid or delay the introduction of potentially allergenic foods beyond age 4 months, irrespective of atopic heredity.[45–47] The WHO and the American Academy of Pediatrics recommend exclusive breastfeeding until age 6 months.[48, 49] Our studies on the effects of breastfeeding and allergenic food introduction do not provide strong evidence to change the contrary feeding guidelines of these expert bodies.

Our observations that vitamin D levels in mid-gestation and at birth are not associated with the risk of eczema, that children introduced to gluten at age ≤ 6 months have a decreased risk of eczema and that children introduced to ≥ 3 allergenic foods at age ≤ 6 months have a decreased risk of physician-diagnosed inhalant allergy require further replication and exploration of underlying pathophysiological mechanisms.

FUTURE PERSPECTIVES

We examined associations of fetal and infant exposures with childhood eczema, allergic sensitization and allergy based on data collected in an observational study. Therefore, we cannot establish causality of the observed associations. Although an RCT is the preferred study design to infer causal relationships, using this study design to assess the causal effects of some of the exposures studied in this thesis might have ethical limitations. Alternative designs, such as the aforementioned PROBIT, LEAP, LEAP-On and EAT trials, might provide additional evidence for causality of the observed associations. The heterogeneous findings from observational studies regarding the effects of maternal and fetal vitamin D levels on childhood eczema emphasize the need for well-designed RCTs. Thus far, two RCTs have showed no effect of vitamin D supplementation in pregnant women on allergy-related outcomes in their offspring at age 3 years.[50, 51] One trial among 158 pregnant women showed that daily supplementation with 800

IU of ergocalciferol or a single bolus of 200,000 IU of cholecalciferol during the third trimester of pregnancy was not associated with eczema, allergic sensitization, allergic rhinitis or food allergy in the child.[50] A recent trial among 581 pregnant women showed that daily supplementation with 2,400 IU of vitamin D3 from 24 weeks of pregnancy until one week after delivery was not associated with the risk eczema or allergic sensitization in the child.[51] Results from the ongoing VDAART trial, which specifically explores the effect of higher dose vitamin D3 supplementation earlier in pregnancy, have not yet been published.[52] Although stress-alleviating interventions during pregnancy has yielded promising results regarding adverse birth outcomes, intervention strategies focused on the reduction of maternal psychiatric symptoms during pregnancy to prevent the occurrence of eczema and allergy should be carefully evaluated using RCTs before widespread implementation is feasible.

Genome-wide association studies represent a relatively new research technique to examine genetic variants involved in human disease. Recently, a large genome-wide association study identified 10 new risk loci related to innate immune signaling and T-cell activation that were robustly associated with eczema.[53] However, the identification of the causal variants that underlie these associations is challenging. Expanding studies to include allele-specific analyses may be a promising strategy to improve the identification of causal variants in a diversity of tissues and cell types.[54] Further evidence for causality may be derived from knockout models, in which disease occurrence after inactivation of the target gene is suggestive of a causal relationship.[55] An *in vitro* study showed that knockout of the filaggrin (*FLG*) gene extensively alters human keratinocyte differentiation and stratum corneum function.[56] Filaggrin is an essential protein for maintaining the epidermal barrier and *FLG* mutations are well known to be associated with eczema.[57] We studied four *FLG* mutations (2282del4, R2447X, R501X and S3247X) that are common in Caucasians, and observed that none of the Moroccan and Surinamese-Creole children carried these mutations. All other children of non-Dutch origin carried a *FLG* mutation less frequently than children of Dutch origin. In non-Caucasian children, specifically those born in the host country as in our study, the prevalence of other *FLG* mutations and their role in the development of childhood eczema is less clear and remains to be further studied.[58] Also, filaggrin plays a central role in the hydration of the stratum corneum, because it is the precursor protein for hygroscopic amino acids and their derivatives, known as natural moisturizing factor (NMF).[59] It was demonstrated that adult carriers of *FLG* mutations have reduced NMF levels in the stratum corneum, compared with non-carriers.[60] Measurement of NMF levels using *in vivo* Raman spectroscopy is much less demanding than genotyping and may serve as a marker of the *FLG* genotype in future research.

