

Original article

Alternaria sensitization and allergic rhinitis with or without asthma in the French Six Cities study

Background: Allergic sensitization to *Alternaria* has been related to asthma in various studies, but its association with allergic rhinitis is still controversial.

Objectives: The aim of this study was to assess at the population level the relationships in childhood between *Alternaria* sensitization and 'past-year rhinoconjunctivitis' (PYRC), 'ever hay fever' (EHF) and 'ever allergic rhinitis caused by allergens other than pollens' (EAR) according to the presence or the absence of asthma.

Methods: This study is part of the Six Cities Study, the French contribution to the International Study of Asthma and Allergies in Childhood (ISAAC) Phase II. Children underwent skin prick test (SPT) to *Alternaria* and parents filled a standardized medical questionnaire.

Results: Some 6726 children with a mean age of 10 years were examined. The overall prevalence of *Alternaria* sensitization was 2.8%, 0.8% for monosensitization. Prevalences of symptoms in sensitized children were 27.7% for PYRC, 27.0% for EHF and 30.4% for EAR. Adjusted Odds Ratios (OR) between *Alternaria* sensitization and allergic rhinitis phenotypes were 2.34 (95% confidence interval: 1.51–3.63) for PYRC, 2.40 (1.65–3.50) for EHF and 2.95 (2.05–4.23) for EAR. The relationship still remained in the case of monosensitization to *Alternaria* for both PYRC and EAR when excluding the asthmatic children [OR = 3.87 (1.54–9.78) and 2.88 (1.10–7.55) respectively].

Conclusion: In our population-based sample of children, we found a link between *Alternaria* sensitization and allergic rhinitis, independently of asthma, which is compatible with the mechanisms of deposition of *Alternaria* in the upper airways.

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Key words: *alternaria*; asthma; monosensitization; rhinitis; sensitization.

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Allergic rhinitis is a worldwide condition with lifetime prevalence estimates ranging from 10 to 30% (1). Allergic rhinitis is the most common form of noninfectious rhinitis (2, 3) and its prevalence is still marginally increasing in most countries in the world (4), including France (5). Allergic rhinitis occurs as a classical allergic reaction where various lengths of exposure to an allergen lead to symptoms in IgE-sensitized individuals. Perennial allergic rhinitis is caused by an allergic response to indoor allergens (mites, animals, cockroaches) present throughout the year. Seasonal symptoms have also been observed

after exposure to outdoor allergens, amongst which are pollens with diameter varying from 10 to 100 µm allowing deposition in nostrils and eyes.

In contrast, the airborne spores, *hyphae* and fragments of fungi are small enough (< 10 µm) to be inhaled into the lower airways (6). Exposure or sensitization to these fungal propagules (especially to *Alternaria*) has been recognized to be a risk factor for asthma (7), including severe asthma (8) and sometimes respiratory arrest in asthma (9). *Alternaria* mould is present both indoors and outdoors (10). Eventhough exposure or sensitization to moulds has been found to be associated with allergic rhinitis in clinical observations (11), evidence about this relationship is insufficient to permit definitive conclusions at the population level because of lack of concordant data (12). In the second National Health and Nutrition Examination Survey, the prevalence of allergic rhinitis was significantly associated with skin reactivity to *Alternaria* (Odds ratio = 2.3; 95% CI: 1.5–3.4 after control-

Abbreviations: EAR, ever allergic rhinitis caused by allergens other than pollens; BMI, body mass index; CA, current asthma; CI, confidence interval; CNIL, National Commission of Informatics and Civil Liberties; EHF, ever hay fever; IgE, Immunoglobulin E; ISAAC, International Study of Asthma and Allergies in Childhood; EA, ever asthma; NS, nonsignificant; OR, Odds Ratio; PYRC, past-year rhinoconjunctivitis; SPT, skin prick test.

ling for confounders. However, the association disappeared when considering the isolated phenotype of allergic rhinitis in the absence of asthma (13).

The aim of this study was to determine the relationships between *Alternaria* sensitization and various phenotypes of allergic rhinitis according to the presence or the absence of asthma in order to disentangle the specific contribution of *Alternaria* to allergic rhinitis. To this extent, data drawn from a large population-based sample of children living in France in whom phenotypes of allergic rhinitis, asthma as well as skin prick test positivity to *Alternaria alternata* had been assessed objectively were used.

