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Role of environmental exposures and filaggrin mutations on associations of ethnic origin with risk of childhood eczema. The Generation R Study

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Ethnic origin, filaggrin and eczema risk

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AUTHOR CONTRIBUTIONS

NE, LD, HD, AS, and SP contributed to the conception and design, acquisition of data, analyses and interpretation of the data, drafted the article, revised it critically for important intellectual content, and gave final approval of the version to be published.

VJ and JJ contributed to the conception and design, acquisition of data, revised the drafted manuscript critically for important intellectual content, and gave final approval of the version to be published.

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FINANCIAL DISCLOSURE

The researchers are independent from the funders. The study sponsors had no role in the study design, data collection, data analysis, interpretation of data, and preparation, review or approval of the manuscript.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ABBREVIATIONS

BMI : body mass index

CI : confidence interval

FLG : filaggrin

OR : odds ratio

ABSTRACT

Role of environmental exposures and filaggrin mutations on associations of ethnic origin with risk of childhood eczema. The Generation R Study; N.J. Elbert, L. Duijts, H.T. den Dekker, V.W.V. Jaddoe, A.M.M. Sonnenschein – van der Voort, J.C. de Jongste, S.G.M.A. Pasmans

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 the age of 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 (FLG) 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 overall and independently.

Results Compared to 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).

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.

KEYWORDS

Eczema, ethnic origin, filaggrin, children, birth cohort.

REQUESTS FOR OFFPRINTS

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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 to 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 to 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 (MEC 217.595/2002/202). Written informed consent was obtained from parents 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 (Figure S1).

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 enrollment. 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 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, parity, pre-pregnancy body mass index (BMI), pet keeping and history of allergy, eczema or asthma was obtained by a questionnaire, completed by the mother at enrollment. Maternal psychological distress in the second trimester of pregnancy was assessed using the Global Severity Index of the Brief Symptom Inventory(15). Information on maternal smoking was obtained by postal questionnaires sent during each trimester of pregnancy. 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 or day care attendance. The child's BMI was calculated using weight and height measured at age 10-13 months during a visit to the routine child health care center. Information on wheezing 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, education, parity, pre-pregnancy BMI, pet keeping, history of allergy, eczema or asthma, psychological distress, and smoking, and child's sex, gestational age, birth weight, breastfeeding, day care attendance, BMI, 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 $\geq 10\%$ (Table 2). Missing covariates were $\leq 20\%$, except for maternal psychological distress (21.8%), and child's day care attendance (26.6%) and wheezing (36.0%) (Table S3). 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 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 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 to children of Dutch origin (p-value <0.001). 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 Table S1.

Ethnic origin and eczema

Crude models showed that compared to 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 Table S2), 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 (OR (95%-CI): 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 psychological distress during pregnancy (OR (95%-CI): 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 model (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 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 to 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 to 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 to 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 to 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 to 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 to 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 origins was low. Furthermore, our results did not materially change when *FLG* mutations were taken into account. This suggest 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 to 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 psychological distress 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 psychological distress levels during pregnancy, compared to mothers of Dutch children. However, precise quantification of attribution of each risk factor separately to the association of ethnic origin with risk of 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 between children of ethnic origins, such as transepidermal water loss(25), natural moisturizing factor(16), stratum corneum lipid composition(26), might have influenced the differences in results. Recently, it was observed in adults that skin barrier characteristics 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 strength of this study is 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 origins. However, some methodological limitations should be considered. First, selection bias in longitudinal studies mainly arises due to 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. Lost 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 sizes. Third, information on eczema was obtained by parental-derived 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 towards health care utilization and different reporting of symptom(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 pre-pregnancy 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 knockdown 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 associations 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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Table 1. Characteristics of mothers and children (n = 5,082).