Although many susceptibility genes for eczema have been identified, only a few studies on gene-gene and gene-environment interactions have been performed. A previous

birth cohort study found interactive effects of single nucleotide polymorphisms in the *IL13* and *STAT6* genes on the occurrence of eczema.[61] Also, early-life cat exposure is suggested to substantially increase the risk of eczema in the first year of life in children with *FLG* mutations, but not in those without these mutations.[62, 63] Both gene-gene and gene-environment interactions require further investigation.

Epigenetics is increasingly receiving attention as a potential mechanism underlying the DOHaD hypothesis.[64] Epigenetic modifications include DNA methylation in promoter regions of specific genes and may affect gene activity, thereby altering epidermal barrier function and the susceptibility for eczema development.[65] Maternal anxiety and depressive symptoms during pregnancy are associated with DNA methylation of the glucocorticoid receptor gene *NR3C1* in newborns and HPA axis stress reactivity at age 3 months.[66] This may be an underlying pathophysiological mechanism for the observed association of maternal psychiatric symptoms during pregnancy with the child's risk of developing atopic diseases. Also, it is suggested that mammalian milk may induce epigenetic modifications of the *FOXP3* gene required for regulatory T-cell maturation and the prevention of atopy.[67] The epigenetic origins of childhood eczema, allergic sensitization and allergy should be further explored in epigenome-wide association and functional genomic studies.

Changes in gut microbiota diversity might affect the risk of developing childhood allergic sensitization and atopic diseases.[68] Maternal stress during pregnancy was found to be associated with the child's intestinal microbiota composition and colonization pattern, predisposing the child to allergic reactions.[69] Also, dietary factors such as human milk oligosaccharides may serve as substrates for the production of microbial metabolites that regulate immune activity and immune tolerance mechanisms.[70, 71] Future studies are needed to explore these complex microbial, dietary and immunological interactions.

Several prediction models have been developed to predict the probability of having eczema at preschool-age based on environmental and genetic factors and other biological markers.[72, 73] Future prediction studies should be extended to school-age children, and it should be assessed whether the risk and protective factors examined in the studies presented in this thesis are of additional value in the prediction models. Subsequently, the newly obtained prediction models should be validated in specific clinical settings such as pediatric hospitals, general practices and youth health centers. There is increasing recognition that eczema, like asthma, is a heterogeneous disease with multiple phenotypes that might have different underlying pathophysiological mechanisms.[74] It would be of additional value to this thesis to focus on the prediction of eczema phenotypes, taking age of onset and persistence over time into account. Similarly, population-based studies on allergic sensitization patterns over time and their relation with comorbidity of eczema and allergy are warranted.[75, 76]

Table. Overview of results of studies on fetal and infant exposures and eczema, allergic sensitization and allergy as presented in this thesis.

	Eczema	Allergic sensitization		Physician-diagnosed allergy	
		Inhalant	Food	Inhalant	Food
Fetal exposures					
Maternal psychiatric symptoms during pregnancy					
Overall psychiatric symptoms	↑	=	=	↑	=
Depressive symptoms	=	=	=	↑	=
Anxiety symptoms	↑	=	=	↑	=
25-hydroxyvitamin D					
Mid-gestation	=	N.S.	N.S.	N.S.	N.S.
At birth	=	N.S.	N.S.	N.S.	N.S.
Infant exposures					
Ethnic origin	↑/=	N.S.	N.S.	N.S.	N.S.
Breastfeeding					
Duration	↑	=	=	=	=
Exclusiveness	↑	=	=	=	=
Allergenic food introduction					
Timing	↓/=	=	=	=	=
Diversity	=	=	=	↓	=

For associations of 25-hydroxyvitamin D levels and ethnic origin with eczema, data on eczema were obtained from age 6 months until age 4 years. For associations of other fetal and infant exposures with eczema, data on eczema were obtained from 6 months until age 10 years. Data on allergic sensitization and allergy were obtained at age 10 years. 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.S. = not studied.

