Genetic predisposition and environmental factors associated with the development of atopic dermatitis in infancy: a prospective birth cohort study

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Abstract

The influence of environmental factors on atopic dermatitis (AD) has been investigated in many cross-sectional studies. It remains however unclear if they could influence AD development early in life. This prospective birth cohort study aimed to monitor aspects of family lifestyle and child's nutrition within a Caucasian population and to assess its association with AD development over the first two years of life. Genetic predisposition was evaluated based on family history and profilaggrin genotyping. Of 149 included children, 36 developed AD. Infants with a family history of atopy developed AD 2.6 times more frequently (30 of 97) than infants without atopic predisposition (6 of 52). Genotyping was carried out on 50% of the children included. Profilaggrin mutations (*R501X, 2282del4, R2447X and S3247X*) were infrequent in our population. Lower incidence of AD was observed in infants exposed to a damp housing environment, lower household income and smoking mothers with a higher but not with a lower education level.

Conclusion : Family history of atopy was a significant risk factor for AD regardless of the most common, currently defined, *FLG* mutations. Humidity at home and passive smoking seem associated with AD development in infancy.

Keywords

atopic dermatitis, environmental factors, lifestyle, nutrition

Abbreviations used

AB, antibiotic

AD, atopic dermatitis

CI, confidence interval

FLG, filaggrin

HR, hazard ratio

What is Known:

- Atopic dermatitis (AD) is associated with mutations in various genes of the immune system and the epidermal barrier complex in particular filaggrin (*FLG*) mutation.
- Inherited factors alone cannot explain the rising AD; environmental factors are therefore likely to play a decisive role in this rise but the exact role that these factors may play in increasing AD risk in infancy remains unclear. Moreover, the relationship between environmental factors and AD has been the focus of mostly cross-sectional studies and not prospective studies.

What is New:

- This prospective birth cohort study, demonstrates that family history of atopy is a significant risk factor for AD regardless of the most common, currently defined, *FLG* mutations.
- A lower incidence of AD was observed in infants exposed to a moist housing environment, lower household income and smoking of mothers with a higher but not with a lower education level.

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Introduction

Atopic dermatitis (AD) is a common multifactorial skin disease in which a large body of evidence suggests a key role of genetic factors. This is underscored by the importance of family predisposition [1–3], the association of AD with mutations in various genes related to the epidermal barrier complex or the immune system [4], and the higher concordance rate for mono- versus dizygotic twins [5]. However, inherited factors alone cannot explain the rising AD incidence that has been observed over the last decades; environmental factors are therefore likely to play a decisive role in this rise [6].

Since the mid-20th century, societies in industrialized countries have undergone major environmental and lifestyle changes, resulting in what is commonly referred to as the "western lifestyle" [7]. Despite the difficulty to firmly establish causality owing to the many potential confounders, some of these changes have been linked to increased AD incidence: smaller family sizes or less crowded homes [8,9], more affluent socioeconomic status [9,10] or decreased exposure to nonpathogenic microbes [11]. Among the myriad other environmental factors that have been implicated in AD [12,13], dietary factors represent another key component of the western lifestyle. A large body of literature suggests that dietary factors may strongly influence early-life gut microbiota and immune system development [14], but to what extent they contribute to AD onset remains poorly

understood. Breastfeeding has long been considered protective against AD, particularly in infants with a family history of atopy [15], but findings from more recent studies have called this protective effect into question [16,17], or even suggested that breastfeeding may be a risk factor for AD [18]. Various dietary supplements (e.g. probiotics, fish oil, trace elements or vitamins) have also been investigated as possibly protective factors, without conclusive results [19,20].

The relationship between environmental factors and AD has been the focus of many, mostly cross-sectional studies [15]. The exact role that these factors may play in increasing AD risk in infancy remains however unclear. To investigate this, we conducted a prospective birth cohort study in a Caucasian population, looking at a wide range of factors related to prenatal exposures, family lifestyle, socioeconomic status, child's nutrition and medical history. Skin changes were monitored over a two-year period or up to AD development, and genetic predisposition was evaluated based on the family history of atopy and filaggrin (*FLG*) genotyping.

Materials and methods

This prospective birth cohort study was approved by the Research Ethics Committee of the University of Lausanne and was conducted according to the Declaration of Helsinki Principles.

