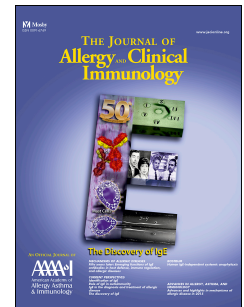


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Early oral exposure to house dust mite allergen through breast milk: a potential risk factor for allergic sensitization and respiratory allergies in children

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CAPSULE SUMMARY

Elevated levels of House Dust Mite allergens in breastmilk are associated with an increased risk of atopic sensitization and respiratory allergy in children. This observation highlights a yet unexplored factor that may contribute to allergy development and interfere with prevention of allergy by breastfeeding .

KEY WORDS

Allergy; allergic sensitization; asthma; allergic rhinitis; *Der p1*, house dust mite; human breast milk; allergy primary prevention; neonate; oral immune tolerance.

ABBREVIATIONS

HDM: House dust mite

Der p: *Dermatophagoides pteronyssinus*

OVA: Ovalbumin

To the Editor,

House dust mites (HDM) are found worldwide and are a major source of perennial aeroallergens. Allergic rhinitis and asthma have been attributed to sensitization to HDM allergens from *Dermatophagoides pteronyssinus* (*Der p*) and *Dermatophagoides farinae* (*Der f*) in up to 80 % of patients ¹. Sensitization to HDM is linked to HDM allergen exposure and is known to occur mostly during the first years of life (reviewed in ²). These observations have led to early life environmental control studies in birth cohorts aimed at reducing domestic HDM exposure to prevent HDM sensitization and allergies; however, results of these studies have been conflicting (reviewed in²). Currently, no efficient strategy is available for prevention of HDM-induced allergy thus highlighting the necessity to better elucidate the source of exposures, risks and protective factors. In recent years, the concept has emerged that early oral exposure to food antigens may be necessary for immune tolerance induction and long-term prevention of inappropriate immune reactions to food and this concept has been tested in recent interventional trials with some promising results ³. Clinical trials have recently showed that introducing solid food while continuing to breastfeed may enhance chances of tolerance induction and prevent food allergy ⁵. We further proposed that oral exposure to aero-allergens through breast milk may affect the risk to develop respiratory allergies ⁶. Recently, we demonstrated the presence of respiratory allergens from *Der p* and *Blomia tropicalis* HDM in human breast milk ^{7, 8}. This surprising observation may be explained by the fact that *Der p* particules are ingested after lung mucociliary clearance of inhaled *Der p* particules and from food contaminated with dust²; *Der p* particules would then follow the same route as dietary antigens to maternal milk⁷. *Der p 1* in maternal milk was found in similar quantities as dietary egg antigen OVA and to be immunoreactive⁷. In a mouse model, we found that *Der p* in breast milk promoted subsequent allergic sensitization in the progeny⁷.

In the present study, our main objective was to assess whether early exposure to *Der p 1* transferred from the mother to the newborn through breast milk would affect child's subsequent development of allergic sensitization, asthma and allergic rhinitis in their first 5 years of life, using prospective population-based data from the French EDEN birth cohort (<http://eden.vjf.inserm.fr>) (See supplementary Table 1, 2 and 3 for mother-children pairs characteristics). *Der p 1* allergen was quantified by ELISA in milk of 255 mothers (supplementary material and ⁷). *Der p 1* was detected in 2/3 of all breast milk samples and median value level was 52.1 pg/ml in the whole population (Figure 1 and Supplementary Table 4). Median *Der p 1* level was higher in mothers with a history of asthma or allergies (Figure 1). In the whole population (n=255), median *Der p 1* level was higher in mothers younger than 34 years compared to those aged more than 34 years (p=0.02), in mothers whose milk was collected during winter compared to the other seasons (p=0.04), and in mothers living in Nancy compared to those living in Poitiers (p=0.0008) (Supplementary Table 5). No significant association was found between *Der p 1* levels in breast milk and proxies of exposure to HDM allergen, such as common HDM sources in the environment where the mother and the child have lived during the follow-up (Supplementary Table 6) as well as the occupancy load (Supplementary Table 7).

To analyze possible associations between *Der p 1* levels in breast milk and health outcomes by age 5, exposure to *Der p 1* was classified as a categorical variable, namely "low" levels below or equal to median vs. "high" levels above the median value obtained in the total population (=52.1 pg/ml) (see supplementary material for detailed statistic analysis method). In the entire population, no significant association was found between *Der p 1* levels and the risk of the studied health outcomes (Figure 2 and Supplementary Table 8). However, children breastfed by mothers with a history of asthma or allergies with high *Der p 1* levels in milk were at significantly higher risk of asthma or allergic rhinitis compared to children from

mothers with low *Der p 1* in milk (OR 3.4, $p=0.02$ (Figure 2 and Supplementary Table 8). In this group, we also found a borderline significance of higher risk for asthma with high milk *Der p1* levels compared to those with low milk *Der p 1* levels (OR 7.8, $p=0.06$). Both relationships persisted after adjustment for confounders (Supplementary Table 8). Among the 255 mother-child pairs with *Der p 1* quantification in breast milk, 142 children had available sera at 5 years for total and *Der p 1* specific IgE assessment (ImmunoCAP technology, Thermo Scientific/Phadia) (see supplementary Table 3 for mother-child pair characteristics). We found that ORs for allergic sensitization in children breastfed by mothers having high compared to low levels of *Der p 1* in milk were higher than 1 in the 3 groups we analyzed (whole population/ children from mother with and without history of asthma and allergy, Figure 2 and supplementary Table 8). High *Der p 1* in milk was associated with a significantly increased risk for having positive total IgE (aOR 3.87, $p=0.003$) and a borderline significant trend for the risk for having specific IgE to *Der p 1* (aOR 2.21, $p=0.07$) (Figure 2 and Supplementary Table 8). An increased OR for having positive total IgE was found in children breastfed by mothers both with and without allergic history (aOR 2.85 and 4.34, respectively); only the latter one being found to be statistically significant ($p=0.2$ and 0.005 , respectively) (Figure 2 and Supplementary Table 8).