CONCLUSIONS

Findings from this thesis suggest that fetal and infant exposures are associated with the development of childhood eczema and allergy, supporting the hypothesis that atopic diseases might have at least part of their origin in fetal life and infancy. Although some of the observed effects were relatively small to moderate, they may be important for the burden of eczema and allergy at the population level. The fetal and infant exposures that were examined in this thesis were not associated with allergic sensitization. Our results may contribute to the identification of high-risk children and the development of primary prevention strategies focused on pregnant women and young children. Further research is needed to explore underlying biological pathways, including the roles of genetic and epigenetic mechanisms and the human microbiota.

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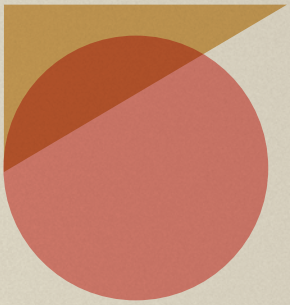
CHAPTER 5



SUMMARIES

SUMMARY

Eczema is a common chronic inflammatory skin disease in childhood that inflicts a substantial physical, psychosocial and economic burden. Eczema may occur solely or coincide with allergic sensitization and symptoms of allergy as part of an atopic constitution. Because the developmental origins of childhood eczema, allergic sensitization and allergy may partly lie in pregnancy and infancy, it is from an etiological perspective crucial to study fetal and infant exposures that may affect the risk of these entities. Also, insight into the role of fetal and infant exposures may provide opportunities for prevention strategies at times when their effect is expected to be greatest.

Chapter 1 is a general introduction and provides the main objectives and the biological hypothesis underlying this thesis. Briefly, we aimed to assess the associations of fetal and infant exposures with childhood eczema, inhalant and food allergic sensitization or allergy. We hypothesized that exposures in fetal life and infancy may affect the child's developing immune system or epidermal barrier function and, subsequently, the risk of eczema, allergic sensitization and allergy. The research was embedded in the Generation R Study, a large multi-ethnic population-based prospective birth cohort in Rotterdam, The Netherlands.

Chapter 2 describes the associations of fetal exposures with eczema, allergic sensitization or allergy in childhood. From *Chapter 2.1*, we concluded that maternal psychiatric symptoms during pregnancy were associated with increased risks of eczema and physician-diagnosed inhalant allergy in school-age children. Results were independent of maternal psychiatric symptoms after delivery and of paternal psychiatric symptoms, suggesting a possible intrauterine programming effect of maternal psychiatric symptoms that increases the child's risk of developing eczema and allergy. In *Chapter 2.2*, we observed no association of vitamin D levels in mid-gestation and at birth with the risk of eczema in preschool-age children.

Chapter 3 describes the associations of infant exposures with eczema, allergic sensitization or allergy in childhood. In *Chapter 3.1*, we observed that Cape Verdean, Dutch Antillean, Surinamese-Creole and Surinamese-Hindustani children had increased risks of eczema in the first 4 years of life. After adjustment for environmental and genetic risk factors, Surinamese-Creole and Surinamese-Hindustani children remained to have increased risks of eczema. The findings described in *Chapter 3.2* suggest that shorter duration or non-exclusiveness of breastfeeding is associated with a modest increased risk of eczema, but not allergic sensitization or physician-diagnosed allergy in school-age children. From *Chapter 3.3*, we concluded that neither timing nor diversity of al-

lergenic food introduction is consistently associated with eczema, allergic sensitization or physician-diagnosed allergy in school-age children. However, children introduced to gluten at age ≤ 6 months had a decreased risk of eczema until age 10 years. Children introduced to ≥ 3 allergenic foods at age ≤ 6 months had a decreased risk of physician-diagnosed inhalant allergy at age 10 years.

Finally, in **Chapter 4**, we provide a general overview of the main findings, highlight methodological considerations in epidemiological studies, discuss the clinical implications of the main findings and suggest directions for future research.

SAMENVATTING

Eczeem is de meest voorkomende chronische inflammatoire huidaandoening bij kinderen en kent aanzienlijke fysieke, psychosociale en economische gevolgen. Eczeem kan afzonderlijk voorkomen of gepaard gaan met allergische sensibilisatie en symptomen van allergie als onderdeel van een atopische constitutie. Omdat de oorsprong van eczeem, allergische sensibilisatie en allergie op de kinderleeftijd mogelijk ten dele in de zwangerschap en de zuigelingenperiode ligt, is het vanuit etiologisch perspectief van belang om foetale en vroegpostnatale factoren te bestuderen die het risico op deze entiteiten kunnen beïnvloeden. Inzicht in de rol van foetale en vroegpostnatale factoren biedt tevens mogelijkheden voor preventieve interventies.

