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Pollen exposure is associated with risk of respiratory symptoms during the first year of life

Short Title: Association between pollen and respiratory symptoms in infancy

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AUTHOR CONTRIBUTIONS

PL, UF, AG, OF and JU designed the study. JU, FD, OG, YS, SY assisted in collection of the clinical and metadata. AG performed the data analysis and wrote the main manuscript with input from the co-authors. JU, OF, ME, OG, JJ and UF contributed to the statistical interpretation of results. ME, RG, DV and KdH provided the environmental exposure data and helped with interpretation of the data. UF is the principal investigator of the BILD cohort. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

PL reports personal fees from OM Pharma, Polyphor, Santhera, Vertex and Vifor, outside the submitted work. JU reports personal fees from Vertex outside the submitted work. OF reports personal fees from OM Pharma, Menarini, ALK, Vertex, Bencard, Medical Tribune, Milupa-Nutricia, Stallergenes Greer and aha! Allergie Zentrum outside the submitted work. The remaining authors declare no conflicts of interest.

Abstract

Background

Pollen exposure is associated with respiratory symptoms in children and adults. However, the association of pollen exposure with respiratory symptoms during infancy, a particularly vulnerable period, remains unclear. We examined whether pollen exposure is associated with respiratory symptoms in infants and if maternal atopy, infant's sex or air pollution modify this association.

Methods

We investigated 14,874 observations from 401 healthy infants of a prospective birth cohort. The association between pollen exposure and respiratory symptoms, assessed in weekly telephone interviews, was evaluated using generalized additive mixed models (GAMM). Effect modification by maternal atopy, infant's sex and air pollution (NO_2, $PM_{2.5}$) was assessed with interaction terms.

Results

Per infant 37±2 (mean±SD) respiratory symptom scores were assessed during the analysis period (January through September). Pollen exposure was associated with increased respiratory symptoms during the daytime (RR [95% CI] per 10% pollen/m^3: combined 1.006 [1.002, 1.009]; tree 1.005 [1.002, 1.008]; grass 1.009 [1.000, 1.23]) and nighttime (combined 1.003 [0.999, 1.007]; tree 1.003 [0.999, 1.007]; grass 1.014 [1.004, 1.024]). While there was no effect modification by maternal atopy and infant's sex, a complex crossover interaction between combined pollen and $PM_{2.5}$ was found (p-Value 0.002).

Conclusion

Even as early as during the first year of life, pollen exposure was associated with an increased risk of respiratory symptoms, independent of maternal atopy and infant's sex. Because infancy is a particularly vulnerable period for lung development, the identified adverse effect of pollen exposure may be relevant for the evolvement of chronic childhood asthma.

Key words

aeroallergen, cohort study, infancy, interaction, longitudinal study

Abbreviations

BILD: Basel-Bern Infant Lung Development, GAMM: generalized additive mixed model, IgE: Immunoglobulin E, NO_2: nitrogen dioxide, $PM_{2.5}$: particulate matter with a diameter ≤ 2.5 µm, RR: risk ratio, ti: tensor product interaction

1 INTRODUCTION

Exposure to pollen has been associated with an increased risk of respiratory symptoms in both allergic[1] and nonallergic[2,3] individuals. This indicates different underlying mechanisms. On one hand, pollen can cause immunoglobulin E (IgE)-mediated respiratory symptoms in sensitized individuals[1]. On the other hand, pollen can negatively impact antiviral immunity, which consequently increases susceptibility to viral infections and related respiratory symptoms independent of allergic predisposition.[2] The recently found positive correlation between pollen concentrations and SARS-CoV-2 infection rates supports this finding.[4] Thereby, the effect of pollen may be modified by genetic and environmental factors. While prepubertal males are at increased risk of sensitization against aeroallergens[5] and offspring of atopic mothers[6,7] are predisposed to develop allergic diseases in general, male sex is additionally associated with a higher risk for pediatric respiratory morbidity in comparison to females[8]. Thus, assuming allergy and viral infection are underlying mechanisms of respiratory symptoms, male sex and maternal atopy may increase the impact of pollen exposure in children. Similarly, this is expected for air pollution, which has been found to aggravate the effect of pollen through various mechanisms. Air pollutants can increase the allergen content of pollen and lead to an extended allergen release by damaging the pollen surface.[9] Particulate pollutants can bind to allergenic fragments and thereby serve as carriers that transport allergens deep into the respiratory tract.[10] In addition, air pollutants suppress antiviral immunity[11,12] and damage the airway epithelium[13,14], increasing susceptibility to respiratory infections and resulting respiratory symptoms.