Our study is the first in literature considering the presence of respiratory HDM *Der p 1* allergen in human breast milk as a potential contributor to the development of allergic sensitization and asthma and allergic rhinitis in children. We observed an increased risk of allergic sensitization at 5 years in children breastfed by mothers with *Der p 1* levels in milk above median value compared to children breastfed by mothers with *Der p 1* levels in milk below median value. We further found that high levels of *Der p 1* in milk were associated with an increased risk of asthma or allergic rhinitis in the first 5 years for children whose mothers had history of asthma or allergy compared to those with low levels of *Der p 1* in milk. This latter

result suggests that a gene-environment interaction between familial asthma or allergy and *Der p 1* in the milk is necessary for increasing the risk of asthma and rhinitis. Our findings are of relevance in the debate about the protective effect on the development of allergies conferred by breast milk, which is still controversial⁹. The data presented in this report highlight that heterogeneity of breastfeeding induced protection from atopic sensitization and respiratory allergies might depend on levels of respiratory *Der p 1* antigen found in mother's milk.

Whether *Der p* is a proxy for other exposures to environmental factors at the origin of the development of child's allergy cannot be excluded. However, high *Der p 1* in breast milk was still a risk factor in subgroups of children when adjusting for environmental exposure to HDM in multivariate models and restricting the investigation to the subgroups with low indoor sources of HDM, indicating that HDM allergen content is an independent risk factor for allergy (data not shown). There were also no differences observed in either breastfeeding behavior or population characteristics between asthmatic or allergic parents, except cat ownership, considered as a confounder. Importantly, data obtained in a mouse model where strict control of maternal and environmental parameters is possible, support the hypothesis of a causal role of *Der p1* in milk in allergic sensitization development.⁷

Identification of factors governing *Der p* levels in breast milk and its pro-allergenic properties is needed to propose early intervention to counteract *Der p* detrimental effects and prevent respiratory allergies. Here, we report significantly higher levels of *Der p 1* in breast-milk from mothers with history of asthma or allergies, mothers living in Nancy versus Poitiers, mothers younger than 35 years and in milk collected during winter. We found no association between *Der p 1* levels in breast milk and proxies of indoor environmental HDM exposure. The apparent lack of association between indoor environmental exposure to *Der p* and levels of *Der p 1* in breast milk is further supported by a report on levels of exposure to *Der p* in 6390 dwellings in France showing that levels of *Der p* exposure were much lower in

Nancy than Poitiers while we found that *Der p 1* levels were significantly higher in milk from Nancy than Poitiers¹⁰. This is consistent with studies showing no direct correlation between maternal food antigen intake and its presence in breast milk¹¹. To elucidate which factors are driving the presence of respiratory allergens in breast milk, we will need to better know how and when we get exposed to respiratory allergens² and to identify factors which affect transfer of antigen from maternal gut to milk. Clarification of mechanisms underlying priming activities of oral exposure to very low amounts of *Der p1* is necessary to delineate under which conditions early oral allergen exposure is safe and beneficial for allergy prevention. Our data obtained from mouse models clearly indicate that the nature of the antigen is critical in deciphering the long-term impact on allergy susceptibility¹². The data presented here highlight the need to be cautious in recommending early oral exposure to allergens. In contrast to antigens such as OVA, *Der p* allergens have strong intrinsic adjuvant properties due to Toll Like Receptor agonist and protease activities which may be responsible for initiation of allergic immune responses¹. Our ongoing studies in neonatal mice assess impacts of milk-transferred *Der p* on gut mucosal immune regulation and ways to counteract *Der p* detrimental effects on allergy susceptibility.

In conclusion, we have identified an environmental factor present in breast milk that may affect the risk of allergic sensitization and respiratory allergies in children. This original observation paves the way for future large cohort analyses of association between oral exposure to aeroallergens and risk of respiratory allergies and for fostering mechanistic studies on factors affecting respiratory allergen levels and allergenicity in breast milk.

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The funding source had no role in data collection, analysis of data, interpretation of results or writing of this manuscript.

AUTHOR CONTRIBUTION

Drs I. Annesi-Maesano and V. Verhasselt had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis

- Conception of the study: V. Verhasselt

- Design of the study: I. Annesi-Maesano and V. Verhasselt

- Acquisition and analysis of biological data: P. Macchiaverni, A. Rekima, M. Tulic and V. Verhasselt

- Statistical analysis: N. Baiz and I. Annesi-Maesano

- data interpretation: all

- Drafting of the manuscript: N. Baiz, P. Macchiaverni, I. Annesi-Maesano and V. Verhasselt

- Critical revision of the manuscript for important intellectual content: all

- Obtaining funding: I. Annesi-Maesano and V. Verhasselt

- Supervision: I. Annesi-Maesano and V. Verhasselt

CONFLICT OF INTEREST DISCLOSURES

The authors have declared that no competing interests exist

ADDITIONAL CONTRIBUTIONS

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REFERENCES

1. Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol* 2011; 32:402-11.
2. Custovic A. To what extent is allergen exposure a risk factor for the development of allergic disease? *Clin Exp Allergy* 2015; 45:54-62.
3. Elizur A, Katz Y. Timing of allergen exposure and the development of food allergy: treating before the horse is out of the barn. *Curr Opin Allergy Clin Immunol* 2016; 16:157-64.
4. Grimshaw KE, Maskell J, Oliver EM, Morris RC, Foote KD, Mills EN, et al. Introduction of complementary foods and the relationship to food allergy. *Pediatrics* 2013; 132:e1529-38.
5. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. *N Engl J Med* 2016; 374:1733-43.
6. Verhasselt V. Neonatal tolerance under breastfeeding influence. *Curr Opin Immunol* 2010; 22:623-30.
7. Macchiaverni P, Rekima A, Turfkruyer M, Mascarell L, Airouche S, Moingeon P, et al. Respiratory allergen from house dust mite is present in human milk and primes for allergic sensitization in a mouse model of asthma. *Allergy* 2014; 69:395-8.
8. Macchiaverni P, Ynoue LH, Arslanian C, Verhasselt V, Condino-Neto A. Early Exposure to Respiratory Allergens by Placental Transfer and Breastfeeding. *PLoS One* 2015; 10:e0139064.