Methods

Population

Out of 9615 children of fourth and fifth grade (9–11 years old) in elementary schools selected in six cities of metropolitan France (Bordeaux, Clermont Ferrand, Créteil, Marseille, Reims and Strasbourg) in the frame of the French Six Cities Study between 1999 and 2000, 7781 participated into the survey that included an epidemiological questionnaire addressed to the parents on respiratory and allergic health, potential risk factors and management, a medical examination including skin prick test positivity to common allergens and skin examination for eczema. Details of the survey have been described elsewhere (14–17). Each centre randomly selected a sufficient number of schools for obtaining a sample of at least 1500 schoolchildren in the appropriate age class according to the International Study of Asthma and Allergies in Childhood (ISAAC) phase II protocol (18). Authorizations by the 'National Commission of Informatics and Civil Liberties (CNIL)' and by the Ethic Committee were sought and obtained before conducting the survey. Children and parents were informed by a letter given to each child at school of the purposes and modalities of the survey, and their written consent was requested.

Questionnaire and health outcomes

Parents filled out a self-administered standardized questionnaire at home. It consisted of an enriched version of the ISAAC questionnaire. Responses were used to assess the prevalence, severity and management of asthma and allied allergic diseases, namely wheezing, allergic rhinitis, and eczema respectively. Such questions had been previously validated and had been translated from English into French by a native French speaker, then back translated into English by a native English speaker. This process had been pursued until the initial English wording had been obtained. Final French wording was provided by a consensus among the Allergy and Respiratory Medicine department heads of the participating centres (19).

Parents' responses to the questionnaire were used in this study to define the following phenotypes of allergic rhinitis and asthma:

- Past-year rhinoconjunctivitis (PYRC): a history of rhinoconjunctivitis in the past year according to a positive answer to the questions: 'In the past 12 months, has your child had a problem with sneezing or a runny or a blocked nose, when he or she did not have a cold or the flu?' and 'In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?'
- Ever hay fever (EHF): a history of hay fever in the past life ('Has your child ever had hay fever?').

- Ever other Allergic Rhinitis (EAR) i.e. caused by allergens other than pollens: a history of any other form of allergic rhinitis ('Has your child ever had another form of allergic rhinitis other than hay fever, i.e. attributable to allergens other than pollens such as cat, house-dust mite, etc.').
- Past-year asthma (PYA) on the basis of an affirmative response to the questions on: 'Have your child had chest wheezing or whistling over the previous 12 months' with a positive history of asthma at some point in life and an affirmative response to the question 'Was your child's asthma confirmed by a doctor?'
- Ever asthma (EA) on the basis of an affirmative response to the question 'Has your child ever had asthma?'

We also considered gender, parental allergy ['had suffered in their life of at least one allergic disease (asthma, allergic rhinitis, eczema)'], parental socio-economic status, overweight [Body Mass Index = weight/(height*height) (kg/m²) lying between 25 kg/m² and 30 kg/m² and obesity when BMI > 30 kg/m² (20)], environmental tobacco exposure ('Do your mother, your father or someone living in the same house smoke?') and humidity ('Is there some moisture visible in your home, or some water damages?') as potential confounders of the relationship between sensitization to *Alternaria* and the health outcomes..

Clinical tests and allergic sensitization

Consent of/on behalf of each of the children was obtained before clinical examination in the classrooms. Children underwent physical examination in the class managed by a physician, which included assessments of weight and height, skin prick tests to common allergens that are the most frequent allergens involved in allergic respiratory symptoms (18). According to the William's protocol, atopic dermatitis was determined by a photo-questionnaire and a physical revision; fieldworkers were trained for clinical tests. Skin prick tests (SPT) were performed using Stallerpoints and Stallergens allergens (Stallergen diagnostics, Antony, France). The allergens used for skin testing were *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* (house-dust mite), cat fur, dog fur, the outdoor mould genus *Alternaria alternata*, *Blatta germanica*, peanut, egg, fish (cod), mixed grass and tree pollens. Histamine (1 mg/ml histamine dihydrochloride) was used as a positive control and an uncoated lancet as the negative control. Tests were performed on the volar surface of the forearm using a standard template and the wheal size was recorded at 15 min as the largest diameter and the diameter at 90° to its midpoint, each to the nearest whole millimetre. Mean wheal diameter was calculated as the average of the two diameters. Skin prick test positivity to a specific allergen was regarded as positive if the corresponding mean wheal diameter was greater than of the negative control.