However, data on the association of pollen with respiratory symptoms and potential effect modification by maternal atopy, infant's sex and air pollution in infants are missing. This is despite the fact that infancy is a particularly vulnerable time period for the development of the lung and later respiratory disease.[15-18] To prevent short- and long-term respiratory health problems, a better understanding of the association between pollen and respiratory symptoms in infants is needed.[19-21] This is particularly relevant with regard to the increasing prevalence of asthma and allergic rhinoconjunctivitis in children and adults[22], which has been observed in the context of the ongoing urbanization and the prolonged and more intense pollen seasons.[23-25]

We hypothesize 1) that pollen exposure increases the risk of respiratory symptoms even during the first year of life and 2) that this risk is increased for infants of atopic mothers, male sex and those exposed to higher air pollution levels. To test our hypotheses, we used longitudinal data from an ongoing birth cohort of healthy infants and assessed 1) the association of pollen exposure with respiratory symptoms during the first year of life and 2) the effect modification by maternal atopy, infant's sex and air pollution (particulate matter with an aerodynamic diameter ≤ 2.5 µm ($PM_{2.5}$), nitrogen dioxide (NO_2)).

2 METHODS

2.1 Study Population

Data were collected from the Basel-Bern Infant Lung Development (BILD) cohort, an ongoing prospective birth cohort of unselected healthy infants. Exclusion criteria for the cohort study are ethnicity other than Caucasian, major birth defects, perinatal diseases and major respiratory disease diagnosed after birth.[26] Because air pollution data were only available from 1st of January 2005 to 1st of January 2017, solely infants enrolled in the BILD cohort between 1st of January 2005 to 1st of January 2016 were included in the present study. For the present study the following additional exclusion criteria applied: preterm-birth (< 37 weeks), < 50 symptoms scores assessed during the 1st year of life (to ensure a comparable number of observations among infants), moved abroad, missing data (Figure S1). The Ethics Committee of Northwest and Central Switzerland (EKNZ, Basel, Switzerland) as well as the Bernese Cantonal Ethics Research Committee (KEK, Bern, Switzerland) approved the study protocol and written informed consent was obtained from the parents at enrolment.

2.2 Outcome

Day- and nighttime respiratory symptoms (cough, wheezing, dyspnea) were assessed weekly throughout the first year of life. Trained study nurses called the parents and evaluated severity of respiratory symptoms during the week preceding the telephone interview with a standardized 5-category severity score, ranging from 0–4, with 0 indicating no symptoms and scores ≥ 1 indicating symptoms with increasing severity. Further details are described elsewhere.[27,28]

2.3 Exposure

All environmental exposures (pollen concentrations, $PM_{2.5}$, NO_2, temperature, relative humidity) were linked to the infant's residential addresses. Addresses were geocoded using the building registry of the Swiss Federal Statistical Office (Neuchâtel). Address changes within the study period were considered for the exposure assignment.

2.3.1 Pollen

Daily pollen concentrations were monitored by the Swiss Federal Office of Meteorology and Climatology (MeteoSwiss) at 14 monitoring stations in Switzerland. Pollen samples were obtained using a Burkard volumetric spore trap.[24] Pollen traps considered for this study do not operate from October through December[24,29], therefore only pollen data from January through September were considered for the analyses. Because air pollution data was only available from 1st of January 2005 to 1st of January 2017, pollen data were also restricted to this time period. Using data from the monitoring station closest to the participant's residential address (mean distance: 10.5 km), 7-day averages were calculated for hazel, birch, alder and grass pollen concentrations for the week prior to each telephone interview. For the analysis three relevant pollen groups were created aggregating the averaged pollen concentrations (pollen/m^3) for the respective species: combined pollen (hazel, birch, alder, grass), tree pollen (hazel, birch, alder) and grass pollen. For each pollen group the analyzed time period was restricted to months with mean aggregated pollen concentrations > 0.1 pollen/m^3. Months below the cut-off were excluded due to minimal variation in pollen concentrations, similar to other studies.[30,31] Since the calculated pollen concentrations were right skewed, combined, tree and grass pollen concentrations were log-transformed with an offset of 1, as done previously.[32,33]

2.3.2 Air pollution

Daily $PM_{2.5}$ and NO_2 concentrations were obtained from previously developed spatiotemporal models.[34,35] In brief, $PM_{2.5}$ concentrations were estimated at a spatial resolution of 100 × 100m with geostatistical hybrid models taking advantage of the Multiangle Implementation of Atmospheric Correction (MAIAC) spectral aerosol optical depth (AOD) data in combination with other spatiotemporal predictor variables.[35] These models explain over 80% of the local variation in $PM_{2.5}$ concentrations. Data from the Copernicus Atmosphere Monitoring Service, Ozone Monitoring Instrument (OMI), land use and meteorological variables were included to estimate daily NO_2 concentrations. To downscale satellite data and incorporate local sources, a multistage framework with mixed-effect and random forest models was applied. With these models, over 70% of the NO_2 variation can be explained.[34] Consistent with the pollen exposure, 7-day averages of $PM_{2.5}$ and NO_2 were calculated for the week prior to each telephone interview.