- 249 9. Victora CG BR, Barros AJ, França GVA, Horton S, Krasevec J, et al. Breastfeeding in
250 the 21st century: epidemiology, mechanisms, and lifelong effect. *The Lancet*
251 2016;475-90.
- 252 10. Rocchi S, Reboux G, Frossard V, Scherer E, Valot B, Laboissiere A, et al.
253 Microbiological characterization of 3193 French dwellings of Elfe cohort children. *Sci*
254 *Total Environ* 2015; 505:1026-35.
- 255 11. Palmer DJ, Gold MS, Makrides M. Effect of maternal egg consumption on breast milk
256 ovalbumin concentration. *Clin Exp Allergy* 2008; 38:1186-91.
- 257 12. Verhasselt V. Is infant immunization by breastfeeding possible? *Philos Trans R Soc*
258 *Lond B Biol Sci* 2015; 370.

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FIGURE LEGENDS

Figure 1. *Der p 1* levels in breast milk

Data represent levels of *Der p 1* measured by ELISA in breast milk samples from mothers of the whole study population (n=255) and from mothers with (n=84) or without (n=171) history of asthma or allergies. Horizontal bar represent median value and dotted line the detection level of *Der p1* by ELISA. For all the milk samples for which *Der p 1* was undetectable by ELISA, we attributed the value of zero. Mann-Whitney U test was used to compare *Der p 1* levels between mothers with and without a history of asthma or allergies.

Figure 2. Prevalence and adjusted associations of respiratory outcomes and allergic sensitization in children according to *Der p 1* levels in milk

Prevalence (Figure 2A) of respiratory outcomes and allergic sensitization in children from mothers with *Der p 1* levels above median value (*Der p 1*; > 52pg/ml, filled column) and below or equal to median value (*Der p*; ≤52 pg/ml, open columns). Fisher test was used to compare prevalence of outcomes.

Adjusted odds ratios (aOR) (Figure 2B) of respiratory outcomes and allergic sensitization in children from mothers with high versus low levels of *Der p 1* in milk in the whole population (black, triangles), in mothers with (red circles) or without (green squares) history of asthma or allergies. Odds ratios were adjusted for city, mother's age, maternal history of asthma or allergies (in non-stratified model-whole population only), smoking during pregnancy, passive smoke exposure during the first 3 years of life, season of birth and newborn's sex.

