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Emilie Burte[a,b,c], Jean Bousquet, MD, PhD,[a,b,d], Valérie Siroux, PhD, [e,f,g], Jocelyne Just, MD, PhD [h,i], Bénédicte Jacquemin, MD, PhD,[a,b,c,j,k*], Rachel Nadif, PhD,[a,b,*]

***: contributed equally to the work**

a. INSERM, U1168, VIMA: Aging and chronic diseases, Epidemiological and Public health approaches, F-94807, Villejuif, France
b. Univ Versailles St-Quentin-en-Yvelines, UMR-S 1168, F-78180, Montigny le Bretonneux, France
c. Univ Pompeu Fabra (UPF), Barcelona, Spain
d. University hospital, Montpellier, France
e. INSERM, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, F-38000 Grenoble, France
f. Univ Grenoble Alpes, F-38000 Grenoble, France
g. CHU de Grenoble, F-38000 Grenoble, France
h. Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, Allergology Department, Paris, France
i. Univ Paris 6 Pierre et Marie Curie, Paris, France
j. ISGlobal- CREAL-Centre for Research in Environmental Epidemiology, Barcelona, Spain
k. CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

Corresponding author:
Emilie Burte,
INSERM, U1168, VIMA: Aging and chronic diseases, Epidemiological and Public health approaches, F-94807, Villejuif, France. Phone number: 33 (0) 145 59 50 22, Fax number: 33 (0) 145 59 51 69
E-mail: emilie.burte @inserm.fr

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Abstract:

Background: Mono- and polysensitization are different IgE-mediated allergic phenotypes in children. Allergic sensitization is associated with both allergic asthma and allergic rhinitis, however, associations between the sensitization pattern and particularly polysensitization with asthma and rhinitis remains poorly studied in adults.

Aim: The aim of this study was to assess how the allergic sensitization pattern associates with asthma, rhinitis and their multimorbidity.

Methods: 1,199 adults from the EGEA study, with extensive phenotypic characterization and all data available on skin prick tests to 10 allergens, total IgE and blood eosinophils were included. Using questionnaires only, participants were classified into 6 groups: asymptomatic (no asthma, no rhinitis), non-allergic rhinitis alone, allergic rhinitis alone, asthma alone, asthma+non-allergic rhinitis and asthma+allergic rhinitis. Mono- and polysensitization were defined by a positive skin prick test to one or more than one allergen respectively.

Results: Asymptomatic participants and those with non-allergic rhinitis alone were mostly non-sensitized (around 72%) while around 12% were polysensitized. Between 32 and 43% of participants with allergic rhinitis alone, asthma alone and asthma+non-allergic rhinitis were non-sensitized and between 37 and 46% of them were polysensitized. 65% of the participants with asthma+allergic rhinitis were polysensitized. The level of total IgE followed a similar trend to that of allergic sensitization. Eosinophils were increased in asthma, especially when associated with rhinitis. Nasal symptoms were more severe and eczema more common in participants with both asthma and allergic rhinitis than in the other groups.

Conclusions: Allergic sensitization and particularly polysensitization rates widely differ according to asthma and rhinitis status. This study emphasized the importance of taking into account multimorbidity between asthma and rhinitis and showed that allergic sensitization is not a dichotomic variable.

Key words: allergic sensitization, asthma, rhinitis, multimorbidity, monosensitization, polysensitization

Abbreviations

AR: Allergic rhinitis

ARIA: Allergic Rhinitis and its Impact on Asthma

BHR: Bronchial Hyperresponsiveness

D.Pteronysinus: Dermatophagoides pteronyssinus

EGEA: Epidemiological study of the Genetics and Environment of Asthma

IgE: Immunoglobulin E

MeDALL: Mechanisms of the Development of ALLergy

NAR: Non-allergic rhinitis

SPT: Skin Prick Test

Introduction

According to the World Allergy Organization (1), IgE-mediated allergic diseases, including allergic respiratory diseases such as rhinitis (2) and asthma are complex (3). These diseases are associated with both allergen-specific IgE and non-allergic mechanisms that may coexist in the same patient. In addition, they tend to cluster and patients may present concomitant or consecutive diseases (allergic multimorbidity) (4) as shown in children within the European MeDALL project (5).