Children were considered as sensitized to *Alternaria* if they had a positive SPT to *Alternaria*: polysensitized if there were at least one other positive SPT in addition to *Alternaria* and monosensitized if there were no other positive SPTs than *Alternaria*. Finally, if children had one or more positive SPT other than *Alternaria*, they were included in the group of 'other sensitization'.

Statistical analysis

Prevalence rates and 95% confidence intervals were calculated and associations of potential determinants with health outcomes were investigated using bivariate and multivariate techniques (21). Conventional methods (21) were used to compare percentages (chi-squared test). Then, in order to obtain the independent effect of the various risk factors of the considered health outcomes, logistic

regression models were applied including all factors that had showed to be related to the health outcomes at univariate analysis ($P < 0.25$). Children without allergic rhinitis and asthma were considered as controls respectively in the comparisons. Odds-ratios (OR) were calculated to quantify the relationships as well as their 95% confidence interval using the logit method according to Woolf.

For the purpose of the analysis, the phenotypes of allergic rhinitis and asthma were analysed and also isolated, i.e. the phenotypes of allergic rhinitis in the absence of asthma (i.e. in nonasthmatics) and the phenotypes of asthma in the absence of allergic rhinitis (i.e. in nonrhinitic individuals). To this extent the following isolated phenotypes were introduced:

- Past-year rhinoconjunctivitis without asthma: having suffered from rhinoconjunctivitis in the past year but not from asthma.
- Hay fever without asthma: having ever suffered from hay fever but not from asthma.
- Other allergic rhinitis without asthma, having ever suffered from other forms of allergic rhinitis than hay fever but not from asthma.
- Past-year asthma without Past-year rhinoconjunctivitis: having reported asthma in the past year without Past-year rhinoconjunctivitis.
- Ever asthma without ever hay fever: having reported ever asthma without ever hay fever.
- Ever asthma without ever other forms of allergic rhinitis.

The analyses were conducted for both sensitization to *Alternaria* and monosensitization to *Alternaria*. The analyses were performed using Epi info 2000 (<http://www.cdc.gov/epiinfo>).

Results

General characteristics of the population-based sample (Table 1)

The final sample included 7781 children, 7223 had a medical examination and 6726 of them had complete records (questionnaire, clinical examination, skin prick tests) (see Fig. 1). Questionnaires response rate was 81%, varying from 72% in Reims to 92% in Clermont-Ferrand. Children's mean age was 10.4 ± 0.6 years.

Association between *Alternaria* sensitization and different phenotypes of rhinitis and asthma (isolated or not)

The overall prevalence of *Alternaria* sensitization was 2.8%, varying from 1.1% in Strasbourg to 4.1% in Bordeaux, 1.8% in Créteil, 3.6% in Reims, 3.0% in Marseille and 3.6% in Clermont Ferrand. Amongst those with *Alternaria* sensitization, 28.6% were monosensitized (0.8% of the total population). 28.1% of the children were atopic, 32.5% amongst boys and 23.80% amongst girls ($P < 0.001$).