Hoofdstuk 1 is een algemene introductie en beschrijft de belangrijkste doelstellingen alsmede de biologische hypothese waarop dit proefschrift is gebaseerd. We stelden ons ten doel onderzoek te doen naar de relaties tussen foetale en vroegpostnatale factoren enerzijds en eczeem, allergische sensibilisatie en allergie op de kinderleeftijd anderzijds. We veronderstelden dat foetale en vroegpostnatale factoren het in ontwikkeling zijnde immuunsysteem van het kind of diens huidbarrière beïnvloeden en, dientengevolge, het risico op eczeem, allergische sensibilisatie en allergie. Het onderzoek maakt deel uit van een grootschalig bevolkingsonderzoek in Rotterdam, genaamd Generation R.

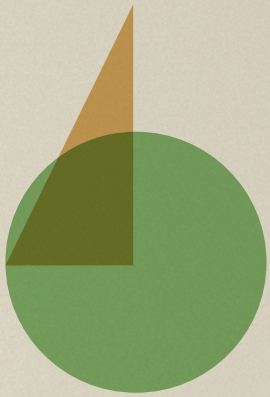
Hoofdstuk 2 beschrijft de relaties tussen foetale factoren en de ontwikkeling van eczeem, allergische sensibilisatie of allergie op de kinderleeftijd. Uit het onderzoek beschreven in *Hoofdstuk 2.1* kunnen we concluderen dat psychiatrische symptomen bij de moeder tijdens de zwangerschap geassocieerd zijn met een verhoogd risico op eczeem en een inhalatieallergie bij het kind tot de leeftijd van 10 jaar. Deze resultaten blijken onafhankelijk van psychiatrische symptomen bij de moeder na de bevalling en van psychiatrische symptomen bij de vader. Dit suggereert dat psychiatrische symptomen bij de moeder mogelijk een programmeringseffect op het kind in de baarmoeder hebben dat het risico op eczeem en allergie op latere leeftijd verhoogt. In *Hoofdstuk 2.2* laten we zien dat vitamine D-serumwaarden gemeten halverwege de zwangerschap en bij de bevalling niet geassocieerd zijn met het risico op eczeem bij kinderen tot de leeftijd van 4 jaar.

Hoofdstuk 3 beschrijft de relaties tussen vroegpostnatale factoren en de ontwikkeling van eczeem, allergische sensibilisatie of allergie op de kinderleeftijd. In *Hoofdstuk 3.1* laten we zien dat kinderen met een Kaapverdische, Antilliaanse, Surinaams-Creoolse of Surinaams-Hindoestaanse achtergrond een verhoogd risico hebben op eczeem tot de leeftijd van 4 jaar. Na correctie voor omgevingsfactoren en genetische factoren

blijven Surinaams-Creoolse en Surinaams-Hindoestaanse kinderen een verhoogd risico op eczeem hebben. De resultaten uit *Hoofdstuk 3.2* suggereren dat kortdurende of niet-exclusieve borstvoeding geassocieerd is met een bescheiden verhoogd risico op eczeem bij kinderen tot de leeftijd van 10 jaar, maar niet met allergische sensibilisatie of allergie. Uit het onderzoek beschreven in *Hoofdstuk 3.3* kunnen we concluderen dat noch de timing van introductie noch de diversiteit van allergene voedingsmiddelen consistent geassocieerd is met eczeem, allergische sensibilisatie of allergie bij kinderen tot de leeftijd van 10 jaar. Kinderen bij wie gluten in de eerste 6 levensmaanden worden geïntroduceerd, hebben echter een verlaagd risico op eczeem tot de leeftijd van 10 jaar. Kinderen bij wie in de eerste 6 levensmaanden 3 of meer allergene voedingsmiddelen worden geïntroduceerd, hebben een verlaagd risico op een inhalatieallergie op de leeftijd van 10 jaar.