Subjects and study design

Our study cohort has been previously described [21]. Briefly, pregnant women planning to give birth at the Lausanne University Hospital (Switzerland) were recruited, and their healthy full-term newborns were enrolled at birth from January 2010 to November 2012. All Caucasian newborns at the CHUV with a first-degree relative (parent or sibling) with atopy will be eligible for this study. Non-Caucasian newborns, those suffering from disorders affecting the epidermal barrier or immune system and those whose cord blood could not be obtained were excluded. The primary aim of this cohort was to compare skin colonization in infants developing AD or not [21]. Enrolled infants having at least one first-degree relative with atopy (physician-diagnosed atopic dermatitis, allergic rhino conjunctivitis and/or allergic asthma) were considered at high risk of AD, whereas those with no family history of atopy were considered at low risk. Written informed consent was obtained from the parents of all infants enrolled.

Clinical follow-up

From birth and throughout the first two years of life, infants were monitored for clinical signs of AD according to the Hanifin and Rajka's diagnostic criteria [22]. Follow-up examinations were scheduled at 1, 3 and 7 days

and at 1, 3, 6, 12 and 24 months, with the study endpoint (final visit) being either the 24-month visit or the time point of AD development. All clinical follow-ups were performed by a physician.

Data collection

Three standardized, partially validated questionnaires were developed based on the recent literature and were used to collect the information on the various environmental factors. The lifestyle and medical questionnaires were completed at birth, 12 months, and at the final visit. The nutrition questionnaire was completed at ages 1, 6, 12 and 18 months, and at the final visit. This information was collected by the physician from the parents patients. All recorded variables are listed in Table 2.

Filaggrin genotyping

Cord blood samples were collected at birth and genomic DNA extracted using standard methods. Twenty-nine AD subjects and 45 healthy controls were tested by Sanger sequencing for four of the most common filaggrin loss-of-function mutations in Caucasians, namely *p.(R501X), c.2282del4, p.(R2447X) and p.(S3247X)*. For cost reasons, these genetic analyses were only performed on high-risk patients who developed AD and low-risk patients who did not. The primers (sense and antisense respectively) used for *R501X* were 5'-CACGGAAAGGCGGGCTGA-3' and 5'-ACCTGAGTGTCCAGACCTATT-3'; for *2282del4* 5'-CACCAGCTCCAGTCAGCAG-3' and 5'-GGGAGGACTCAGACTGTTT-3'; for *R2447X* 5'-ACGTGGCCGGTCAGCA-3' and 5'-CCTGACCCTCTTGGGACGT-3'; and for *S3247X* 5'-CCAGAAACCATCGTGGATCTG-3' and 5'-TGCCTGATTGTCTGGAGCG-3'.

Statistical analysis

Hazard ratios for potential AD risk factors were calculated using Cox regression (R function 'coxph'), and their significance was assessed using the log-rank (function 'survdiff') or Wald test for the uni- and multivariate regressions, respectively. The proportional hazards assumption was tested for each variable by examining Schoenfeld residuals (function 'cox.zph'). The variables entered as potential confounders in the multivariate model were limited in number owing to the relatively small number of events in our cohort, hence their selection based on prior knowledge from the literature. All analyses were conducted with R version 3.3.0 [23] and the 'survival' package [24]. $P < 0.05$ was considered statistically significant. Due to the small sample size and, hence, exploratory nature of our data, we did not perform any correction for multiple comparisons. We considered it more important not to miss potentially interesting associations [25].

Results

We identified 1,433 white pregnant women, of whom 605 agreed to participate. Among the newborns, 416 did not meet the inclusion criteria and 40 were lost to follow-up. Of the 149 included infants (78 boys and 71 girls), 97 belonged to the high-risk and 52 to the low-risk group regarding AD development. Demographic and clinical characteristics of the study population were summarized in supplementary Table 1. AD developed 2.6 times more frequently in the high-risk group (31%; 30 of 97) compared to the low-risk group (12%; 6 of 52). The mean age at AD onset was 9.4 months (median: 6 months; range: 1–24 months) (Fig.1). Filaggrin genotyping was done in 29 high-risk infants with AD and 45 low-risk children without AD at age two years. Only one AD infant and one control carried the *R501X* mutation; similarly, one AD infant and one control had the 2282del4 mutation; one AD infant carried the R2447X mutation, and one control had the S3247X mutation.