Most epidemiological studies define allergic status as being sensitized or not (thus as having at least one positive skin prick test or at least one specific IgE>0.35kU/L). Nevertheless, sensitization to an allergen does not necessary imply nasal symptoms (6) and, conversely, nasal symptoms may possibly be due to a non-allergic rhinitis despite an allergic sensitization. Over 70% of symptomatic patients are sensitized to more than one allergen i.e polysensitized as found in both children and adults (7-9). Important clinical and immunological differences exist between mono and polysensitized patients suggesting that polysensitization is the expression of a distinct disease both in children and adults (5,10,11). Moreover, persistence of allergic diseases over time is associated with multimorbidity and/or allergic polysensitization (2). A recent study in Finnish adults showed that polysensitization -but not monosensitization- was associated with asthma (12). All of these studies emphasize phenotypic differences between mono and poly sensitized subjects, as recently summarized in a review (6). However, to our knowledge, no study has ever specifically assessed the sensitization pattern (mono- vs polysensitization, total IgE rate, eosinophil counts, severity of the symptoms) according to asthma and rhinitis status in adults.

In adults, using an unsupervised approach, we have previously identified three clusters of rhinitis with similar characteristics similar to those known by clinicians but differing in term of allergic sensitization, and this whatever the asthma status (13). Furthermore, in the cluster combining asthma and allergic rhinitis, participants showed a particularly high rate of polysensitization compared to the other clusters. This finding prompted us to perform a study assessing allergic sensitization in relation to asthma and rhinitis. Our hypothesis is that allergic sensitization, and particularly polysensitization, differ according to asthma and rhinitis status comorbidity and, in adults, this confirms the MeDALL concept that has previously been shown in children (5).

The aim of this study was to assess how the allergic sensitization pattern, assessed by mono- vs polysensitization, total IgE, eosinophil counts and severity of the symptoms, associates with asthma, rhinitis and their multimorbidity in 1199 adults of the EGEA (Epidemiological study of the Genetics and Environment of Asthma) study.

Methods

Study design

The EGEA study is a French case-control and family study based on an initial group of asthma cases and their first-degree relatives, as well as a group of controls (EGEA1, n=2047; https://egeanet.vjf.inserm.fr).

Setting and participants

The protocol and descriptive characteristics of the EGEA study have been previously published (14). Briefly, EGEA is a 20-year follow-up study combining a case-control study with a family study of asthma cases (children or adults). 2047 children (<16 years) and adults from five French cities were enrolled between 1991 and 1995. The participants included 348 cases with current asthma recruited in chest clinics, their 1244 first-degree relatives, and 415 population-based controls. A follow-up of the initial cohort was conducted between 2003 and 2007 (EGEA2) (15). Among the alive cohort (n = 2,002), 92% (n = 1,845) completed a short self-administered questionnaire and among them 1,601 had a complete examination (1570 adults). All participants responded to questionnaires based on international standardized tools to characterize asthma, respiratory and allergic symptoms and treatments, and environmental exposures.

Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris). Written informed consent was signed by all participants.

Variables

Allergic sensitization

Skin-prick tests (SPTs) to 10 of the most commons aero-allergens (cat, *Dermatophagoides pteronyssinus*, olive, birch, *Parieteria judaica*, timothy grass, *Cupressus*, ragweed pollen, *Cladosporium herbarum*, *Alternaria tenuis*, Stallergènes, Antony, France) were selected for the analysis (16,17). Negative (uncoated) and positive (histamine) SPT controls were assessed. SPT with a mean wheal diameter 3mm ≥ than the negative control was considered as positive (16). SPTs assessment was performed by trained professionals and in the same way for all adult participants, whatever the center. SPTs to *Blattela germanica* and *Aspergillus* were also available but not included in the analysis as the quality of the reagents was insufficient.