2.3.3 Meteorological variables

Daily average temperature at a 100 × 100m resolution was obtained from a spatiotemporal model described in detail elsewhere.[36] In summary, variations in ground-based air temperature data from measurement stations operated by MeteoSwiss were explained in a random forest framework using a range of predictor variables. This included satellite-derived Moderate Resolution Imaging Spectroradiometer (MODIS) land surface temperature, satellite-derived normalised difference vegetation index (NDVI), elevation, land use and meteorological data. A 7-day average of temperature was then calculated for the week preceding the telephone interview. Weekly average relative humidity was assigned from the meteorological station closest to the infant's residential address.

2.5 Risk factors

The following data on known perinatal, hereditary and environmental risk factors for respiratory morbidity were derived from hospital records, questionnaires and weekly telephone interviews: sex[27], siblings[37], childcare[37], maternal education[38], maternal atopy[27,39], maternal smoking during pregnancy[37], cats[40], dogs[41], breastfeeding (any breastfeeding/no breastfeeding)[37], delivery mode[42], age[37], birth weight[43], season of birth[44].

2.6 Statistical analysis

The association of pollen exposure with day- and nighttime respiratory symptom scores was assessed using generalized additive mixed models (GAMM) with quasi-Poisson distribution. GAMMs provide a general framework for extending linear mixed models. They allow the assessment of non-linear relationships between exposure and outcome by using smooth functions.[45] Random effect and autoregressive correlation structure (lag 1) were employed to account for correlation of respiratory symptoms within each subject. Estimates are expressed as risk ratios (RR) per 10% increase in pollen/m^3 ($RR = 1.10^{\beta}$). An RR of 1.006 can be interpreted as a 0.6% increase in respiratory symptom score for an increase in pollen concentration by 10%. Separate models were built for combined, tree and grass pollen. All models were adjusted for a priori selected risk factors identified in previous studies of this cohort[19,27,37]: $PM_{2.5}$, NO_2, siblings, childcare, sex, breastfeeding, age, maternal smoking during pregnancy, temperature and month (to control for seasonal variation of respiratory symptoms) (Figure 1). In addition, models (except combined pollen models) were mutually adjusted for tree pollen and grass pollen. Pollen groups and covariates were included as smoothing terms in case of a non-linear relationship with respiratory symptom scores. If a non-linear relationship has been identified, the sample was restricted to the range of pollen concentrations linearly associated with the outcome in order to obtain interpretable coefficient estimates.

To evaluate effect modification by maternal atopy or infant's sex, separate models including factor-smooth interaction terms were fitted. In order to assess effect modification by NO_2 and $PM_{2.5}$, separate models were fitted including tensor product interaction terms (ti).

In addition, three sensitivity analyses were performed. First, the association between pollen and respiratory symptoms was investigated considering additional risk factors: cat, dog, delivery mode, birth weight, maternal education, maternal atopy, season of birth, study center (to control for inter-center differences) and relative humidity (Figure 1). Second, the sample was restricted to observations collected after six months of age, to evaluate the effect of pollen on respiratory symptoms in the absence of maternal IgE in the infant's blood.[46,47] Third, we assessed the association of combined, tree and grass pollen with respiratory symptoms without restricting the analyzed time period to months with > 0.1 pollen/m^3. This was to evaluate whether the inclusion of several days with zero pollen impacts the results.

Data pre-processing and descriptive statistics were performed with STATA (version 16.1, STATA Corp, Texas). GAMM models were executed in R (version 4.0.3).[48]

3 RESULTS

3.1 Study population

We included in total 401 term-born infants with complete data for 14,874 measurement time points to analyze the association between pollen and respiratory symptoms during the first year of life (Figure S1). Details on the study population are outlined in Table 1.

Respiratory symptom scores were overall low (Table 1 and Table S2). The mean±SD symptom score from January through September across all years was 0.13±0.13 for daytime respiratory symptoms and 0.12±0.14 for nighttime respiratory symptoms. Within these months, infants on average±SD had daytime respiratory symptoms for 3.92±3.77 weeks and nighttime respiratory symptoms during 3.03±3.01 weeks. Temporal development and seasonal fluctuations in daytime respiratory symptom scores and pollen concentrations are shown in Figure 2. Analysis periods (months with mean pollen concentrations > 0.1 pollen/m^3) lasted from January through September for combined pollen, January through July for tree pollen and April through September for grass pollen. Descriptive statistics for weekly pollen concentrations and other environmental exposures are given in Table 2 and Table S1.

3.2 Association of pollen and respiratory symptoms

We found that exposure to combined pollen, as well as tree and grass pollen separately, was associated with respiratory symptoms during the first year of life. Results for each pollen group are summarized in Figure 3 and Table 3.