	Dutch n = 3,756	Cape Verdean n = 125	Dutch Antillean n = 156	Moroccan n = 295	Surinamese- Creole n = 168	Surinamese- Hindustani n = 147	Turkish n = 435	P-value for difference ³
Maternal characteristics								
Age (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
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
Pre-pregnancy BMI (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
Pet keeping, 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
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
Psychological distress ¹	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
Smoking, yes (%)	23.2 (872)	25.6 (32)	29.5 (46)	6.8 (20)	36.9 (62)	19.0 (28)	37.2 (162)	<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 (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
BMI (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, 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 (%) ²	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). BMI = body mass index.

¹ 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) level of psychological distress(37).

² Missing data on filaggrin mutations (n = 1,986).

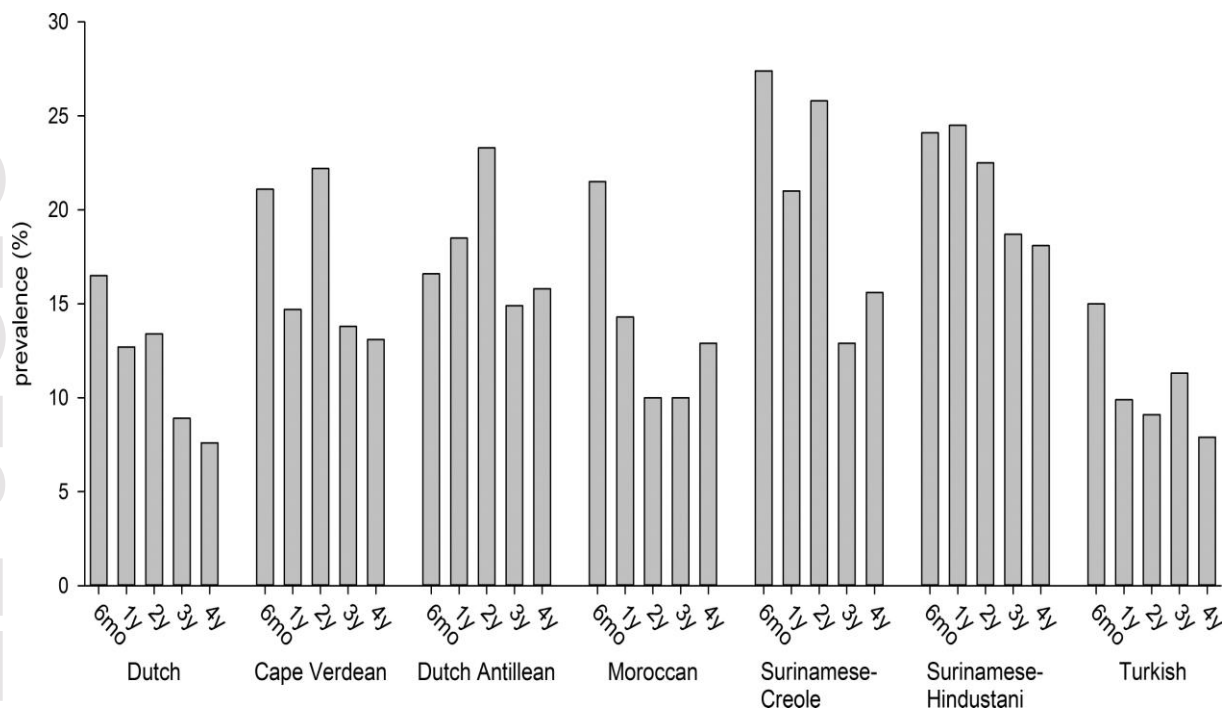
³ P-values for difference were calculated by one-way ANOVA for continuous variables with a normal distribution, the Kruskal-Wallis test for continuous variables with a skewed distribution, and Pearson's Chi-square test for categorical variables.