Figure 1. *Der p1* levels in breast milk

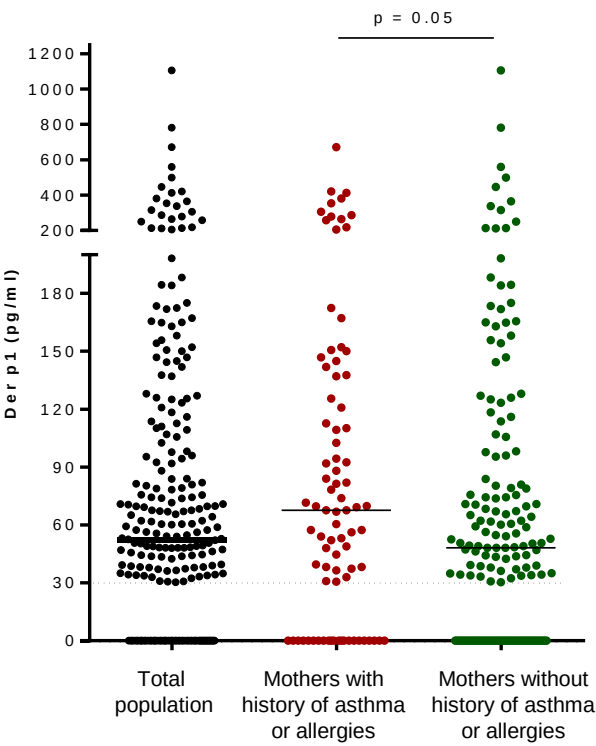
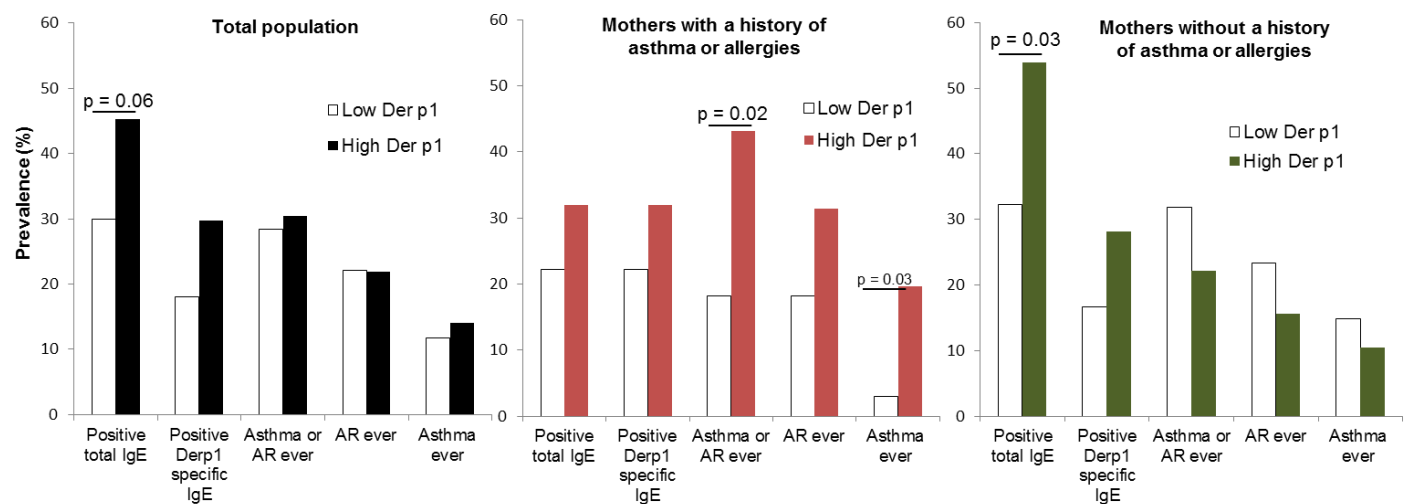
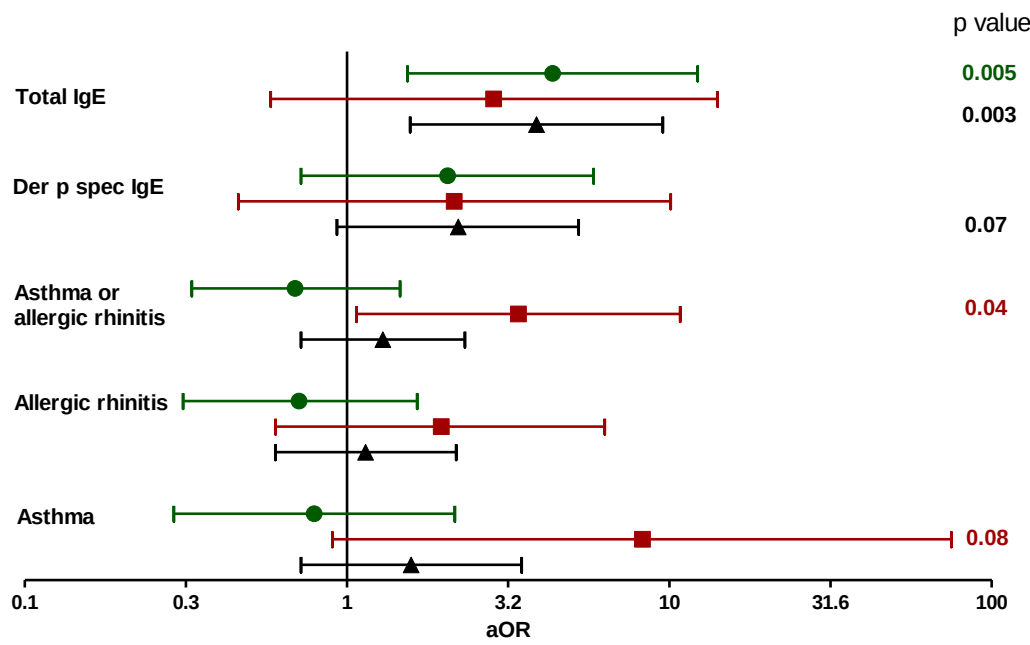


Figure 2. Prevalence and adjusted associations of respiratory outcomes and allergic sensitization according to *Der p1* levels.

A.



B.



Supplementary material

METHODS

Study design and participants

Mother-child pairs were drawn from the French EDEN (“Etude des Déterminants pré et post-natal du développement et de la santé de l’Enfant”) prospective birth cohort study (<http://eden.vjf.inserm.fr>).¹ Among 621 mother-child pairs with breast milk collected during the first week postpartum and their health status followed-up, 255 pairs whose breast milk was collected between day 3 and day 7 postpartum were randomly selected. The study received approval from the ethics committee (CCPPRB) of Kremlin Bicêtre University Hospital on December 12th, 2002 and from CNIL (Commission Nationale Informatique et Liberté), the French data privacy institution. Written consent was obtained from all mothers.

Health outcomes

Children were follow-up from birth to 5 years of age. Parents completed an enriched version of the International Study of Asthma and Allergies in Childhood phase-I questionnaire^{2, 3} filled at different periods of the follow-up.⁴ Asthma was defined as parental report of doctor-diagnosis of asthma plus either one or more attacks of wheeze or use of asthma medication in the last 12 months. Allergic rhinitis was defined as having problem with sneezing or a runny nose or blocked nose without a cold or a flu, accompanied by itchy-watery eyes in the past 12 months.⁵ Ever prevalence of asthma, allergic rhinitis and asthma or allergic rhinitis (combined variable) was assessed as the presence of each health event at least once during the first 5 years of child’s life. Maternal and paternal history of asthma or allergies was defined on the basis of a positive answer to a questionnaire administrated by a nurse on physician-diagnosed asthma, allergic rhinitis, eczema or food allergies.

In a subgroup of 142 children for whom blood serum was available, allergic sensitization was defined by assessing total and *Der p1* specific immunoglobulin E (IgE) using ImmunoCAP technology (Thermo Scientific/Phadia). Total IgE were considered positive when > 60 IU/mL and specific IgE to *Der p1* when ≥ 0.1 IU/mL.