All three questionnaires were well completed, with less than 5% missing data for most of the recorded variables. Household income and parental level of education were the two factors having the highest missing response rate (16 % and 11 % of cases, respectively). Variables for which more than 95% of subjects had the same value (see Table 2) were not analyzed further. Cox regression was used to calculate crude and atopy risk-adjusted hazard ratios (HRs) for each environmental factor. For all factors except those related to nutrition, adjusted HRs were further computed using a multivariate model which included atopy risk, gender, delivery mode and presence or not of siblings. We decided not to enter household income or parental education level into the multivariate model due to the high proportion of missing data.

Family history of atopy, higher humidity at home and exposure to tobacco smoke are significantly associated with AD development in infancy

In our cohort, a family history of atopy was a major risk factor for AD development regardless of the most common filaggrin mutations (Table 3). Higher household income appeared to increase AD risk more than twofold, but did not reach significance. By contrast, we found that a higher humidity level in the home (HR 0.27, 95% CI 0.08-0.89) and passive smoking (HR 0.42, 95% CI 0.21-0.86) were associated with reduced AD risk. Overall, results from the uni- and multivariate models led to similar conclusions (Fig. 2), but the apparently protective effect of an urban setting (HR 0.51, 95% CI 0.26–1.03) in the univariate model was noticeably attenuated by adjusting (HR 0.62, 95% CI 0.30–1.27). The other environmental factors showed no significant association with AD development.

Since socioeconomic factors could confound the effect of passive smoking on AD risk, we proceeded with additional subgroup analyses, stratifying for maternal and paternal education level, and for household income. Surprisingly, these analyses revealed no major confounding. We however found evidence of a strong interaction

between passive smoking and maternal education: passive smoking was associated with reduced AD risk in infants whose mother had a higher education level (HR 0.21, 95% CI 0.069-0.62), but increased risk in infants whose mother had a lower education level (HR 2.8, 95% CI 0.60-13).

We also assessed whether the effect of household income was confounded by other environmental factors. In an alternate multivariate model including pet ownership, urban setting and both paternal and maternal education level, we could attenuate the HR for income by almost 40%. By contrast, the effect of income was unaffected by adjusting for various prenatal exposures (alcohol, tobacco smoke and antibiotics), delivery mode or mother age. These findings suggest that at least part of the effect of household income on AD risk may be due to other socioeconomic indicators and lifestyle-related factors.

Duration of breastfeeding is not associated with AD development in infancy

We observed no association between AD risk and breastfeeding (exclusive or not) duration (Table 4). Of note, mothers of high-risk children did not have a longer duration of exclusive breastfeeding than those of low-risk children, indicating that awareness of a risk of atopic disorders in the family did not cause mothers to breastfeed for a longer period.

Food diversification was uniform between groups and occurred for all children between four and six months. We therefore did not study this parameter but we analyzed the age of introduction of allergenic foods. Regarding the age at solid food introduction, we had to set the cutoff at age one year in order to have relatively well balanced groups, despite most AD in our cohort occurring before age six months ; this led to serious loss of available cases, and a corresponding loss of power to detect significant associations. We found no evidence that introduction of any of the foods mentioned in Table 4 before age one year was associated with AD development before age two years ; only one subject had wheat introduced after age one year.

Discussion

In our study, patients with a positive family history of atopy had almost threefold greater risk of developing AD, which is consistent with previous research [1–3]. It is clear that hereditary factors are an important component of AD pathogenesis [15]. AD has been associated with mutations in various genes of the epidermal barrier complex and the immune system. One of those genes, *FLG*, which encodes a structural protein that plays an essential role in the proper function of the cutaneous barrier, has been extensively studied [26–29]. Currently the filaggrin (FLG) gene as the most notable so far [30]. Skin barrier dysfunction is of particular relevance to AD pathogenesis since it increases transepidermal water loss and penetration of allergens and bacteria through the skin [31]. The four most prevalent filaggrin mutations (*c.2282del4*, p.*R501X*, *p.R2447X* and *p.S3247X*) [26] found in Caucasian populations were surprisingly infrequent in our study, suggesting that atopic disorders in parents or siblings may be a major risk factor regardless of these *FLG* mutations. It is conceivable that other *FLG* mutations, or mutations in other genes of relevance to AD, for instance in the Th2 signalling pathway, may have been missed. Additionally, there is probably a strong influence of pre- and perinatal environmental factors, which might alter the expression of *FLG* and other important genes [4]. It is likely that AD pathogenesis involves a constellation of both genetic and epigenetic alterations, particularly in genes related to the epidermal barrier and immune system, as well as complex gene-environment interactions. Our data suggest that the common *FLG* mutations may not be used on their own as a reliable predictor of subsequent AD development.