Ten slotte geven we in **Hoofdstuk 4** een overzicht van de belangrijkste resultaten uit voornoemde studies, benadrukken we de methodologische beperkingen van epidemiologische studies, bediscussiëren we de klinische implicaties van onze bevindingen en doen we suggesties voor toekomstig onderzoek.

CHAPTER 6



APPENDICES

LIST OF PUBLICATIONS

Elbert NJ, Duijts L, den Dekker HT, de Jong NW, Nijsten TE, Jaddoe VW, de Jongste JC, Gerth van Wijk R, Tiemeier H, Pasmans SG. Maternal psychiatric symptoms during pregnancy and risk of childhood atopic diseases. Clin Exp Allergy. 2017;47(4):509-19.

Gazibara T, **Elbert NJ**, den Dekker HT, de Jongste JC, Reiss I, McGrath JJ, Eyles DW, Burne TH, Tiemeier H, Jaddoe VW, Pasmans SG, Duijts L. Associations of maternal and fetal 25-hydroxyvitamin D levels with childhood eczema: The Generation R Study. Pediatr Allergy Immunol. 2016;27(3):283-9.

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Elbert NJ, Kiefte-de Jong JC, Voortman T, Nijsten TE, de Jong NW, Jaddoe VW, de Jongste JC, Gerth van Wijk R, Duijts L, Pasmans SG. Allergenic food introduction and risk of childhood atopic diseases. *Submitted*.

Paternoster L, Standl M, Waage J, Baurecht H, Hotze M, Strachan DP, Curtin JA, Bønnelykke K, Tian C, Takahashi A, Esparza-Gordillo J, Alves AC, Thyssen JP, den Dekker HT, Ferreira MA, Altmaier E, Sleiman PM, Xiao FL, Gonzalez JR, Marenholz I, Kalb B, Pino-Yanes M, Xu CJ, Carstensen L, Groen-Blokhuis MM, Venturini C, Pennell CE, Barton SJ, Levin AM, Curjuric I, Bustamante M, Kreiner-Møller E, Lockett GA, Bacelis J, Bunyavanich S, Myers RA, Matanovic A, Kumar A, Tung JY, Hirota T, Kubo M, McArdle WL, Henderson AJ, Kemp JP, Zheng J, Smith GD, Rüschendorf F, Bauerfeind A, Lee-Kirsch MA, Arnold A, Homuth G, Schmidt CO, Mangold E, Cichon S, Keil T, Rodríguez E, Peters A, Franke A, Lieb W, Novak N, Fölster-Holst R, Horikoshi M, Pekkanen J, Sebert S, Husemoen LL, Grarup N, de Jongste JC, Rivadeneira F, Hofman A, Jaddoe VW, Pasmans SG, **Elbert NJ**, Uitterlinden AG, Marks GB, Thompson PJ, Matheson MC, Robertson CF; Australian Asthma Genetics Consortium (AAGC), Ried JS, Li J, Zuo XB, Zheng XD, Yin XY, Sun LD, McAleer MA, O'Regan GM, Fahy CM, Campbell L, Macek M, Kurek M, Hu D, Eng C, Postma DS, Feenstra B, Geller F, Hottenga JJ, Middeldorp CM, Hysi P, Bataille V, Spector T, Tiesler CM, Thiering E, Pahukasahasram B, Yang JJ, Imboden M, Huntsman S, Vilor-Tejedor N, Relton CL, Myhre R, Nystad W, Custovic

A, Weiss ST, Meyers DA, Söderhäll C, Melén E, Ober C, Raby BA, Simpson A, Jacobsson B, Holloway JW, Bisgaard H, Sunyer J, Probst-Hensch NM, Williams LK, Godfrey KM, Wang CA, Boomsma DI, Melbye M, Koppelman GH, Jarvis D, McLean WH, Irvine AD, Zhang XJ, Hakonarson H, Gieger C, Burchard EG, Martin NG, Duijts L, Linneberg A, Jarvelin MR, Nöthen MM, Lau S, Hübner N, Lee YA, Tamari M, Hinds DA, Glass D, Brown SJ, Heinrich J, Evans DM, Weidinger S; EArly Genetics and Lifecourse Epidemiology (EAGLE) Eczema Consortium. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. Nat Genet. 2015;47(12):1449-56.

van Meel ER, den Dekker HT, **Elbert NJ**, Jansen PW, Moll HA, Reiss IK, de Jongste JC, Jaddoe VW, Duijts L. A population-based prospective cohort study examining the influence of early-life respiratory tract infections on school-age lung function and asthma. The Generation R Study. *In revision*.

van Meel ER, de Jong M, **Elbert NJ**, den Dekker HT, Reiss IK, de Jongste JC, Jaddoe VW, Duijts L. Duration and exclusiveness of breastfeeding and school-age lung function and asthma. Ann Allergy Asthma Immunol. 2017;119(1):21-6, e2.