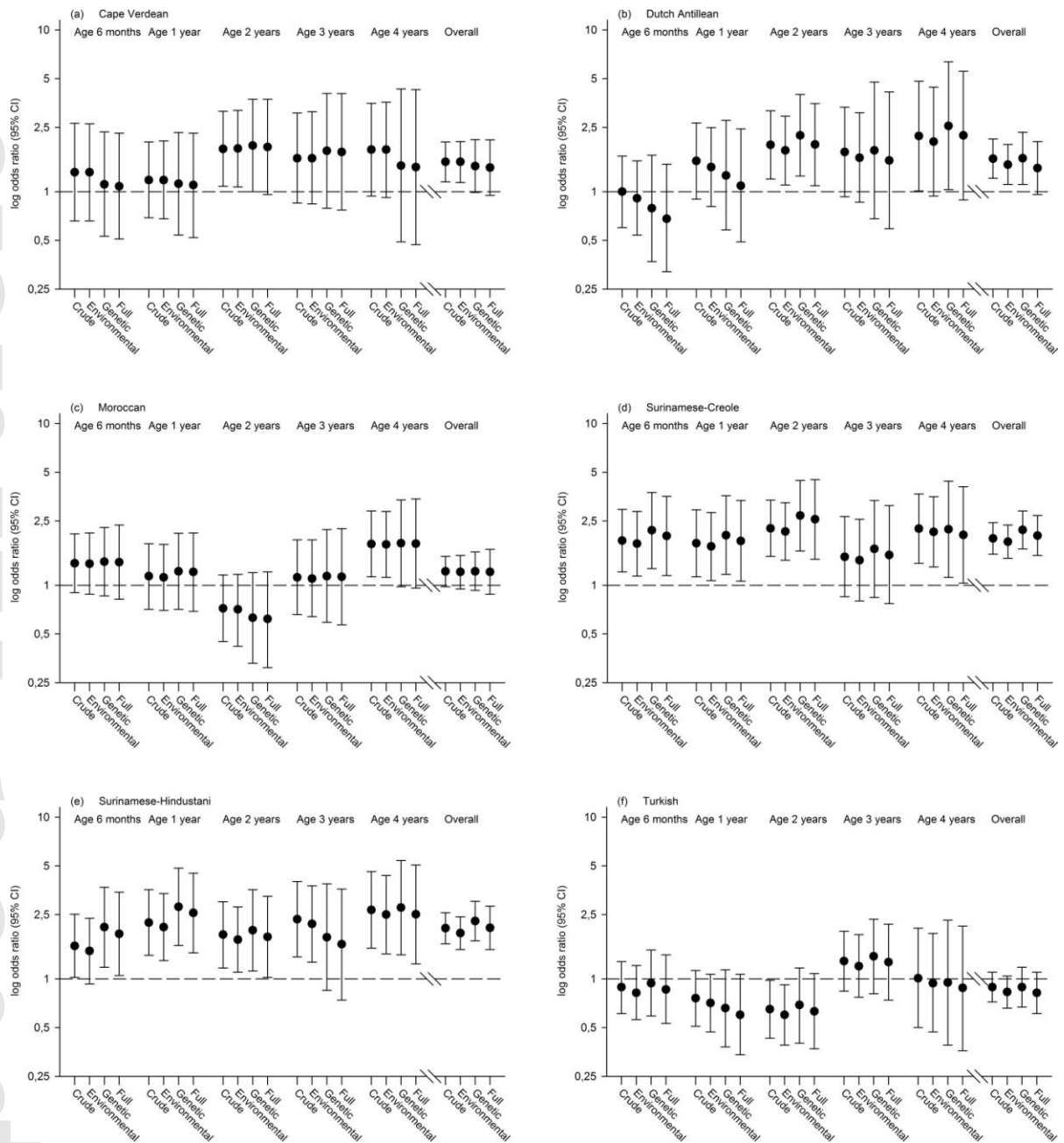
Table 2. Effects of known environmental and genetic risk factors on the overall risk of eczema in children of different ethnic origin.