Within the whole population (Table 2), 11.5% of the children reported past-year rhinoconjunctivitis (PYRC), 12.7% ever hay fever (EHF), 12.6% ever allergic rhinitis (EAR) caused by allergens other than pollens, 12.1% past-year asthma (PYA) and 9.4% ever asthma (EA). In *Alternaria*-sensitized children, the prevalence of the phenotypes of allergic rhinitis was 27.7% for PYRC, 27.0% for EHF, 30.4% for EAR, and the prevalence of asthma

Table 1. General characteristic of the study population ($n = 6726$) in the French Six Cities Study

Factors	Prevalence (%)	95% CI
Gender		
Male	49.7	(48.5–50.9)
Body corpulence		
Overweight	1.8	(1.7–2.3)
Obese	0.3	(0.1–0.4)
Skin prick positivity to at least one allergen		
All	28.1	(27.1–29.2)
Boys	32.5	(30.9–34.1)
Girls	23.8	(22.4–25.3)
Parental education		
Primary	8.3	(7.6–9.0)
Secondary	41.8	(40.5–43.1)
University	47.8	(46.5–49.1)
Other	2.0	(1.7–2.5)
Parental allergy		
Father	18.0	(17.1–19.0)
Mother	25.3	(24.2–26.4)
Both	7.0	(6.4–7.6)
Exposure to passive smoking		
All	44.5	(43.3–45.8)
Mother	27.7	(26.6–28.8)
Father	29.6	(28.5–30.8)
Both parents	14.6	(13.7–15.5)
Other	4.9	(4.4–5.4)
Exposure to humidity and dampness		
Yes	20.5	(19.5–21.6)

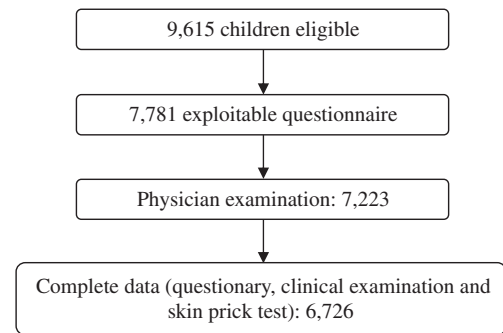


Figure 1. Result of each sampling step in the French Six Cities Study.

was 22.8% for PYA and 20.0% for EA. In nonsensitized children, prevalence of rhinitis was 11.1% for PYRC, 12.3% for EHF, 12.1% for EAR, whereas the prevalence of asthma was 11.8% for PYA and 9.1% for EA.

The prevalence of the phenotypes of allergic rhinitis and asthma was more important within *Alternaria*-sensitized children than within the rest of the population. The crude ORs were 3.08 (95% Confidence Interval: 2.07–4.56) for PYRC, 2.65 (1.82–3.84) for EHF, 3.18 (2.22–4.55) for EAR, 2.20 (1.50–3.22) for PYA, and 2.50 (1.65–3.75) for EA. These trends were still observed (Fig. 2) after adjusting the ORs for potential confounders

Table 2. Relationships between *Alternaria* sensitization and phenotypes of allergic rhinitis and asthma in the French Six Cities Study

	Percentage prevalence (95% Confidence Interval) of the disease			Unadjusted Odds Ratio (95% CI)
	All (<i>n</i> = 6726)	Children nonsensitized to <i>Alternaria</i> (<i>n</i> = 6537)	Children sensitized to <i>Alternaria</i> (<i>n</i> = 189)	
Past-year rhinoconjunctivitis	11.5 (10.7–12.4)	11.1 (10.2–11.9)	27.7 (20.5–35.8)	3.08 (2.07–4.56)
Ever hay fever	12.7 (11.9–13.6)	12.3 (11.4–13.2)	27.0 (20.3–34.7)	2.65 (1.82–3.84)
Ever other allergic rhinitis	12.6 (11.8–13.5)	12.1 (11.3–13.0)	30.4 (23.4–38.2)	3.18 (2.22–4.55)
Current asthma	12.1 (11.3–13.0)	11.8 (11.0–12.7)	22.8 (16.7–29.8)	2.20 (1.50–3.22)
Ever asthma	9.4 (8.7–10.2)	9.1 (8.4–9.9)	20.0 (14.2–26.9)	2.50 (1.65–3.75)