Nguyen AN, **Elbert NJ**, Pasmans SG, Kiefte-de Jong JC, de Jong NW, Moll HA, Jaddoe VW, de Jongste JC, Franco OH, Duijts L, Voortman T. Diet quality throughout early life in relation to allergic sensitization and atopic diseases in childhood. Nutrients. 2017;9(8):e841.

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PORTFOLIO

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Erasmus MC department	Dermatology
Research school	Netherlands Institute of Health Sciences
PhD period	January 2014 – October 2017
Promotor	Prof. dr. S.G.M.A. Pasmans
Copromotor	Dr. L. Duijts

	Year	Workload (ECTS)
1. PhD training		
General courses		
Master of Science in Health Sciences, Clinical Epidemiology, Netherlands Institute of Health Sciences, Rotterdam, The Netherlands	2014–2016	70
Core curriculum		
Principles of Research in Medicine and Epidemiology	2015	0.7
Methods of Public Health Research	2015	0.7
Clinical Trials	2015	0.7
Health Economics	2015	0.7
The Practice of Epidemiologic Analysis	2015	0.7
Fundamentals of Medical Decision Making	2015	0.7
Study Design	2015	4.3
Biostatistical Methods I: Basic Principles	2015	5.7
Clinical Epidemiology	2014	5.7
Methodologic Topics in Epidemiologic Research	2015	1.4
Biostatistical Methods II: Classical Regression Models	2015	4.3
Advanced elective courses		
Epidemiology of Infectious Diseases	2016	1.4
Repeated Measurements in Clinical Studies	2016	1.4
Missing Values in Clinical Research	2016	0.7
Methods of Clinical Research	2016	0.7
Conceptual Foundation of Epidemiologic Study Design	2016	0.7
Methods of Health Services Research	2016	0.7
History of Epidemiologic Ideas	2016	0.7
Causal Mediation Analysis	2016	0.7
Social Epidemiology	2016	0.7
Quality of Life Measurement	2016	0.9
Courses for the Quantitative Researcher	2016	0

	Year	Workload (ECTS)
Specific courses		
Center for Patient Oriented Research course, Erasmus Medical Center, Rotterdam, The Netherlands	2014	0.3
Integrity in Science course, Erasmus Medical Center, Rotterdam, The Netherlands	2014	0.3
Systematic literature retrieval in PubMed, Erasmus Medical Center, Rotterdam, The Netherlands	2014	0.5
Systematic literature retrieval in other databases, Erasmus Medical Center, Rotterdam, The Netherlands	2014	0.25
Endnote course, Erasmus Medical Center, Rotterdam, The Netherlands	2014	0.25
Basic Course on R, Erasmus Postgraduate School Molecular Medicine, Rotterdam, The Netherlands	2015	1.4
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (eBROK), Nederlandse Federatie van Universitair Medische Centra, Utrecht, The Netherlands	2016	1.5
Seminars and workshops		
CK-CARE Summer School – Eczema/Dermatitis, Davos, Switzerland	2014	1.0
Research meetings, Generation R, Erasmus Medical Center, Rotterdam, The Netherlands	2014–2017	4.0
Maternal & Child Health meetings, Generation R, Erasmus Medical Center, Rotterdam, The Netherlands	2014–2017	4.0
Research meetings, Department of Dermatology, Erasmus Medical Center, Rotterdam, The Netherlands	2014–2017	4.0
Journal Club, Department of Dermatology, Erasmus Medical Center, Rotterdam, The Netherlands	2014–2017	2.0
Skintermezzo, Department of Dermatology, Erasmus Medical Center, Rotterdam, The Netherlands	2014–2017	1.0
Seminars, Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands	2014–2017	4.0
Seminar Career Orientation, Erasmus Medical Center, Rotterdam, The Netherlands	2017	0.2