***Der p 1* measurement**

Transitional breast milk was collected using a sterile breast-pump from day 3-7 post-delivery. The samples were aliquoted and stored at -80°C . Before analysis, milk samples were thawed and centrifuged for 20 min at 160 g at 4°C . The top layer of fat and the pellet were discarded and the intermediate fluid fraction used for *Der p 1* measurement using a modified protocol of *Der p 1* ELISA kit 5H8/4Cl (EL-DP1, Indoor Biotechnologies) as previously described.⁶ A typical dose response curve of *Der p 1* detection in PBS-Tween 0.05%-BSA % is shown in Figure 1. *Der p 1* recovery in undiluted human milk or in milk diluted 2 or 4 times in PBS-Tween 0.05%- BSA 1% is also shown in Supplementary Figure 1. For the analysis of *Der p 1* levels in milk from this cohort, milk sample were diluted twice and an internal positive control was used in each ELISA plate. Samples with *Der p 1* levels greater than 30 pg/ml were considered positive. For all the milk samples for which *Der p1* was undetectable by ELISA, we attributed the value of zero.

HDM sources

The questionnaire allowed us to assess indoor environment sources of HDM as proxies of environmental HDM⁷⁻¹⁰ to which mothers and children could have been exposed to at least once during the follow-up. As an additional proxy of exposure to HDM in the dwelling, the occupancy load, which has been demonstrated to be a strong indicator of exposure to HDM allergen sources indoors¹¹ and defined by the mean of the number of occupants divided by the number of rooms in the dwelling during the follow-up, was also used. A 2-class variable

was introduced for the occupancy load in order to indicate low (< median value) vs. high (> median value) exposure to indoor sources of HDM.

Statistical analyses

The sample was described using frequencies of categorical variables, means and standard deviations of continuous variables (median and interquartile range for *Der p1*). We used Chi-square tests to compare categorical data, T-tests for normally-distributed data and Mann-Whitney U tests for skewed data.

To quantify the relationships between exposure to *Der p1* allergen through lactation and health outcomes, and because of the non-normal distribution of *Der p1* levels, exposure to *Der p1* was classified as a categorical variable, namely “low” levels below or equal to median vs. “high” levels above the median value obtained in the total population. Crude odd ratios (ORs) between *Der p1* exposure and health outcomes and 95% confidence intervals (CI) were evaluated with univariate logistic regression models and adjusted ORs and 95% CI with multivariate logistic regression models. In addition to the inclusion of confounders based on their statistically significant association with health outcomes, we selected other adjustment variables, on the basis of their known relationship to asthma and allergic rhinitis. To examine effect modification of maternal asthma or allergies in the association of health outcomes with *Der p1* levels, an interaction term between *Der p1* and maternal history of asthma or allergies was included in the multivariate models. A stratified analysis was also performed in order to assess whether the associations between *Der p1* exposure and health outcomes differed according to maternal history of asthma or allergies.

All statistical analyses were performed using SAS statistical software version 9.3 (SAS Institute Inc, Cary, North Carolina).

1. Baiz, et al. Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. *The Journal of allergy and clinical immunology* 2014; 133:147.
2. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J* 1998; 12:315.
3. Williams, et al. Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. *J Allergy Clin Immunol* 1999; 103:125.
4. Heude, et al. Cohort Profile: The EDEN mother-child cohort on the prenatal and early postnatal determinants of child health and development. *International journal of epidemiology* 2015.
5. Asher, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8:483.
6. Macchiaverni, et al. Mother to child transfer of IgG and IgA antibodies against *Dermatophagoides pteronyssinus*. *Scandinavian journal of immunology* 2011; 74:619.
7. Bemt, et al. Influence of mattress characteristics on house dust mite allergen concentration. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 2006; 36:233.
8. Uchikoshi, et al. A study of the ecology of the house dust mite in dwelling houses. *The Tokai journal of experimental and clinical medicine* 1982; 7:233.
9. Abbott, et al. House dust mite counts in different types of mattresses, sheepskins and carpets, and a comparison of brushing and vacuuming collection methods. *Clinical allergy* 1981; 11:589.
10. Tobias, et al. Exposure to indoor allergens in homes of patients with asthma and/or rhinitis in southeast Brazil: effect of mattress and pillow covers on mite allergen levels. *International archives of allergy and immunology* 2004; 133:365.
11. Hasselaar E. How Healthy Is The Bedroom? . *Indoor Air Exposure: Healthy Buildings*, 2006:185.

Supplementary Table 1. Characteristics of the study population with *Der p I* assessed in breast milk compared to the rest of the population