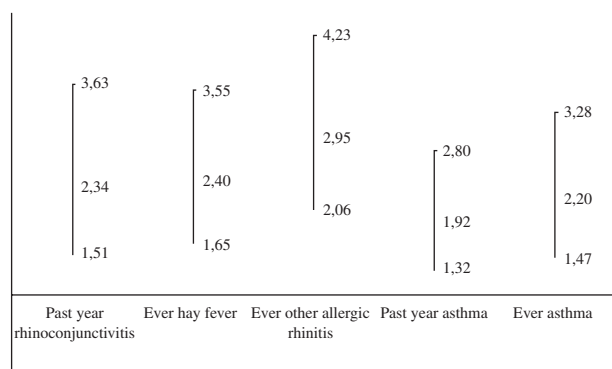


Figure 2. Relationship between *Alternaria* sensitization and phenotypes of allergic rhinitis and asthma. Adjusted Odds-Ratios for gender, parental allergy, socioeconomic status, humidity, and passive smoking (*n* = 6726).

such as gender, parental history of allergy, parental socioeconomic status, overweight, environmental tobacco smoking, and humidity.

A significant relationship among *Alternaria* sensitization and isolated phenotypes of rhinitis without asthma and of asthma without allergic rhinitis was still observed after adjusting data for potential confounders (Table 3).

Association between *Alternaria* monosensitization and phenotypes of isolated rhinitis and asthma

Out of the 189 (2.8%) children that were sensitized to *Alternaria*, 54 (28.6%) were monosensitized. *Alternaria* monosensitization was statistically associated with PYRC without asthma [adjusted OR = 3.87 (1.54–9.78)] and EAR without asthma [adjusted OR = 2.88 (1.10–7.55)] respectively (Table 4). Although the odd-ratio between EHF in the absence asthma and *Alternaria* sensitization was still higher than 1, the relationship was not statistically significant (*P* = 0.07).

Discussion

After adjusting for confounders, *Alternaria* sensitization was associated in our general population of children not

only as expected with asthma but also with allergic rhinitis, especially when this was isolated from asthma. The relationship between *Alternaria* sensitization and allergic rhinitis was even stronger in monosensitized children.

In our population-based sample of children, the conventional association between *Alternaria* sensitization and asthma was confirmed, with adjusted odds-ratio (OR) values ranging between 1.92 and 2.20 according to the type of the asthma phenotype, in agreement with other transversal epidemiological studies (18–20).

In warm countries with high *Alternaria*-sensitization rate, as in coastal Australia or in Colorado desert environments, the adjusted OR for the relationship between *Alternaria* sensitization and current asthma or diagnosed persistent asthma was 3.4 in 805 children aged 8–11 years (22) or 5.6 in 912 11-year-old children respectively (23). In the US National Health and Nutrition Examination Survey III survey, adjusted OR for children diagnosed as having asthma by physician was 1.9 in a population of 5384 children aged 6 to 16 years old (24).

On the contrary, the relationship of *Alternaria* sensitization to allergic rhinitis remains still controversial. Existing population studies involving a high number of subjects found that the association between sensitization to *Alternaria* and rhinitis did not persist after adjustment for potential confounders. Cross-sectional as well as birth cohort studies have often found significant positive associations in univariate analyses, but negative associations in multivariate analysis after adjusting for potential confounders. In a South American study involving 3294 young adults, no association was found between sensitization to *Alternaria* and rhino-conjunctivitis after adjusting for other allergens (25). Two North European studies conducted in children and in adults reported a significant association between sensitization to any mould and allergic rhinitis, but only in univariate analysis (26, 27). In the second National Health and Nutrition Examination Survey (13), conducted in a sample of 4877 persons 6 to 24 years old living in the USA, the prevalence of allergic rhinitis was associated with skin reactivity to *Alternaria* after controlling for confounders (odds ratio = 2.3; 95% CI: 1.5–3.4). However, when considering isolated allergic rhinitis, in the absence of asthma,

Table 3. Relationships of *Alternaria* sensitization to isolated phenotypes of allergic rhinitis and asthma in the French Six Cities Study