We found a negative association between exposure to cigarette smoke and AD development, even after adjusting for family history of atopy or socioeconomic factors. Whereas passive smoking has a well-established positive association with asthma, its relationship with AD remains controversial [32,33]. Two recent meta-analyses concluded that both passive and active smoking slightly increased AD risk in the general population, despite the limitations raised by the authors [13,34]. An obvious limitation of meta-analyses is publication bias, which cannot be excluded [34]. Most studies analyzing the association between AD and passive smoking were cross-sectional and thus did not allow causal inference. Additionally, studies were very heterogeneous, with a risk of AD misclassification as the disease was not always diagnosed by trained dermatologists. The discrepancy of our findings with the conclusions of these meta-analyses may also be due to our study population consisting of infants (0–2 years) rather than children or adults. Albeit detrimental at all ages, exposure to environmental tobacco smoke could have some differential effects according to the stage of immune system maturation, an issue that was beyond the scope of the present work.

It is noteworthy that some studies reached similar conclusions as ours [9,16,35,36], and some of them (e.g. the one of Linneberg and colleagues [35]) were also prospective birth cohort studies. In line with our results, the study of Hjern et al. reported that parental tobacco smoking was a protective factor even after controlling for confounding factors such as sex, age and socioeconomic factors [36]. Ludvigsson et al. reported that parental smoking was associated with a lower risk of AD, and their conclusion was reinforced by urine cotinine measurements in part of the study population [16]. In the Taylor-Robinson study, smoking during pregnancy also seemed to be a protective factor, a finding that was confirmed by grouping estimates from other studies [9]. Conclusions from that study were based on a large cohort and might apply as well to other countries with high standard of living such as ours.

To explain the unexpected finding of a negative association between passive smoking and AD, most authors brought up residual confounding [9,35,36], but to date this has not been investigated in detail. It is conceivable that smokers may have specific behaviours that could be protective against AD development, such as ventilating the housing more frequently or going for a walk outside with the infant while smoking [36]. The strong interaction which we observed between passive smoking and maternal education supports the idea that putative behavioral patterns associated with cigarette smoking could further depend on socioeconomic factors. Aside from residual confounding, biological mechanisms might underlie the negative association between passive smoking and AD risk in infancy [35,36]. Some investigators proposed an immunosuppressive mechanism and an alteration of T lymphocytes, reducing the immune response to inhaled antigens [35–37]. In respiratory and other diseases, several studies have indeed reported immunomodulatory effects of tobacco smoke exposure [38–40]. Furthermore, there is some evidence of an inverse association between exposure to tobacco smoke and risk of certain diseases like Parkinson's, endometrial cancer or ulcerative colitis [41]. One major limitation of our study is that tobacco smoke exposure was not objectively measured, like in most studies investigating the relationship between tobacco smoke and AD. This issue has been previously raised [33] and may be complicated by a nonlinear dose-response relationship.

Another postnatal environmental factor that appeared protective against AD in our study was a higher level of humidity in the housing. This finding appears compatible with AD pathophysiology: a higher humidity level in the environment may attenuate the increased transepidermal water loss typically observed in AD [42,43]. Humidity level might also be associated with substantial changes in the indoor microbiota. Most studies to date have focused on the role of climate [44], but the role of indoor humidity before AD onset has not received much

attention. In the prospective birth cohort study of Bisgaard et al., wherein humidity was measured in the bedroom, no association was found with AD onset before age 3 years [45]. This discrepancy with our finding may be explained by our subjective assessment of humidity. Indeed, we only indirectly assessed humidity levels based on a question asking about the presence of molds at home, although we have no reason to believe that this assessment was performed differently by parents of infants who later developed AD compared to parents of unaffected infants, this does not allow us to measure the humidity rate.