Variable	Whole population (n = 621)	Study Population (n = 255)	Rest of the population (n = 366)	p **
City, %				
Poitiers	38.1	39.6	36.9	
Nancy	61.9	60.4	63.1	0.77
Maternal age, Mean $\pm$ SD (yrs.) *	31.5 $\pm$ 4.6	30.3 $\pm$ 4.4	30.7 $\pm$ 4.7	0.50
< 25 yrs., %	10.4	10.6	10.4	
25-34 yrs., %	72.4	71.8	72.5	
> 34 yrs., %	17.2	17.7	17.0	0.99
BMI, Mean $\pm$ SD (kg/m²)	25.8 $\pm$ 4.4	26.3 $\pm$ 4.7	25.5 $\pm$ 4.1	0.19
Normal, %	50.2	46.8	52.3	
Overweight, %	35.7	35.7	36.1	
Moderate obesity, %	10.3	12.3	8.8	
Severe obesity, %	3.7	5.2	2.8	0.55
Maternal history of asthma or allergies, %	29.7	33.0	27.1	0.26
Paternal history of asthma or allergies, %	24.8	23.9	25.5	0.91
Maternal gestational diabetes, %	7.0	5.9	7.9	0.59
Maternal educational level, %				
Primary school or less	3.3	2.8	3.6	
Secondary school	53.6	54.0	53.4	
University degree or higher	43.2	43.2	42.8	0.99
Paternal educational level, %				
Primary school or less	5.6	4.8	6.2	
Secondary school	60.2	62.7	58.9	
University degree or higher	34.2	32.5	34.8	0.92
Any smoking during pregnancy, %	32.3	31.0	33.5	0.73
Household income (Euros), %				
$\leq$ 2300	65.4	66.3	65.0	
> 2300	34.6	33.7	35.0	0.97
Presence of a pet in the house during pregnancy, %	53.9	53.3	54.4	0.96
Presence of a pet in the house (0 – 5 years), %	64.4	64.7	64.4	0.99
Presence of a cat in the house during pregnancy, %	25.8	25.1	26.2	0.95
Presence of a cat in the house (0 – 5 years), %	35.8	33.7	37.2	0.68
Any smoking (0–3 years), %	43.8	42.8	44.5	0.91
Dampness in housing (0–3 years), %	7.7	8.2	7.4	0.94
Daycare attendance first started, %				
Before 1 year	67.0	67.5	66.7	0.97
Older siblings, %				
0	49.4	51.8	47.5	
1-2	46.2	44.7	47.5	
$\geq$ 3	4.3	3.5	4.9	0.79
Gestational age at birth, Mean (weeks) $\pm$ SD	39.3 $\pm$ 1.6	39.2 $\pm$ 1.8	39.3 $\pm$ 1.5	0.99
Preterm birth †, %	5.4	5.5	5.2	0.95
Birth weight, Mean (kg) $\pm$ SD	3.3 $\pm$ 0.5	3.3 $\pm$ 0.6	3.3 $\pm$ 0.5	0.58
Low birth weight ‡, %	5.4	5.5	5.2	0.95
Caesarian delivery, %	14.9	16.5	13.7	0.60
Sex of the new-born, %				
Female	49.1	45.5	51.4	0.41
Season of birth, %				
Summer	24.8	23.5	25.4	
Autumn	19.2	20.0	18.6	
Winter	23.7	24.3	23.2	
Spring	32.3	32.2	32.8	0.99
Mean duration of breastfeeding (months) $\pm$ SD	4.7 $\pm$ 3.7	4.8 $\pm$ 3.7	4.7 $\pm$ 3.7	0.96
Exclusively breastfed for $\geq$ 6 months, %	12.3	12.2	12.6	0.98
Respiratory outcomes, %				
Asthma ever	11.2	12.9	10.1	0.59
Allergic rhinitis ever	19.8	22.0	18.3	0.52
Asthma or allergic rhinitis ever	27.4	29.3	25.9	0.65

*SD: standard deviation;** p-value for Chi-2 tests (categorical variables) or Mann-Whitney U tests (continuous variables) comparing the three groups; †<37 completed weeks of gestation; ‡ < 2500 g

Supplementary Table 2. Characteristics of mothers with and without history of asthma or allergies

Variable	Mothers with history of asthma or allergies (n = 84)	Mothers without history of asthma or allergies (n = 171)	p **
City, %			
Poitiers	41.7	38.6	
Nancy	58.3	61.4	0.64
Maternal age, Mean $\pm$ SD (yrs.) *	30.3 $\pm$ 4.2	30.3 $\pm$ 4.6	0.99
< 25 yrs., %	9.5	11.1	
25-34 yrs., %	75.0	70.2	
> 34 yrs., %	15.5	18.7	0.72
BMI, Mean $\pm$ SD (kg/m²)	26.3 $\pm$ 4.6	26.3 $\pm$ 4.7	0.82
Normal, %	43.9	48.2	
Overweight, %	39.0	34.1	
Moderate obesity, %	12.2	12.4	
Severe obesity, %	4.9	5.3	0.89
Paternal history of asthma or allergies, %	29.8	21.1	0.13
Maternal gestational diabetes, %	4.8	6.4	0.59
Maternal educational level, %			
Primary school or less	2.4	3.0	
Secondary school	50.0	56.0	
University degree or higher	47.6	41.1	0.62
Paternal educational level, %			
Primary school or less	1.2	6.6	
Secondary school	69.5	59.3	
University degree or higher	29.3	34.1	0.10
Any smoking during pregnancy, %	32.5	30.2	0.70
Household income (Euros), %			
$\leq$ 2300	54.8	61.9	
> 2300	45.2	38.1	0.37
Presence of a pet in the house during pregnancy, %	51.2	54.4	0.63
Presence of a pet in the house (0 – 5 years), %	59.5	67.3	0.22
Presence of a cat in the house during pregnancy, %	17.2	28.1	0.02
Presence of a cat in the house (0 – 5 years), %	33.3	33.9	0.93
Any smoking (0–3 years), %	39.3	44.4	0.43
Dampness in housing (0–3 years), %	10.7	7.0	0.31
Daycare attendance first started, %			
Before 1 year	63.1	69.6	0.30
Older siblings, %			
0	58.3	48.5	
1-2	40.5	46.8	
$\geq$ 3	1.2	4.7	0.17
Gestational age at birth, Mean (weeks) $\pm$ SD	39.5 $\pm$ 1.7	39.1 $\pm$ 1.8	0.27
Preterm birth †, %	4.8	5.9	0.72
Birth weight, Mean (kg) $\pm$ SD	3.3 $\pm$ 0.6	3.3 $\pm$ 0.5	0.44
Low birth weight ‡, %	3.6	6.4	0.35
Caesarian delivery, %	15.5	17.0	0.76
Sex of the newborn, %			
Female	46.8	42.9	0.55
Season of birth, %			
Summer	24.0	22.6	
Autumn	17.0	26.2	
Winter	24.6	23.8	
Spring	34.5	27.4	0.34
Mean duration of breastfeeding (months) $\pm$ SD	4.7 $\pm$ 3.7	4.8 $\pm$ 3.7	0.96
Exclusively breastfed for $\geq$ 6 months, %	14.3	11.1	0.47
Respiratory outcomes, %			
Asthma ever	13.1	12.9	0.96
Allergic rhinitis ever	26.2	19.9	0.25
Asthma or allergic rhinitis ever	33.3	27.5	0.34