	Overall risk for eczema					
	Cape Verdean	Dutch Antillean	Moroccan	Surinamese- Creole	Surinamese- Hindustani	Turkish
Odds ratio (95% CI) 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)
<i>Odds ratio (95% CI) when additionally</i>						
<i>adjusted for:</i>						
Environmental risk factors - mother						
Age	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)
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)
Pre-pregnancy body mass index	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)
Pet keeping	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)
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)
Psychological distress	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)
Smoking	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)
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	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	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	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) or the percentage change of odds ratios of the crude model when additionally adjusted for risk factors using generalized estimating equation models. The crude model is unadjusted. The environmental model (all environmental risk factors) is adjusted for maternal age, education, parity, pre-pregnancy body mass index (BMI), pet keeping, history of allergy, eczema or asthma, psychological distress, and smoking, and child's sex, gestational age, birth weight, breastfeeding, day care attendance, BMI 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.





**Role of environmental exposures and filaggrin mutations on associations of ethnic origin
with risk of childhood eczema. The Generation R Study**

Supplementary material

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14 **Table S1.** Characteristics of mothers and children included and not included in the study.

	Included n = 5,082	Not included n = 518	P-value for difference ¹
Maternal characteristics			
Age (years)	30.9 (4.9)	27.6 (5.7)	<0.001
Missing	0 (0)	0.2 (1)	
Education (%)			<0.001
Primary or secondary	47.3 (2,406)	74.9 (388)	
Higher	48.8 (2,479)	13.5 (70)	
Missing	3.9 (197)	11.6 (60)	
Parity (%)			<0.001
0	55.3 (2,809)	43.1 (223)	
≥1	42.0 (2,136)	55.0 (285)	
Missing	2.7 (137)	1.9 (10)	
Pre-pregnancy BMI (kg/m ²)	23.7 (18.8-35.9)	24.6 (18.3-38.2)	<0.001
Missing	9.4 (480)	4.6 (24)	
Pet keeping (%)			<0.001
No	52.2 (2,653)	59.1 (306)	
Yes	27.7 (1,409)	20.3 (105)	
Missing	20.1 (1,020)	20.6 (107)	
History of allergy, eczema or asthma (%)			0.36
No	51.6 (2,623)	45.5 (236)	
Yes	32.5 (1,652)	31.7 (164)	
Missing	15.9 (807)	22.8 (118)	
Psychological distress	0.13 (0-1.31)	0.33 (0.02-2.07)	<0.001
Missing	21.8 (1,108)	42.9 (222)	
Smoking (%)			<0.001
No	68.9 (3,502)	56.4 (292)	
Yes	21.7 (1,105)	30.7 (159)	
Missing	9.4 (475)	12.9 (67)	
Child characteristics			
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)	
<i>Missing</i>	0 (0)	0 (0)	
Sex (%)			<0.05
Male	50.3 (2,556)	56.0 (290)	
Female	49.7 (2,526)	43.8 (227)	
<i>Missing</i>	0 (0)	0.2 (1)	
Gestational age (weeks)	40.1 (36.0-42.3)	40.0 (35.5-42.3)	<0.05
<i>Missing</i>	0.3 (14)	0.4 (2)	
Birth weight (grams)	3,455 (556)	3,334 (546)	<0.001
<i>Missing</i>	0.1 (3)	0.4 (2)	
Breastfed ever (%)			0.68
No	8.2 (416)	7.0 (36)	
Yes	87.2 (4,431)	68.7 (356)	
<i>Missing</i>	4.6 (235)	24.3 (126)	
Day care attendance (%)			<0.05
No	29.8 (1,513)	2.1 (11)	
Yes	43.6 (2,214)	0.4 (2)	
<i>Missing</i>	26.6 (1,355)	97.5 (505)	
BMI (kg/m ²)	17.4 (14.8-20.4)	17.5 (14.8-20.8)	0.19
<i>Missing</i>	20.2 (1,027)	39.8 (206)	
Wheezing (%)			0.07
No	33.2 (1,685)	0 (0)	
Yes	30.8 (1,566)	0.6 (3)	
<i>Missing</i>	36.0 (1,831)	99.4 (515)	

Values are means (SD), medians (2.5-97.5th percentile) or percentages (absolute numbers) based on observed data. BMI = body mass index.