Prior research has shown that a low level of relative humidity could alter cutaneous barrier function and increase susceptibility to atopic lesions [44,46]. Previous reports have also shown that low environmental humidity results in downregulation of filaggrin expression and increased water loss [44]. Such changes may not only aggravate established AD, but also promote AD onset in infants already at risk because of innate or acquired deficiency of genes (especially filaggrin) playing an important role in AD pathogenesis.

Finally, during our study, no evidence was found concerning the relationship between food introduction before 12 months and AD. Most AD was developed before, so this way of categorizing caused the exclusion of many AD patients. However, the link between AD, sensitization and food allergies is well established [47]. For example, AD patients seem more sensitized than control patients to various foods, such as milk, egg, sesame, peanut or wheat [48]. Moreover, some studies have shown that AD precedes the development of food sensitization and allergy [47,49].

However, the causal link between exposure to certain foods and AD remains an open question. Several studies could suggest that food is an AD trigger [50]. The mechanism could be mediated by IgE and could involve T-cells of the skin [50]. Apparently, there could also be unmediated IgE mechanisms [50]. To our knowledge there is no food involved in the onset of AD.

The strengths of our study is its prospective design, the close follow-up, and the fact that we only considered exposures occurring before the diagnosis of AD was made, which limited reverse causality. Further, AD diagnosis was made by trained dermatologists, which avoided misclassification. Our work also has some limitations. For coast reasons, genotyping was performed only half of our population. Moreover, we did not objectively measure exposure to second-hand smoke and indoor humidity. Several analysis could not be performed due to lack of data. Finally, many newborns had exclusion criteria and the relatively small sample size did not allow full adjustment for all potential confounders using a single multivariate model. Nonetheless, we

carefully evaluated how our conclusions were influenced by the most likely confounders based on the literature.

In summary, our study demonstrates that family history of atopy is a significant risk factor for AD regardless of the most common, currently defined, *FLG* mutations. We also show that infant's exposure to a higher humidity level and to parental tobacco smoke at home is associated with a reduced AD risk. Our findings highlight the likely contribution of both genetic and epigenetic factors to AD pathogenesis, as well as complex gene-environment interactions. The crucial influence of a positive family history of atopy on AD risk indicates that early preventive care may be beneficial in offspring at risk.

Authors' contributions

SCZ conceived and designed the study and data collection performa. SM and CL collected the data. CG, PM and SCZ analysed the data. CG, PM and SCZ wrote the first manuscript draft. All authors provided intellectual input, had access to the complete dataset, contributed to manuscript revisions and approved of the final version. SCZ is the guarantor.

Compliance with Ethical Statements

Conflict of Interest: The authors declare that they have no conflict of interest.

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This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Informed consent was obtained from all individual participants included in the study

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453 **Table 1.** Demographic and clinical characteristics of the study population

	Patients, n (%)			
Characteristics	**All (n = 149)**	**High risk[1] (n = 97)**	**Low risk (n = 52)**	**P-Value[2]**
Sex, n(%)				
Female	71 (48)	47 (46)	24 (48)	0.79
Male	78 (52)	50 (54)	28 (52)	
Gestational age, weeks				
Mean	39.2	39.1	39.2	0.93[3]
SD	1.09	1.04	1.19	
Birth mode				
Vaginal	103 (69)	67 (69)	36 (69)	0.98
Cesarean	46 (31)	30 (31)	16 (31)	
Season of birth, n (%)				
Spring	30 (20)	21 (22)	9 (17)	0.14
Summer	29 (19)	22 (23)	7 (13)	
Fall	56 (38)	30 (31)	26 (50)	
Winter	34 (23)	24 (25)	10 (19)	
AD development, n (%)				
Yes	36 (24)	30 (31)	6 (12)	**0.008**
No	113 (76)	67 (69)	46 (88)	
Season of AD onset, n (%)				