	Year	Workload (ECTS)
Conferences		
23rd European Academy of Dermatology and Venereology Congress, Amsterdam, The Netherlands	2014	1.0
13th European Academy of Dermatology and Venereology Spring Symposium, Athens, Greece	2016	1.0
10th World Congress on Developmental Origins of Health and Disease, Rotterdam, The Netherlands	2017	1.0
2. Teaching		
S.E.P. van Odijk, MSc student, Erasmus Medical Center, Rotterdam, The Netherlands	2015–2016	3.0
A.E.M. Nouwen, MSc student, Erasmus Medical Center, Rotterdam, The Netherlands	2017	3.0
Teaching assistant, Biostatistical Methods I: Basic Principles, Netherlands Institute of Health Sciences, Rotterdam, The Netherlands	2017	0.5
Teaching assistant, Principles of Research in Medicine and Epidemiology, Netherlands Institute of Health Sciences, Rotterdam, The Netherlands	2017	0.5
3. Other		
PhD representative Management Team, Generation R, Erasmus Medical Center, Rotterdam, The Netherlands	2014–2015	1.0
Member Student Panel, Netherlands Institute of Health Sciences, Rotterdam, The Netherlands	2015–2016	1.0
Peer review of articles for scientific journals (Clinical & Experimental Allergy, Journal of Allergy and Clinical Immunology, Journal of European Academy of Dermatology Venerology and Pediatric Allergy and Immunology)	2015–2017	1.0

1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours.

ABOUT THE AUTHOR

Niels Jan Elbert was born on the 23rd of January 1985 in Oss, The Netherlands. He grew up in the polder village of Wieringerwerf, where he completed secondary school at the Regionale Scholengemeenschap Wiringherlant in 2003. In the same year, he commenced his studies in psychology and physical education at Neosho County Community College in Chanute, Kansas, USA, and joined the NCCC Panthers Men's Soccer Team, NCCC Forensics and Debate Team and NCCC Academic Excellence Challenge Team. Upon his return to the Netherlands in 2004, he was admitted to study medicine at Utrecht University. During his studies, he held full-time positions as treasurer and vice-president on the board of the Erasmus Student Network Utrecht. After his graduation in 2012, he worked as a medical doctor at the Department of Internal Medicine at the Diakonessenhuis in Utrecht and as an editor of the Dutch Journal of Medicine. In 2014, he started a PhD trajectory leading to this thesis at the Department of Dermatology and within the Generation R Study Group at the Erasmus Medical Center under supervision of prof. dr. S.G.M.A. Pasmans, who had previously supervised his student research project on pediatric vascular tumors and malformations at University Medical Center Utrecht, and co-supervision of dr. L. Duijts. He holds a master's degree in health sciences with specialization in clinical epidemiology from the Netherlands Institute for Health Sciences. Soon after his PhD defence, Niels will cycle around the globe with his girlfriend Elvira.

DANKWOORD

In 1664 schreef dichter en kunstrechter Nicolas Boileau-Despréaux (1636–1711), behept met een scherpe satirische pen: *"si j'écris quatre mots, j'en effacerai trois"*. Het tekent mijns inziens het lot van jonge schrijvers en wetenschappers in hun zoektocht naar betekenis en perfectie. Vier stappen vooruit, drie achteruit, als een dansende processie zonder einde. Omkijkend, zie ik de hoge pieken en diepe dalen. Tijd vloog bij vermaak, kroop bij verveling en stond stil bij verdriet. Een worsteling met de wijzers van de klok, met de onwrikbare omgeving en, bovenal, met mezelf. Meermaals liep ik tegen de beperkingen van eigen lichaam en geest aan, maar telkens hervond ik het doorzettingsvermogen, de veerkracht en de kunst van het relativeren. Overstelpt door het gevoel van opluchting en een zekere trots nu de klus geklaard is, besef ik me dat dit proefschrift er nooit was geweest zonder de steun en toewijding van anderen. Deze mensen verdienen het om bij naam genoemd te worden.

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