Asthma and Allergic rhinitis definitions

Asthma status was based on a positive answer to either *''Have you ever had attacks of breathlessness at rest with wheezing?''* or *''Have you ever had asthma attacks?''* or as being recruited as an asthma case. Allergic Rhinitis (AR) ever was defined by a positive answer to nasal symptoms: *"Have you had a problem with sneezing or runny or blocked nose when you did not have a cold or the flu?"* and a positive answer to *"Have you ever had allergic rhinitis?"* or *"Have you ever had hay fever?"*. Non-allergic Rhinitis (NAR) ever was defined by a positive answer to nasal symptoms and a negative

answer to *"Have you ever had allergic rhinitis?"* and *"Have you ever had hay fever?"*.

The quantitative asthma symptom score, as defined by Pekkannen *et al.* was used to describe the phenotype of asthma and as a proxy of severity of asthma (18).

Participants were classified into 6 groups, based only on their responses to the questionnaire: no asthma and no rhinitis (Reference group), non-allergic rhinitis (NAR) only, allergic rhinitis (AR) only, asthma only (As+), asthma+NAR (As+NAR), and asthma+AR (As+AR). These groups are similar to those highlighted by a clustering approach, but using only two questions on rhinitis and not using allergic sensitization (13).

Nasal symptoms were considered, similarly to the ARIA guidelines (2), as intermittent if they occur more than one month per year but less than 4 days per week or as persistent if they occur more than a month per year and more than 4 days per week. Moreover, if the symptoms occurred less than one month per year, persistence of nasal symptoms was considered as rare. Severity of nasal symptoms was assessed using the answers to the question "*Have these nose problems disturbed you daily activities?*". This enabled a score of disturbance to be obtained from 0 to 3 (0: no, 1: a little bit, 2: moderately, 3: a lot).

Other phenotypes - definition

Eczema, conjunctivitis or sinusitis were defined as a positive answer to *"Have you ever had eczema?"* (respectively conjunctivitis or sinusitis).

Biological phenotypes

Total IgE were assessed by the UniCAP system (Pharmacia®) from blood samples in a centralized laboratory, and expressed in international units (IU) per milliliter.

Eosinophil cell counts were obtained from white blood cell counts.

Study size

The present analysis was conducted in 1199 adult participants of EGEA2 who had available data on asthma status, rhinitis status, SPT, total Immunoglobulin E (IgE), and blood eosinophils. Since this is an exploratory study, no power calculation was needed.

Bias

Analyses were also performed using the 12 allergens including *Aspergillus* and *Blatta Germanica*, and results were very similar, with similar percentages of mono- and polysensitization according to the groups (data not shown).

Due to the familial design of the study, a sensitivity analysis was conducted in a sub-sample of the population with one randomly-selected member per family. These analyses with 566 participants have shown very similar results to those of the study on the 1199 participants (data not shown).

Statistical analysis

To test whether general, phenotypic and allergic characteristics differ among the groups and differs from the reference group (no asthma no rhinitis), the Chi2 test and univariate polytomic logistic regression with no further adjustment were performed. For variables available only in subjects with rhinitis (such as age of onset, persistence or severity) or asthma (such as age of onset), these tests were performed only among the adequate population (i.e subjects with rhinitis or asthma).

To test whether some groups tend to be more non-sensitized (no positive SPT) or monosensitized (1 positive SPT) than poly-sensitized (>=2 SPTs), a polytomic logistic regression was used, adjusting results on several variables: age, sex, smoking status and educational level, chosen as they differed significantly according to the six groups. The reference class was the group with neither asthma nor rhinitis. This same methodology was used to compare sensitization to each of the 10 allergens among the groups. Severity and persistence of nasal symptoms, total IgE level and eosinophil count were compared group by group using logistic regression adjusted for age, sex, smoking status and educational level.