	Percentage prevalence (95% Confidence Interval) of the disease			Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio* (95% CI)
	All (n = 6726)	Children nonsensitized to <i>Alternaria</i> (n = 6537)	Children sensitized to <i>Alternaria</i> (n = 189)		
Past year					
Rhinoconjunctivitis in the absence of asthma	7.5 (6.8–8.2)	7.2 (6.5–8.0)	18.0 (12.0–25.4)	3.44 (2.17–5.45)	3.27 (2.06–5.20)
Asthma in the absence of rhinoconjunctivitis	6.5 (5.9–7.2)	6.4 (5.7–7.1)	12.2 (7.3–18.9)	2.66 (1.56–4.53)	2.38 (1.39–4.08)
Both	4.1 (3.6–4.7)	4.0 (3.5–4.5)	10.1 (5.6–16.3)	3.50 (1.95–6.27)	3.03 (1.68–5.49)
Ever					
Hay fever without asthma	9.0 (8.3–9.8)	8.7 (8.0–9.5)	19.6 (13.6–26.8)	3.08 (2.02–4.70)	2.84 (1.85–4.36)
Asthma without hay fever	5.6 (5.0–6.3)	5.4 (4.9–6.1)	12.4 (7.6–18.7)	3.14 (1.89–5.21)	2.79 (1.67–4.65)
Both	3.6 (3.1–4.1)	3.5 (3.0–4.0)	7.8 (4.1–13.3)	3.10 (1.67–5.75)	2.68 (1.44–5.01)
Other allergic rhinitis without asthma	7.5 (6.8–8.2)	7.2 (6.5–7.9)	18.7 (12.9–25.8)	3.61 (2.35–5.54)	3.45 (2.24–5.31)
Asthma without other allergic rhinitis	4.2 (3.7–4.8)	4.1 (3.6–4.7)	9.0 (5.0–14.7)	3.05 (1.71–5.44)	2.68 (1.50–4.80)
Both	5.1 (4.5–5.7)	4.9 (4.3–5.5)	11.6 (7.0–17.7)	3.29 (1.96–5.52)	2.89 (1.71–4.88)

In each analysis the control group is composed of children without allergic rhinitis and asthma respectively.

*Odds-ratios adjusted for gender, parental history of allergy, parental socioeconomic status, overweight, environmental tobacco smoking and humidity.

Table 4. Relationship of *Alternaria* sensitization and monosensitization to isolated phenotypes of allergic rhinitis and asthma in the French Six Cities Study

	Nonsensitized	Polysensitized including to <i>Alternaria</i>	Monosensitized to <i>Alternaria</i>	Other sensitizations excluding <i>Alternaria</i>
Past-year rhinoconjunctivitis without asthma (n = 5434)				
Prevalence, %	5.3	25.3	18.2	17.9
Unadjusted OR (95% CI)	1.00	6.09 (3.55–10.46)	3.99 (1.63–9.78)	3.91 (3.15–4.84)
Adjusted OR* (95% CI)	1.00	4.37 (2.28–8.39)	3.87 (1.54–9.78)	3.90 (3.10–4.91)
Ever hay fever without asthma (n = 5495)				
Prevalence, %	6.4	29.4	13.5	20.2
Unadjusted OR (95% CI)	1.00	6.10 (3.76–9.89)	2.29 (0.88–5.92)	3.71 (3.06–4.49)
Adjusted OR 95% CI)	1.00	5.73 (3.44–9.53)	2.46 (0.94–6.45)	3.68 (3.02–4.49)
Ever other allergic rhinitis without asthma (n = 5495)				
Prevalence, %	5.5	27.6	13.9	15.7
Unadjusted OR (95% CI)	1.00	6.48 (3.97–10.58)	2.74 (1.06–7.13)	3.17 (2.57–3.91)
Adjusted OR (95% CI)	1.00	6.14 (3.68–10.22)	2.88 (1.10–7.55)	3.13 (2.53–3.88)

In each analysis the control group is composed of children without allergic rhinitis and asthma respectively.

*Odds-ratios adjusted for gender, parental history of allergy, parental socioeconomic status, overweight, environmental tobacco smoking, and humidity.

this was no longer associated with *Alternaria*. Lastly, in two British birth cohorts of 4- to 5-year-old children, sensitization to *Alternaria*, as assessed by skin prick test and/or specific IgE, was associated with the presence of allergic rhinitis in univariate analyses only (28, 29). Differences among studies might be attributable to variations in populations, classes of age of the participants, methods and exposure to *Alternaria*. In our population-based study, the relationship of *Alternaria* sensitization persisted after adjustment for a large variety of confounders, after exclusion of children suffering also from asthma and was stronger in children who were monosensitized to *Alternaria alternata* so supporting the existence of a link between sensitization to *Alternaria* and allergic rhinitis, independently of asthma.