Spring	10 (28)	7 (23)	3 (50)	0.29[4]
Summer	5 (14)	4 (13)	1 (17)	
Fall	8 (22)	7 (23)	1 (17)	
Winter	13 (36)	12 (40)	1 (17)	
Age at AD onset in months, n (%)				
0-6	20 (56)	17 (57)	3 (50)	0.44[3]
7-12	5 (14)	5 (17)	0	
13-18	4 (11)	2 (6.7)	2 (33)	
19-24	7 (19)	6 (20)	1 (17)	

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455 *Bold text indicates significance.*

456 *Abbreviations: AD, atopic dermatitis; SD, standard deviation.*

457 [1]*At least one first-degree relative with atopy.*

458 [2]*High- versus low-risk comparison, using Pearson chi-square test, except as noted.*

459 [3]*Wilcoxon-Mann-Whitney test.*

460 [4]*Pearson chi-square test on all AD patients, with the null hypothesis that all seasons of onset are equally likely.*

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465 **Table 2**. Summary of all demographic, clinical and lifestyle factors recorded

Lifestyle questionnaire
Presence of carpets at home
Microwave use
High level of humidity at home (as estimated by the presence of mold at home)
Exposure to second-hand smoke
Pet ownership
Fabric softener use
Urban versus rural setting
Mother's education level
Father's educational level
Household income
Frequency of cleaning
Newly built house
Newly renovated house
Medical questionnaire
Family history of physician-diagnosed atopic disorders
Prenatal AB exposure
Prenatal alcohol exposure
Gender
Mode of delivery
Presence of siblings
Age of mother
AB intake in the first month of life
Smoking during pregnancy
Fish oil intake during pregnancy
Renovation of the home during pregnancy
Duration of hospitalization of the neonate
Invasive procedures at birth
Varicella in the first month of life
Antifungal treatment of the study subject (topical or systemic)
Nutrition questionnaire
Duration of exclusive breastfeeding
Duration of breastfeeding (exclusive or not)
Age at introduction of fish
Age at introduction of egg
Age at introduction of seafood
Age at introduction of celery
Age at introduction of peanuts
Age at introduction of kiwi
Age at introduction of milk
Age at introduction of nuts

Age at food diversification

italics : variables not analyzed further because of too narrow a distribution (at least one category with < 5% of all subjects)

Table 3. Cox regression results for lifestyle and medical factors

Variable		**No AD, n (%)**	**AD infants, n (%)**	**Crude HR (95% CI)**	**P-value[1]**	**Adjusted[2] HR (95% CI)**	**P-value[3]**	**Adjusted[4] HR (95% CI)**	**P-value[3]**
Family history of atopy	No	46 (41%)	6 (17%)	Reference				Reference	
	Yes	67 (59%)	30 (83%)	3.02 (1.26-7.26)	0.01	-	-	2.77 (1.14-6.73)	0.02
Socioeconomic factors									
Urban setting	No	20 (18%)	12 (34%)	Reference		Reference		Reference	
	Yes	89 (82%)	23 (66%)	0.51 (0.26-1.03)	0.06	0.60 (0.29-1.23)	0.16	0.62 (0.30-1.27)	0.19
Household income	< 90 000 CHF	39 (40%)	6 (21%)	Reference		Reference		Reference	
	> 90 000 CHF	58 (60%)	22 (79%)	2.29 (0.92-5.68)	0.07	2.08 (0.84-5.18)	0.11	2.33 (0.91-5.9)	0.08
Father's education level	Mandatory school or college	52 (51%)	13 (42%)	Reference		Reference		Reference	
	University or high school	49 (49%)	18 (58%)	1.35 (0.66-2.78)	0.4	1.18 (0.57-2.44)	0.66	1.02 (0.49-2.13)	0.2
Mother's education level	Mandatory school or college	44 (44%)	11 (35%)	Reference		Reference		Reference	
	University or high	57 (56%)	20 (65%)	1.37 (0.65-2.86)	0.4	1.23 (0.59-2.63)	0.58	1.18 (0.55-2.5)	0.68