An increase in combined pollen exposure was linearly associated with increased risk of daytime respiratory symptom scores (RR [95% CI] per 10% pollen/m^3: 1.006 [1.002, 1.009]). Similarly, an increase in exposure to tree pollen was linearly associated with increased risk of daytime respiratory symptoms (RR [95% CI] per 10% pollen/m^3: 1.005 [1.002, 1.008]). We identified a non-linear relationship between grass pollen and daytime respiratory symptoms (Figure 3). For grass pollen concentrations < 10 pollen/m^3, we observed no association with respiratory symptoms, whereas the risk of respiratory symptoms increased linearly for pollen concentration $>$ 10 pollen/m^3. After restricting the sample to grass pollen concentrations > 10 pollen/m^3 (5047 observations), an increase in pollen was associated with increased risk of daytime respiratory symptoms (RR [95% CI] per 10% pollen/m^3: 1.009 [1.000, 1.018]) and increased risk of nighttime respiratory symptoms (RR [95% CI] per 10% pollen/m^3: 1.014 [1.004, 1.024]). No associations could be found for combined pollen and tree pollen with nighttime respiratory symptoms (Table 3).

In addition, we performed several interaction analyses to assess the effect modification by maternal atopy, infant's sex and air pollution ($PM_{2.5}$, NO_2). We found no interaction between combined pollen and maternal atopy (Figure S2, upper panel). Furthermore, we found no interaction between combined pollen and infant's sex (Figure S2, lower panel). However, we found a complex crossover interaction between combined pollen and $PM_{2.5}$ (p-Value 0.002) (Figure S3). Pollen had the opposite effect on respiratory symptoms dependent on the level of $PM_{2.5}$, although there was no main effect of $PM_{2.5}$ (Table S3). We found no interactions between $PM_{2.5}$ and tree pollen or grass pollen. Furthermore, we found no interactions between combined pollen, grass pollen or tree pollen and NO_2 (data not shown), although NO_2 itself was positively associated with daytime respiratory symptoms (RR [95% CI] per μg/m^3: 1.011 [1.002, 1.020] (Table S3).

3.3 Sensitivity Analyses

The three sensitivity analyses confirmed the robustness of the observed association between pollen exposure and respiratory symptoms.

The models extended by additional covariates (Table S4), as well as those excluding observations collected before six months of age (Table S5) and those without restriction to months with mean pollen concentrations > 0.1 pollen/m^3 (Table S6) yielded comparable results to the main models.

4 DISCUSSION

This is the first longitudinal study to show novel surprising evidence that pollen exposure is associated with risk of respiratory symptoms in healthy infants. Interestingly, the effect of pollen was not enhanced by infant's sex and maternal atopy. Our results extend the findings of previous studies that show a positive association between pollen exposure and respiratory symptoms in older children and adults[1-3] to infancy, which is an important window of opportunity.

An increase in exposure to combined pollen as well as to tree pollen and grass pollen seperately, was associated with an increase in respiratory symptoms. This clearly agrees with our first hypothesis that pollen exposure—even as early as during the first year of life—is related to an increased risk of respiratory symptoms. The large number of observations enabled us to detect an association even when effect sizes were relatively small. The effect sizes identified herein are comparable to those previously reported for the assocation between pollen and lower respiratory tract symptoms in children and adults.[1] Because we have examined the association between pollen and lower respiratory tract symptoms (wheeze, cough, dyspnea), the effects might be stronger for upper respiratory tract symptoms (e.g. rhinitis, sneezing, sore throat) typically evoked by pollen exposure in predisposed individuals.[1]

Until now, it was assumed that pollen-related allergic respiratory symptoms rarely occur in infants because sensitization to pollen usually develops after the first year of life.[49-51] It has been found that allergen-specific IgE in cord blood[46] and in neonates[47] is most likely a result of a transfer of maternal IgE to the fetus and does not indicate a sensitization of the fetus or the infant itself.[46] To reduce the possibility of contamination by maternal IgE, we excluded observations from the first six months of life. Thereby, we again found a significant association between pollen and respiratory symptoms. This finding increases the likelihood that respiratory symptoms result from a pollen sensitization of the infant itself. Yet, contrary to what would be expected for sensitized infants, we found no effect modification by maternal atopy. To further clarify if respiratory symptoms are related to a pollen sensitization and if maternal allergy status modifies an allergic reaction of the child, additional data on maternal and offspring IgE levels would be needed.