As a sensitivity analysis, we also adjusted the results using occupation instead of educational level, adjusting on parental asthma and childhood spent on a farm.

All the analyses were performed using the R statistical software (19).

Results

Characteristics of the participants

Participants were classified into 6 groups: no asthma no rhinitis (Reference group, N=362), NAR alone (NAR, N=169), AR alone (AR, N=167), asthma alone (As+, N=65), asthma+NAR (As+NAR, N=78) and asthma+AR (As+AR, N=358). The characteristics are presented in Table I. The participants of the groups with asthma were younger (p-value As vs non-As: <0.001), and more likely to be male (p-value As vs non-As: 0.015). The participants who had asthma and rhinitis – allergic or non-allergic - declared a younger age of onset than those without asthma (p-value rhinitis vs rhinitis+As: <0.001). The participants with As+AR had a higher prevalence of eczema to those in the other groups (p-value<0.05 whatever the group).

Allergic sensitization evaluated by SPT

Participants without symptoms of rhinitis or asthma and those with NAR had no allergic sensitization in over 71%, and less than 14% were sensitized to over 2 allergens (Figure 1). Participants with AR alone or As+ alone had no allergic sensitization in about 33% of cases whereas about 42% of them were sensitized to over 2 allergens. Participants with As+NAR had no positive SPT in 43.6% of cases and 37.0% of them were sensitized to over 2 allergens. Participants with As+AR had no positive SPT in 14.8% of cases and 65% of them were sensitized to over 2 allergens.

Compared to the participants without asthma and rhinitis, polysensitization (*versus* non or mono-sensitized) was highly associated with AR alone and even more so with As+AR (crude and adjusted odds-ratios in Table 2). Lower aORs were observed for As+ and As+NAR and no significant association was found for NAR alone. Using different levels of adjustment did not modify the results (see Table E1 in the Online Repository)

Sensitization according to different allergens

The repartition of the allergic sensitization according to the group and to the 10 allergens is given in figure 2. *D.pteronyssinus*, cat, and allergens related to hay/pollen were the most common allergens. The sensitization rate to *D.pteronyssinus* was higher in all groups of symptomatic participants i.e. AR alone, As+ alone, As+NAR and As+AR groups as compared to the reference group (no asthma no rhinitis). The sensitization rate to cat was higher in all groups of symptomatic participants except for the NAR alone group. For hay/pollen allergens, the sensitization rate was particularly high for participants with AR alone and As+AR, whatever the allergen. Sensitization to timothy grass was the most common allergen for hay/pollen, followed by Olive tree. Sensitization rates to *Parietaria* and Cypress were low in all groups. Sensitization to *Cladosporium* and *Alternaria* was over 10% only in the As+ alone and As+AR groups.

Persistence and severity of nasal symptoms

Nasal symptoms were more persistent in As+AR participants compared to As+NAR (p-value adjusted <0.001) or NAR alone (adjusted p-value=0.018) and slightly more persistent compared to AR alone (adjusted p-value=0.14). There was no difference between NAR alone and As+NAR (adjusted p-value=0.81). Nasal symptoms were more severe in participants with As+AR compared to As+NAR (p-adjusted<0.001), NAR alone (p-value<0.001) or AR alone (adjusted p-value=0.010). Nasal symptoms were also more severe in participants with As+NAR than in those with NAR alone (adjusted p-value=0.036) (Table 1).

Blood eosinophils and total IgE

Blood eosinophil counts were higher in all symptomatic groups compared to the reference group (no asthma, no rhinitis). AR alone and As+ alone had a similar level whereas eosinophils were even higher when asthma was associated with rhinitis, allergic or non-allergic. Total IgE levels followed a similar trend to allergic sensitization, with a higher value in participants with As+AR, compared to participants without asthma and rhinitis or NAR alone, whereas participants with As+ alone, As+NAR and As+AR had intermediate levels (Table 1).