	school								
Household environmental factors									
Passive smoking	No	45 (41%)	23 (64%)	Reference		Reference		Reference	
	Yes	64 (59%)	13 (36%)	0.43 (0.22-0.84)	0.01	0.42 (0.21-0.84)	0.01	0.42 (0.21-0.86)	0.02
High level of humidity	No	86 (76%)	33 (92%)	Reference		Reference		Reference	
	Yes	27 (24%)	3 (8%)	0.32 (0.10-1.05)	0.05	0.31 (0.09-1.01)	0.05	0.27 (0.08-0.89)	0.03
Microwave use	No	45 (40%)	20 (56%)	Reference		Reference		Reference	
	Yes	68 (60%)	16 (44%)	0.58 (0.30-1.11)	0.1	0.55 (0.29-1.08)	0.08	0.57 (0.28-1.13)	0.11
Fabric softener use	No	53 (50%)	22 (65%)	Reference		Reference		Reference	
	Yes	53 (50%)	12 (35%)	0.572 (0.283-1.16)	0.11	0.59 (0.29-1.20)	0.15	0.64 (0.31-1.33)	0.23
Pet ownership	No	61 (56%)	24 (67%)	Reference		Reference		Reference	
	Yes	47 (44%)	12 (33%)	0.67 (0.33-1.31)	0.22	0.67 (0.33-1.35)	0.26	0.66 (0.32-1.37)	0.27
Carpets at home	No	50 (46%)	20 (57%)	Reference		Reference		Reference	
	Yes	59 (54%)	15 (43%)	0.68 (0.35-1.33)	0.25	0.62 (0.32-1.23)	0.17	0.65 (0.32-1.33)	0.24
Obstetric factors									
Age of mother, yrs	Mean	32.2	33.1	1.04[5] (0.97-1.12)	0.24	1.05[5] (0.98-1.14)	0.19	1.05[5] (0.97-1.15)	0.25
	SD	4.9	4						

Prenatal alcohol exposure	No	89 (82%)	25 (71%)	Reference		Reference		Reference	
	Yes	20 (18%)	10 (29%)	1.68 (0.81-3.5)	0.16	1.86 (0.88-3.93)	0.1	1.75 (0.82-3.72)	0.15
Mode of delivery	Cesarean	37 (33%)	9 (23%)	Reference		Reference		Reference	
	Vaginal	76 (67%)	27 (75%)	1.39 (0.65-2.95)	0.39	1.40 (0.65-2.99)	0.39	1.41 (0.63-3.13)	0.4
Prenatal AB exposure	No	80 (71%)	27 (77%)	Reference		Reference		Reference	
	Yes	32 (29%)	8 (23%)	0.74 (0.34-1.63)	0.46	0.79 (0.36-1.76)	0.57	0.71 (0.30-1.65)	0.42
Personal and medical history of the study subject									
Gender	Female	57 (50%)	13 (36%)	Reference		Reference		Reference	
	Male	56 (50%)	23 (64%)	1.66 (0.84-3.27)	0.14	1.73 (0.87-3.44)	0.12	1.69 (0.82-3.45)	0.15
Presence of siblings	No	64 (60%)	16 (47%)	Reference		Reference		Reference	
	Yes	43 (40%)	18 (53%)	1.59 (0.81-3.12)	0.17	1.63 (0.83-3.23)	0.15	1.52 (0.77-3.03)	0.23
AB intake in the first month of life	No	103 (93%)	32 (94%)	Reference		Reference		Reference	
	Yes	8 (7%)	2 (6%)	0.78 (0.18-3.28)	0.73	0.87 (0.21-3.70)	0.86	0.96 (0.22-4.11)	0.95

AB, antibiotic ; HR, hazard ratio; CI, confidence interval

[1]log-rank test ; [2]adjusted for family history of atopy; [3]Wald test; [4]adjusted for family history of atopy, gender, delivery mode and presence or not of siblings; [5]HR for each additional year

Table 4. Cox regression results for nutrition-related factors

Variable		**No AD, n (%)**	**AD infants, n (%)**	**Crude HR (95% CI)**	**P-value[1]**	**Adjusted[2] HR (95% CI)**	**P-value[3]**
Breastfeeding							
Duration of breastfeeding	< 3 months	17 (15%)	3 (11%)	Reference		Reference	
	≥ 3 months	96 (85%)	24 (89%)	1.30 (0.39-4.35)	0.67	1.12 (0.33-3.77)	0.85
Duration of exclusive breastfeeding, mo	Mean	3.8	3.4	0.92[4] (0.79-1.08)	0.33	0.92[4] (0.79-1.08)	0.31
	SD	2.7	2.3	-	-	-	-
Food diversification							
Milk	Before 12 months	54 (49%)	7 (50%)	Reference		Reference	
	After 12 months	56 (51%)	7 (50%)	1.01 (0.35-2.88)	0.99	1.08 (0.38-3.11)	0.88
Egg	Before 12 months	28 (25%)	2 (14%)	Reference		Reference	
	After 12 months	85 (75%)	12 (86%)	0.53 (0.12-2.39)	0.4	0.53 (0.12-2.36)	0.4
Nuts	Before 12 months	60 (59%)	6 (60%)	Reference		Reference	
	After 12 months	41 (41%)	4 (40%)	1 (0.28-3.56)	1	1.01 (0.28-3.60)	0.99