In our case, the observed respiratory symptoms may also reflect a nonallergic response to a viral infection. This is supported by the study of Gilles et al., which suggests that pollen suppresses antiviral immunity by downregulation of type I and III interferons in nasal mucosa, increasing the susceptibility to respiratory viral infections independent of sensitization.[2] Although our results suggest an overall effect of pollen, mediation analyses—including data for infant's sensitization status and viral infection—would be needed to evaluate the mechanisms underlying the association between pollen and respiratory symptoms. This may further help to elucidate the modifying effect of infant's sex. Previous studies have shown an increased risk of allergic respiratory symptoms[5,52] and higher susceptibility for respiratory infections[8] in boys compared to girls. Therefore, we hypothesized that the effect of pollen on respiratory symptoms is enhanced in male infants. However, our results suggest that the demonstrated effect of pollen is not only independent of maternal atopy but also independent of the infant's sex. Moreover, air pollution did not clearly impact the effect of pollen, which disagrees with our second hypothesis. However, it supports the results from previous studies which also found no significant interaction between pollen and air pollution in their association with respiratory symptoms or asthma hospital admissions.[53]

4.1 Strengths and limitations

To the best of our knowledge, this is the first longitudinal study investigating the association of pollen and respiratory symptoms in healthy infants. One major strength of our study is the amount of observations (14,874) available to investigate the association between pollen and respiratory symptom scores. This large number of observations increases the possibility of capturing some variation between NO_2 and $PM_{2.5}$, which are obviously correlated as they are emitted by similar sources. However, the fact that background levels of NO_2 and $PM_{2.5}$ can vary regionally as well as temporally and that the pollutants have different health effects, limits their correlation.[9,10] Using a multipollutant model adjusted for several co-exposures and including interactions, we follow the emerging exposome concept.[54] This approach is appropriate for the study of health outcomes that can be caused by a complex interaction of environmental, lifestyle and genetic factors.[54,55] Our capability to control for major confounders increases the likelihood of detecting a marginal effect of pollen on respiratory symptoms. Among others, this includes the adjustment for temperature, humidity and month of assessment, which are associated with both respiratory health and pollen concentrations. Cold temperatures and low humidity are associated with increased respiratory symptoms[56,57] and viral infections[58], while warmer temperatures and low humidity are associated with increased pollen concentrations[59,60]. Because these meterological variables correlate with seasonal viral infections, they serve us as surrogate control variables for the latter. By adjusting the models for the month of assessment, temperature and humidity we reduced the risk of confounding by pollen-independent viral infections. Furthermore, the association we found between grass pollen and respiratory symptoms, using data from April through September, suggests that respiratory symptoms are not only driven by viruses and meteorological conditions typical for winter and early spring. This supports the novel evidence that pollen exposure is another risk factor for respiratory symptoms in healthy infants. Other strengths of our study include the prospective design which reduces the risk of a recall bias, and the high-quality exposure data (pollen, air pollution, temperature and relative humidity) estimated for each interview time point and individual, increasing the accuracy of the assocation studied.

Limitations of this study include the use of 7-day average respiratory symptom scores and the subsequent 7-day averages for pollen exposure. This could result in blurring of the results, as pollen concentration varies from day to day and the effect of pollen on respiratory symptoms is generally expected within 1 day after exposure.[2] Additional blurring could emerge through the measurement error expected for Burkard traps.[61] Being aware of potential imprecision of our effect estimates, we have refrained from defining a threshold of minimum pollen concentration to cause respiratory symptoms. Other than that, due to its invasive assessment and limited resources, no data on sensitization to pollen or viral infections were obtained for this study. Such data would allow investigation of the underlying mechanisms of the association between pollen and respiratory symptoms. As with regard to our interaction analysis between air pollution and pollen, the relatively low variation of air pollution and respiratory symptoms within and between subjects should be considered. Beside the overall low effect sizes, this complicates the detection of an interaction effect.[62] Our results and the controversial evidence from previous studies both underpin the recognition that the assessment of an air pollution-pollen interaction in an epidemiological study is methodologically challenging.[53] In addition, spatiotemporal data were available for $PM_{2.5}$ and NO_2 only. Further studies are needed to evaluate the interaction between other relevant air pollutants (e.g. ozone, sulphur dioxide, carbon monoxide) and pollen in their association with respiratory symptoms in infants.

5 CONCLUSION

In this study, we show first evidence that—even as early as during the first year of life—increased exposure to combined pollen and to tree and grass pollen separately, is associated with increased respiratory symptoms. Importantly, we found that this is even the case in healthy term-born infants. The association was independent of the atopic status of the mother and infant's sex. Because infancy is a particularly vulnerable period for lung development, the identified adverse effect of pollen exposure may be relevant to the evolvement of chronic childhood asthma. Additional larger studies including data on infant's sensitization status and presence of viral infections may help to further elucidate the underlying mechanisms of the association between pollen and respiratory symptoms.