*SD: standard deviation;** p-value for Chi-2 tests (categorical variables) or Mann-Whitney U tests (continuous variables); †<37 completed weeks of gestation; ‡ < 2500 g

Supplementary Table 3. Characteristics of children with and without immunoglobulin E (IgE) assessments

Variable	Sample with IgE assessment (n = 142)	Sample without IgE assessment (n = 113)	p **
City, %			
Poitiers	62.0	11.5	
Nancy	38.0	88.5	<0.0001
Maternal age, Mean $\pm$ SD (yrs.) *	30.5 $\pm$ 4.8	30.0 $\pm$ 4.0	0.72
< 25 yrs., %	12.0	8.9	
25-34 yrs., %	67.6	77.0	
> 34 yrs., %	20.4	14.2	0.60
BMI, Mean $\pm$ SD (kg/m²)	26.7 $\pm$ 4.8	25.7 $\pm$ 4.5	0.11
Normal, %	40.0	55.4	
Overweight, %	41.4	28.6	
Moderate obesity, %	12.9	11.6	
Severe obesity, %	5.7	4.5	0.39
Maternal history of asthma or allergies, %	30.3	36.3	0.60
Paternal history of asthma or allergies, %	21.1	27.4	0.50
Maternal gestational diabetes, %	6.3	5.3	0.94
Maternal educational level, %			
Primary school or less	3.6	1.8	
Secondary school	58.6	48.2	
University degree or higher	37.9	50.0	0.40
Paternal educational level, %			
Primary school or less	6.6	2.7	
Secondary school	65.7	58.9	
University degree or higher	27.7	38.4	0.34
Any smoking during pregnancy, %	33.8	27.3	0.54
Household income (Euros), %			
$\leq$ 2300	71.1	60.2	
> 2300	28.9	39.8	0.18
Presence of a pet in the house during pregnancy, %	58.5	46.9	0.19
Presence of a pet in the house (0 – 5 years), %	72.5	54.9	0.01
Presence of a cat in the house during pregnancy, %	26.1	23.9	0.69
Presence of a cat in the house (0 – 5 years), %	38.7	27.4	0.06
Any smoking (0–3 years), %	45.8	38.9	0.55
Dampness in housing (0–3 years), %	7.8	8.9	0.95
Daycare attendance first started, %			
Before 1 year	63.4	72.6	0.30
Older siblings, %			
0	48.6	55.8	
1-2	47.9	40.7	
$\geq$ 3	3.5	3.5	0.85
Gestational age at birth, Mean (weeks) $\pm$ SD	39.3 $\pm$ 1.8	39.2 $\pm$ 1.7	0.65
Preterm birth †, %	4.2	7.1	0.61
Birth weight, Mean (kg) $\pm$ SD	3.3 $\pm$ 0.6	3.3 $\pm$ 0.5	0.62
Low birth weight ‡, %	6.3	4.4	0.80
Caesarian delivery, %	11.3	23.0	0.04
Sex of the newborn, %			
Female	45.1	46.0	0.99
Season of birth, %			
Summer	23.9	23.0	
Autumn	20.4	19.5	
Winter	22.5	26.6	
Spring	33.1	31.0	0.99
Mean duration of breastfeeding (months) $\pm$ SD	4.3 $\pm$ 3.6	5.4 $\pm$ 3.8	0.06
Exclusively breastfed for $\geq$ 6 months, %	10.6	14.2	0.68
Respiratory outcomes, %			
Asthma ever	12.7	13.3	0.99
Allergic rhinitis ever	22.5	21.2	0.97
Asthma or allergic rhinitis ever	28.9	30.1	0.98

*SD: standard deviation;** p-value for Chi-2 tests (categorical variables) or Mann-Whitney U tests (continuous variables); †<37 completed weeks of gestation; ‡ < 2500 g

Supplementary Table 4. *Der p1* levels in breast milk according to maternal history of asthma or allergies

	Total population (n = 255)	Mothers with a history of asthma or allergies (n = 84)	Mothers without a history of asthma or allergies (n = 171)	p*
% of positive samples (n)	69.8 (178)	73.8 (62)	67.8 (116)	0.30
Median (IQR), pg/mL	52.1 (0.0 – 109.3)	67.6 (0.0 – 137.4)	48.2 (0.0 – 95.4)	0.05
Median (IQR) among positive samples, pg/mL	73.8 (49.0 – 146.8)	90.0 (57.4 – 150.7)	67.8 (48.1 – 127.5)	0.05
% of samples (n) above median value obtained from the total population	50.20 (128)	60.7 (51)	45.03 (77)	0.02

* p-value of Mann-Whitney U test and chi-square test for comparison of continuous variables and percentages, respectively, between mothers with or without a history of asthma or allergies.

Supplementary Table 5. Factors associated with *Der p1* concentration in breast milk according to maternal history of asthma or allergies

Variable	Total population (n = 255)		Mothers with a history of asthma or allergies (n = 84)		Mothers without a history of asthma or allergies (n = 171)	
	<i>Der p1</i> (pg/mL) Median (IQR)	p*	<i>Der p1</i> (pg/mL) Median (IQR)	p*	<i>Der p1</i> (pg/mL) Median (IQR)	p*
City						
Poitiers	44.4 (0.0; 70.6)	0.008	48.9 (0.0; 102.6)	0.16	38.5 (0.0; 64.2)	0.03
Nancy	61.4 (0.0; 125.5)		71.5 (33.0; 141.9)		50.3 (0.0; 113.6)	
Maternal age						
< 25 yrs.	50.6 (0.0; 106.9)	0.02	64.7 (19.2; 149.6)	0.29	50.6 (0.0; 106.9)	0.08
25-34 yrs.	58.9 (0.0; 123.3)		69.8 (30.9; 144.9)		49.2 (0.0; 108.3)	
> 34 yrs.	34.3 (0.0; 65.2)		37.4 (0.0; 67.6)		34.1 (0.0; 60.8)	
Smoking during pregnancy						
No smoking	54.5 (0.0; 116.0)	0.06	67.3 (15.4; 139.8)	0.95	50.6 (0.0; 106.9)	0.01
Smoking	44.4 (0.0; 73.9)		70.8 (0.0; 111.7)		30.8 (0.0; 50.3)	
Season of birth						
Summer	41.6 (0.0; 86.2)	0.04	69.2 (33.0; 167.2)	0.49	34.3 (0.0; 61.8)	0.01
Autumn	48.1 (0.0; 81.9)		60.4 (0.0; 84.9)		35.0 (0.0; 73.7)	
Winter	64.9 (42.4; 144.9)		69.2 (37.8; 115.1)		60.3 (42.4; 158.1)	
Spring	54.4 (0.0; 116.0)		67.6 (0.0; 152.1)		49.0 (0.0; 98.2)	