Peanuts	Before 12 months	56 (64%)	4 (67%)	Reference		Reference	
	After 12 months	32 (36%)	2 (33%)	1.12 (0.20-6.12)	0.9	1.01 (0.18-5.56)	0.99
Kiwi	Before 12 months	53 (54%)	6 (55%)	Reference		Reference	
	After 12 months	45 (46%)	5 (45%)	0.98 (0.30-3.22)	0.97	0.89 (0.27-2.94)	0.85
Celery	Before 12 months	42 (43%)	5 (43%)	Reference		Reference	
	After 12 months	56 (57%)	7 (57%)	0.90 (0.29-2.86)	0.86	0.87 (0.27-2.74)	0.81
Fish	Before 12 months	8 (7%)	2 (14%)	Reference		Reference	
	After 12 months	105 (93%)	12 (86%)	2.18 (0.48-9.83)	0.3	1.93 (0.43-8.78)	0.39
Seafood	Before 12 months	60 (73%)	5 (83%)	Reference		Reference	
	After 12 months	22 (27%)	1 (17%)	1.80 (0.21-15.51)	0.59	1.63 (0.19-14.16)	0.66

HR, hazard ratio; CI, confidence interval

[1]log-rank test; [2]adjusted for family history of atopy; [3]Wald test; [4]HR for each additional month

Fig. 1 Cumulative incidence of AD according to the family history of atopic disorders

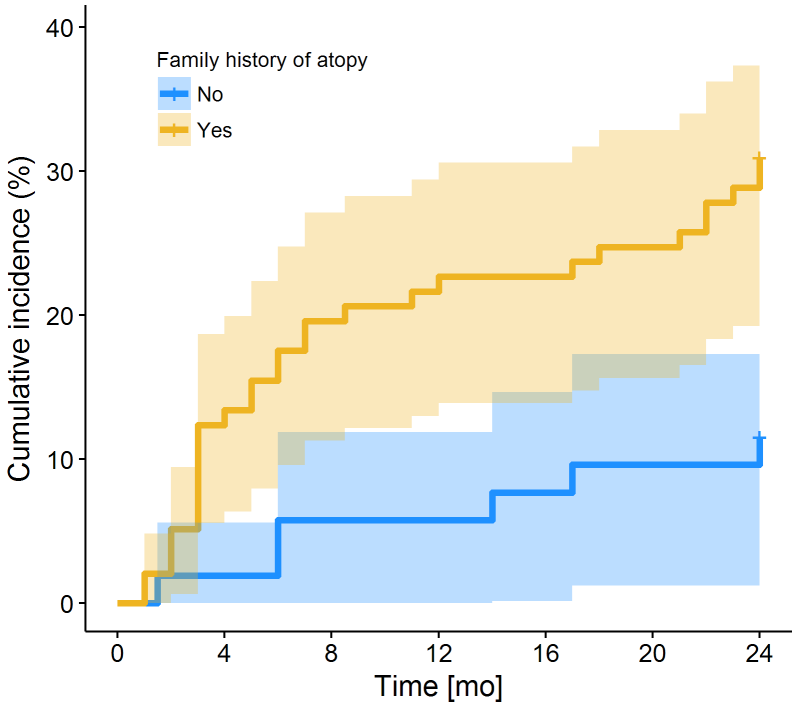

Fig. 2 Forest plot showing the crude and adjusted hazard ratio (HR) for all variables with P < 0.10 in the univariate Cox models. Adjusted HRs were calculated from a model including family history of atopy, delivery mode, gender and presence or not of siblings. Error bars represent 95% confidence interval.