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Table 1 Characteristics of the study population

Sample size, n	401
Anthropometrics	
Boys, n (%)	201 (50)
Gestational age at birth in weeks, mean±SD	39.67±1.12
Birth weight in kg, mean±SD	3.37±0.45
Risk factors	
Any breastfeeding in 1st yr of life, n (%)	397 (99)
Weeks with any breastfeeding in 1st yr of life, mean±SD	33.52±14.52
Presence of siblings, n (%)	215 (54)
Childcare in 1st yr of life, n (%)	133 (33)
Maternal smoking in pregnancy, n (%)	16 (4)
Education mother †, n (%)	
Low	62 (15)
Middle	123 (31)
High	216 (54)
Cesarean section, n (%)	89 (22)
Maternal atopy, n (%)	128 (32)
Cats at home, n (%)	61 (15)
Dogs at home, n (%)	20 (5)
Season of birth ‡	
Winter (December–February), n (%)	89 (22)
Spring (March–May), n (%)	103 (26)
Summer (July–August), n (%)	108 (27)
Autumn (September–November), n (%)	101 (25)
Respiratory symptoms §	
Daytime symptom score, mean±SD	0.13±0.13
Nighttime symptom score, mean±SD	0.12±0.14
Weeks with daytime symptoms, mean±SD □	3.92±3.77
Weeks with nighttime symptoms, mean±SD □	3.03±3.01

† Low: < 4 years apprenticeship, Middle: ≥ 4 years apprenticeship, High: tertiary education

‡ Meteorological season

§ Assessed from January through September

□ Weeks with respiratory symptom score ≥ 1

Table 2 Distribution of weekly pollen concentrations, air pollution and meteorological conditions for the respective analysis period

	Combined pollen†			Tree pollen‡			Grass pollen§		
	Mean±SD	Median	IQR	Mean±SD	Median	IQR	Mean±SD	Median	IQR
Pollen (pollen/m^3) □	77.4±137.7	24.3	73.0	71.2±195.1	5.6	50.7	32.7±50.8	10.0	38.6
Air pollution (µg/m^3)									
$PM_{2.5}$	16.0±8.0	14.2	8.6	16.8±8.6	14.8	9.6	13.7±5.0	12.9	6.3
NO_2	19.0±11.4	16.9	16.0	20.2±11.9	18.4	16.9	14.6±8.5	13.0	11.9
Relative humidity (%)	72.1±8.7	72.3	11.7	71.6±9.1	71.9	12.8	70.0±8.3	70.4	11.4
Temperature (°C)	11.7±7.3	13.4	12.1	10.1±7.5	10.7	12.4	15.8±4.2	16.1	5.7

† Months with mean combined pollen concentration (pollen/m^3) > 0.1: January through September

‡ Months with mean tree pollen concentration (pollen/m^3) > 0.1: January through July

§ Months with mean grass pollen concentration (pollen/m^3) > 0.1: April through September

Table 3 Association of pollen exposure with respiratory symptom scores during infancy

	Daytime symptom score			Nighttime symptom score		
	RR	95% CI	p-Value	RR	95% CI	p-Value
Combined pollen, (n=14874) †	1.006	1.002, 1.009	0.002	1.003	0.999, 1.007	0.170
Tree pollen, (n=11483) †	1.005	1.002, 1.008	0.003	1.003	0.999, 1.007	0.088
Grass pollen, (n=5047) ‡	1.009	1.000, 1.018	0.049	1.014	1.004, 1.024	0.005

GAMM adjusted for NO_2, $PM_{2.5}$, siblings, childcare, sex, breastfeeding, age, maternal smoking during pregnancy, temperature, month

† risk ratio per 10% increase in pollen/m^3: $(1.10)^\beta$, e.g. an estimate of 1.006 can be interpreted as a 0.6% increase in respiratory symptom score for an increase in pollen concentration by 10%.

‡ risk ratio per 10% increase in pollen/m^3: $(1.10)^\beta$, sample restricted to pollen concentrations (pollen/m^3) > 10

Figure 1 Directed acyclic graph (DAG) of the association between pollen exposure and respiratory symptoms during the first year of life. Exposure of interest and outcome are connected with a bold arrow. Dashed arrows show unobserved associations and solid arrows show observed associations. Orange arrows represent potential effect modifications. Filled boxes are covariates included in the main models. Clear boxes with a solid outline are covariates additionally included for the sensitivity analysis. Boxes with a dashed outline show unobserved variables.

Figure 2 Temporal development and seasonal fluctuations of mean daytime respiratory symptom scores and mean pollen concentrations across study participants in Bern (January 2005 to January 2017) and Basel (March 2012 to January 2017). Areas shaded in light blue represent months excluded from the analysis due to missing data (no pollen data from October through December; start of cohort study in Basel in 2012).