It has been observed that children who are sensitized to *fungi* are more likely to be sensitized also to other aeroallergens (30). Thereafter, monosensitization to *Alternaria* has seemed to us a necessary criterion to ensure that the observed findings were specific to *Alternaria*. This had not been explored previously at the population level. An Italian study found that 1.3% of 6840 Italian children attending a clinical division were monosensitized to *Alternaria* (31). Amongst them, a higher incidence of asthmatic manifestations was found (31). In a clinical study involving 28 children monosensitized to *Alternaria* in Spain, and selected for immunotherapy, only three patients had exclusively nasal symptoms (32). However, the groups of children evaluated in clinical studies reflect those who require medical

assistance because of more severe allergic symptoms. In our population-based sample, monosensitization to *Alternaria* was associated significantly with PYRC and EAR.

Alternaria spores are among the largest spores in common outdoor fungi ($> 10 \mu\text{m}$) (33) and may therefore induce allergic rhinitis as it is assumed that virtually all particles with an aerodynamic diameter in excess of $10 \mu\text{m}$ are trapped in the upper airways (34). *Alternaria* spores present seasonal variation in temperate regions, because of increased growth on rotting leaves and other biomaterials, as assessed by aerobiological collectors placed on the roof tops of high building (35). *Alternaria* spores are considered to be present in high concentrations in summer, but they are also present throughout the year in lower concentrations (36). As a consequence of its distribution, *Alternaria* may induce both perennial and seasonal rhinitis, as observed in our population of children through the exploration of various phenotypes of allergic rhinitis.

Our findings confirm that upper and lower airways may have common risk factors. *Alternaria* is an independent factor for both asthma and rhinitis. Yet, the present state of knowledge does not yet allow stating whether or not the connections observed between upper and lower airway disease are different manifestations of one or two basic disease processes (37). In our investigation conducted in children, *Alternaria* was more related to allergic rhinitis than to asthma, which suggests different pathophysiological mechanisms and timing for the two diseases occurrence. These mechanisms include genetic factors, as shared or not shared linked regions have been found for allergic rhinitis and asthma (38). However, existing results do not prevent allergic rhinitis from being a risk factor for asthma as previously shown (37). *Ad hoc* studies are needed to disentangle the relationships according to the various phenotypes of these diseases.

The prevalence of allergic sensitization to *Alternaria* that we observed is comparable to previous figures so validating the quality and the representativeness of our data. In other population-based studies, the prevalence of sensitization to *Alternaria* appears to vary with climate and age. In Sweden, 3.8% of 7- to 8-year-old children have been found to be sensitized to *Alternaria* (26) whereas in Great Britain, 4.8% of 4-year-old infants reacted to *Alternaria* (39). In the European Community Respiratory Health Study conducted amongst young adults, the median prevalence of sensitization to *Alternaria* was 3.3%, with a lower prevalence in both southern and northern Europe (40) and a higher median cumulative prevalence in France 5.8%. In our study conducted in children aged 10 years on average living in six cities of France with different meteorological characteristics, this amounted to 2.80% of the subjects.

Other advantages of our study consist in the large population-based sample randomly selected, the use of a standardized protocol to assess allergic sensitization, the

appropriate statistical analyses taking into account several potential confounders and in the exclusion of children with asthma, which allowed investigating the specific contribution of *Alternaria* sensitization to allergic rhinitis only. One limitation of our study is the lack of objective measurements of airborne *Alternaria* spores throughout the duration of the Six Cities study. However, we do not think that this would have brought crucial information as the children could have moved in their city and among cities during their life.

Conclusion

The main finding of our study is that, in our population-based study of children, *Alternaria* sensitization was found to be associated with allergic rhinitis in the absence of asthma, which is compatible with the mechanisms of deposition of *Alternaria* spores in the upper airways. Further investigations conducted in other population-based samples are needed to confirm and better explain our results.

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All authors declare that the answer to the questions on your competing interest form are all No and therefore have nothing to declare.

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