¹ P-values for difference were 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.

20 **Table S2.** Association of ethnic origin with eczema per year and overall (n = 5,082).

	Odds ratio (95% confidence interval) for eczema					
	0.5 years	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)
Surinamese-Hindustani						
Crude model (n = 147)	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 = 147)	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)

21 Values are odds ratios (95% confidence interval) using longitudinal generalized estimating equation models. Reference group is
22 children of Dutch origin. Missing data on filaggrin (*FLG*) mutations in the genetic and full model (n = 1,986). The crude model is
23 unadjusted. The environmental model is adjusted for maternal age, education, parity, pre-pregnancy body mass index (BMI), pet
24 keeping, history of allergy, eczema or asthma, psychological distress, and smoking, and child's sex, gestational age, birth weight,
25 breastfeeding, day care attendance, BMI, and wheezing. The genetic model is adjusted for *FLG* genotype. The full model is adjusted
26 for both environmental and genetic risk factors.

	Observed	Imputed
Maternal characteristics		
Age (years)	30.9 (4.9)	30.9 (4.9)
<i>Missing</i>	0 (0)	0 (0)
Education (%)		
Primary or secondary	47.3 (2,406)	50.4 (2,559)
Higher	48.8 (2,479)	49.6 (2,523)
<i>Missing</i>	3.9 (197)	0 (0)
Parity (%)		
0	55.3 (2,809)	55.8 (2,837)
≥1	42.0 (2,136)	44.2 (2,245)
<i>Missing</i>	2.7 (137)	0 (0)
Pre-pregnancy BMI (kg/m ²)	23.7 (18.8-35.9)	23.8 (18.8-35.9)
<i>Missing</i>	9.4 (480)	0 (0)
Pet keeping (%)		
No	52.2 (2,653)	64.0 (3,253)
Yes	27.7 (1,409)	36.0 (1,829)
<i>Missing</i>	20.1 (1,020)	0 (0)
History of allergy, eczema or asthma (%)		
No	51.6 (2,623)	62.2 (3,163)
Yes	32.5 (1,652)	37.8 (1,919)
<i>Missing</i>	15.9 (807)	0 (0)
Psychological distress	0.13 (0-1.31)	0.15 (0-1.38)
<i>Missing</i>	21.8 (1,108)	0 (0)
Smoking (%)		
No	68.9 (3,502)	76.0 (3,860)
Yes	21.7 (1,105)	24.0 (1,222)
<i>Missing</i>	9.4 (475)	0 (0)
Child characteristics		
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)
<i>Missing</i>	0 (0)	0 (0)
Sex (%)		
Male	50.3 (2,556)	50.3 (2,556)
Female	49.7 (2,526)	49.7 (2,526)
<i>Missing</i>	0 (0)	0 (0)
Gestational age (weeks)	40.1 (36.0-42.3)	40.1 (36.0-42.3)
<i>Missing</i>	0.3 (14)	0 (0)
Birth weight (grams)	3,455 (556)	3,455 (556)
<i>Missing</i>	0.1 (3)	0 (0)
Breastfed ever (%)		
No	8.2 (416)	8.8 (449)
Yes	87.2 (4,431)	91.2 (4,633)
<i>Missing</i>	4.6 (235)	0 (0)
Day care attendance (%)		
No	29.8 (1,513)	44.4 (2,256)
Yes	43.6 (2,214)	55.6 (2,826)
<i>Missing</i>	26.6 (1,355)	0 (0)
BMI (kg/m ²)	17.4 (14.8-20.4)	17.4 (14.9-20.4)
<i>Missing</i>	20.2 (1,027)	0 (0)
Wheezing (%)		
No	33.2 (1,685)	54.5 (2,771)
Yes	30.8 (1,566)	45.5 (2,311)
<i>Missing</i>	36.0 (1,831)	0 (0)

28 Values are means (SD), medians (2.5-97.5th percentile) or percentages (absolute numbers)
29 based on observed and imputed data. BMI = body mass index.