Figure 3 Marginal effects of pollen exposure on the risk of daytime respiratory symptoms (pollen exposure shown on ln scale and backtransformed scale: exp(ln)-1). Shaded areas represent the 95% confidence interval. Models were adjusted for NO_2, $PM_{2.5}$, siblings, childcare, sex, breastfeeding, age, maternal smoking during pregnancy, temperature and month. a) Association between combined pollen and daytime respiratory symptoms. b) Association between tree pollen and daytime respiratory symptoms. c) Association between grass pollen and daytime respiratory symptoms. The red line indicates the cut-off used to assess linear relationship between grass pollen and respiratory symptoms.

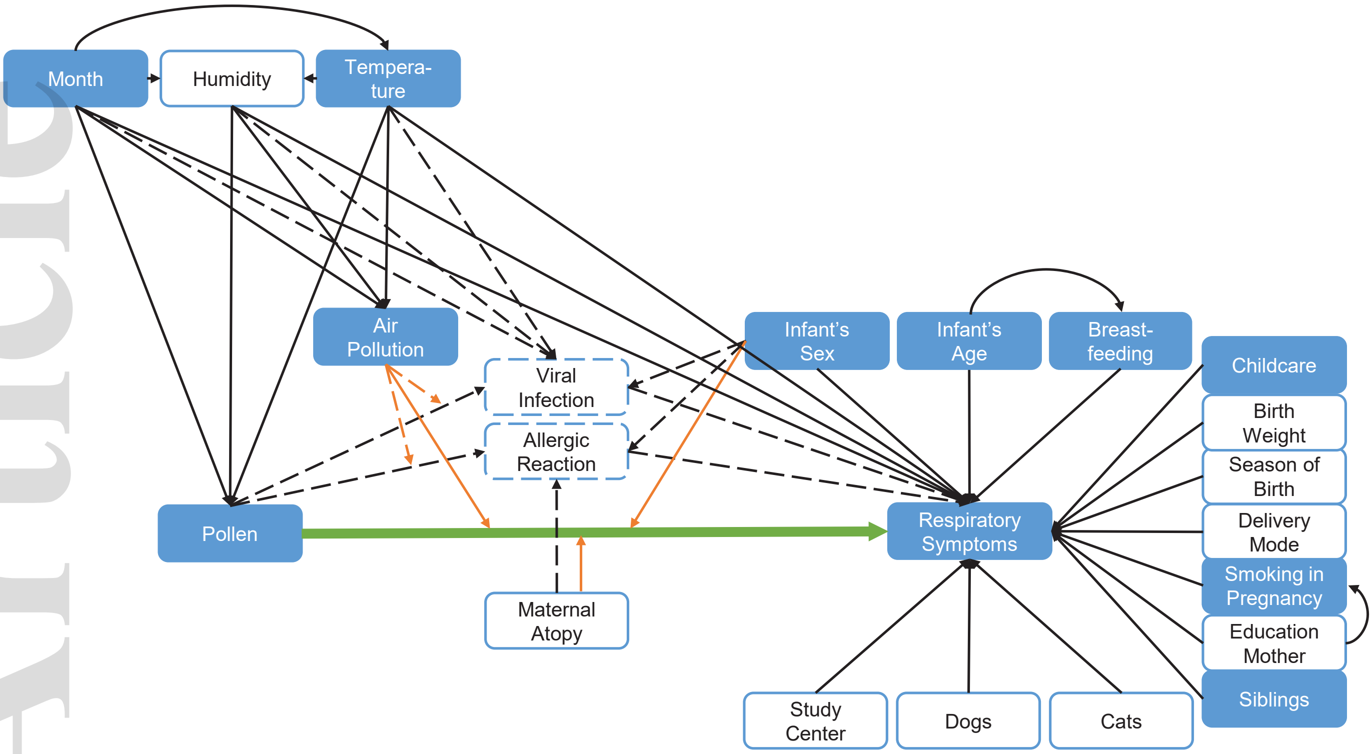

Figure 1_Gisler et al

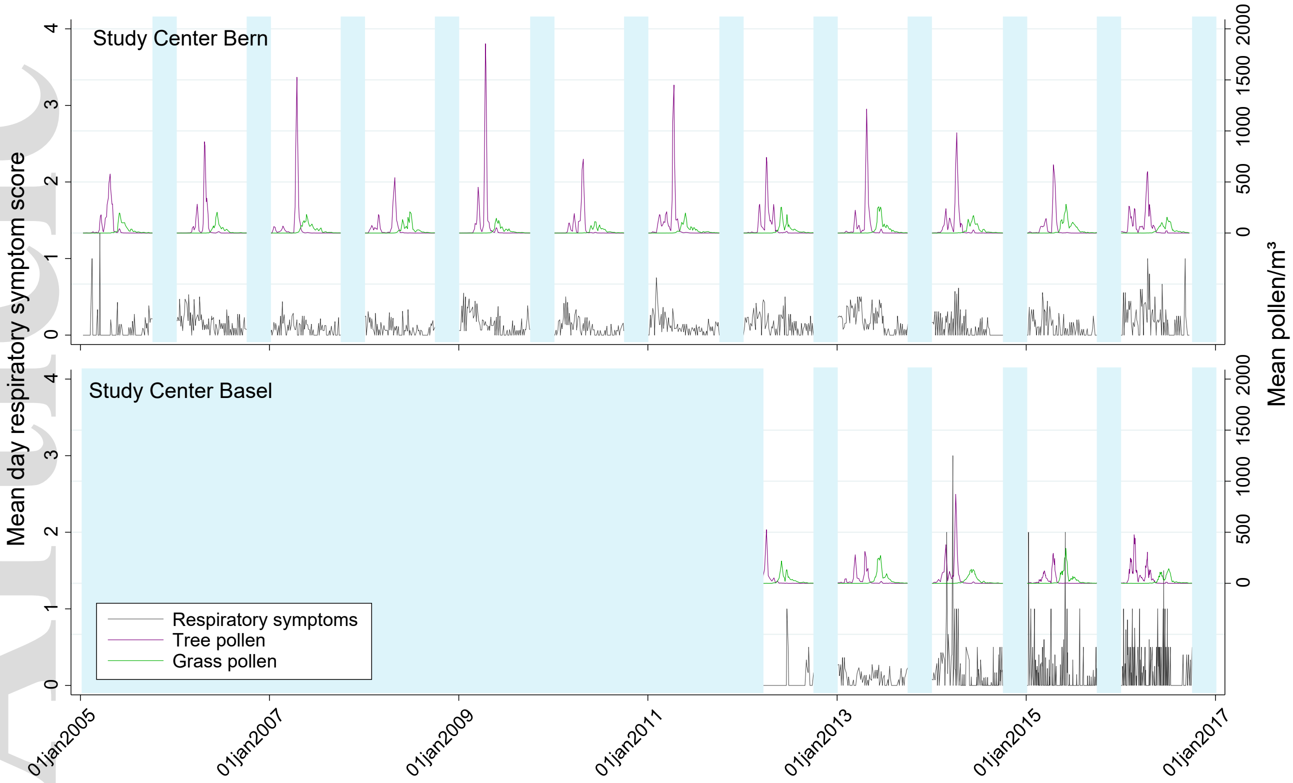

Figure 2_Gisler et al

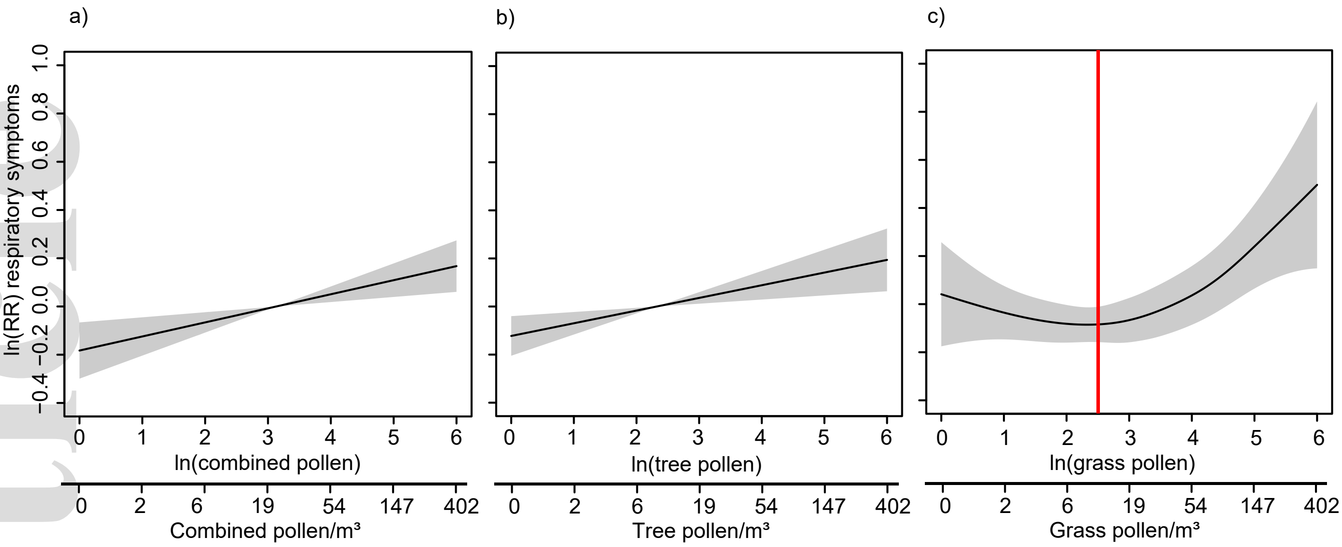

Figure 3_Gisler et al