*p-value of Mann-Whitney U test or Kruskal-Wallis test

Supplementary Table 6. Relationships between house dust mites (HDM) sources in the environment where the mother and the child lived during the follow-up and *Der p 1* levels in breast milk

Potential HDM source	Low HDM in milk % (n)	High HDM in milk % (n)	p-chi2/Fisher
Do it yourself activities (n = 251)			
Never	54.0 (68)	44.8 (56)	
Rarely	34.9 (44)	42.4 (53)	
Often	11.1 (14)	12.8 (16)	0.35
Presence of a carpet at home (n = 239)	37.1 (43)	26.0 (32)	0.07
Double curtains or wall fabrics (n = 236)	22.2 (26)	19.3 (23)	0.58
Child's mattress (n = 52)			
New	54.6 (12)	50.0 (15)	
Bought 1 to 3 years ago	18.2 (4)	33.3 (10)	
Bought more than 3 years ago	22.7 (5)	13.3 (4)	
Do not know	4.6 (1)	3.3 (1)	0.61
Child's type of pillow (n = 51)			
Synthetic	18.2 (4)	6.9 (2)	
Do not use pillow	81.8 (18)	93.1 (27)	0.22
Child's type of blanket (n = 51)			
Synthetic	22.7 (5)	10.3 (3)	
Wool	4.6 (1)	0.0 (0)	
Other	9.1 (2)	10.3 (3)	
Do not use blanket	63.6 (14)	79.3 (23)	0.39

Supplementary Table 7. Relationship between the occupancy load in the dwellings where the mother and the child have lived and *Der p1* levels in the breast milk

Occupancy load	Low HDM in milk % (n)	High HDM in milk % (n)	p-chi2
n = 255			
<i>Low</i>	42.4 (53)	42.2 (51)	
<i>High</i>	57.6 (72)	57.9 (70)	0.97
n = 142			
<i>Low</i>	39.5 (30)	45.0 (27)	
<i>High</i>	60.5 (46)	55.0 (33)	0.52

Occupancy load was defined as the mean of the number of occupants divided by the number of rooms in the dwelling during the follow-up (Hasselaar E. How Healthy Is The Bedroom? . Indoor Air Exposure: Healthy Buildings, 2006:185-8)

Table 8. Unadjusted and adjusted relationships of *Der p1* levels (above versus below median value; 52.1pg/ml) in breast milk to respiratory outcomes and total and specific IgE levels

Maternal status Health outcome	cOR*	95% CI**	p**	aOR†	95% CI**	p**
Whole population (n=255)						
Total population						
Asthma ever	1.22	0.59; 2.55	0.59	1.58	0.72; 3.48	0.26
AR ever	0.99	0.55; 1.80	0.97	1.14	0.60; 2.18	0.69
Asthma or AR ever	1.11	0.65; 1.90	0.71	1.29	0.72; 2.32	0.40
Mothers with a history of asthma or allergies (n = 84)						
Asthma ever	7.80	0.95; 64.17	0.06	8.25	0.90; 74.95	0.08
AR ever	2.06	0.71; 5.96	0.18	1.96	0.60; 6.34	0.26
Asthma or AR ever	3.41	1.20; 9.70	0.02	3.40	1.07; 10.8	0.04
Mothers without a history of asthma or allergies (n = 171)						
Asthma ever	0.66	0.26 ; 1.67	0.38	0.79	0.29; 2.16	0.64
AR ever	0.60	0.28; 1.32	0.21	0.71	0.31; 1.65	0.43
Asthma or AR ever	0.60	0.30; 1.21	0.15	0.69	0.33; 1.46	0.33
Sub-population with IgE assessments (n=142)						
Total population						
Positive total IgE	1.95	0.97; 3.89	0.06	3.87	1.57; 9.54	0.003
Positive <i>Der p1</i> IgE	1.93	0.88 ; 4.25	0.10	2.21	0.93; 5.23	0.07
Mothers with a history of asthma or allergies (n = 43)						
Positive total IgE	1.67	0.41; 6.63	0.48	2.85	0.58; 14.12	0.20
Positive <i>Der p1</i> IgE	1.65	0.41; 6.63	0.48	2.15	0.46; 10.10	0.33
Mothers without a history of asthma or allergies (n = 99)						
Positive total IgE	2.46	1.07; 5.65	0.03	4.34	1.54;12.24	0.005
Positive <i>Der p1</i> IgE	1.81	0.67; 4.91	0.25	2.05	0.72; 5.82	0.18

AR: Allergic rhinitis; *cOR: crude odds ratio (unadjusted); ** 95% confidence interval and p-value; † aOR: adjusted odds ratio for city, mother's age, maternal history of asthma or allergies (in non-stratified model-whole population only), any smoking during pregnancy, any passive smoke exposure during the first 3 years of life, season of birth and new-born's sex.

Supplementary Material Figure 1