Discussion

In the present study, using new analyses, we showed that polysensitization was the highest among participants with asthma and allergic rhinitis multimorbidity by comparison to asthma or rhinitis

alone. Asymptomatic participants or those with non-allergic rhinitis are in the vast majority, non-sensitized or sensitized to one allergen. Levels of total IgE followed a similar trend to allergic sensitization. Eosinophil counts were increased in asthma alone, and the greatest number was found when asthma was associated with rhinitis. Nasal symptoms were more severe in participants with As+AR than in participants from other groups.

This study presents several strengths and limitations. It was performed among over 1000 adults from the EGEA study that is not representative of the French population, but enriched in participants with asthma, allowing a good statistical power to address allergic multimorbidities. This particular design (case control and family study) and the age differences at inclusion between cases, relatives and controls explains in part that participants with asthma were younger than participants without asthma (20). The age of onset of nasal symptoms differs according to the group, and is significantly lower in participants with allergic rhinitis. This result is not surprising because allergic rhinitis often appears at a younger age than non-allergic rhinitis whereas non-allergic rhinitis is often characterized by onset after the age of 20 years (21). The age of onset of nasal symptoms is also lower in participants with asthma, and this can be explained by the concomitance of two facts: (i) rhinitis and asthma are strongly related, often coexist, and one often leads to the other; (ii) the mean age of onset of asthma is generally lower than 20 years and, even more, often occurs during childhood. Thereupon, the age of onset of nasal symptoms was the lowest in participants with asthma+AR. The extensive phenotypic characterization regarding respiratory health, and particularly rhinitis and asthma, is clearly a strength. Rhinitis was not diagnosed by a physician but was defined by self-reported symptoms, as is mostly the case in epidemiological studies. Thereby, using self-reported questionnaires leads to a possible misclassification of the subjects due to a poor knowledge of the disease. However, to classify our participants we used their answers to questions from an interviewer-based, standardized and validated questionnaire from the European Community Respiratory Health Study (ECRHS). Several epidemiological studies have already used these self-reported symptoms to define rhinitis (22-24). Using self-reported questionnaires also leads to another possible misclassification due to recall bias, as is often the case in epidemiology. The differentiation between allergic and non-allergic rhinitis was also based on self-reported symptoms and did not take allergic sensitization into account. This classification could be surprising at first glance, as some participants have unusual characteristics such as in the NAR or no rhinitis groups where some reported hay fever or allergic rhinitis or in the AR group where some were not sensitized to any of the 10 allergens. This definition, although unusual, enabled us to refine questionnaire-based phenotypes and our results support that choice. In our previous unsupervised study, we found 3 clusters of rhinitis (13) whatever the asthma status. Whereas characteristics of the participants were similar to the phenotypes of rhinitis known by clinicians, the allergic sensitization differed strongly among the three phenotypes. In this study, we have put forward 3 groups based only on two frequent rhinitis questions. The level of allergic sensitization was similar

to the one found in the cluster analysis as opposed to the classical phenotypes, and this confirms the interest of taking this particular definition of rhinitis. Another limitation of our study is the difficulty to distinguish allergic asthma from non-allergic asthma phenotypes. First, because we stratified asthma sub-groups according to rhinitis, and secondly because of the inherent difficulty to differentiate between both types of asthma in epidemiological settings. However, participants with co-occurrence of allergic asthma and non-allergic rhinitis should exist and this may be one explanation as to why participants with asthma+NAR were sensitized.

In this study, we decided to define allergic sensitization using SPTs rather than specific-IgE – because the SPTs have a better predictive value for rhinitis (25). Thus, some differences may be found with other studies, since the two methods are not exactly comparable (2). Furthermore, SPTs were defined at the extract level (i.e. IgE reactivity to several non-related - or not obviously related - allergenic source materials) and not at the molecular level (i.e. IgE reactivity to several nonrelated - or non-obviously related - allergenic molecules) (5). This could have changed the way of defining polysensitization and may have increased the number of allergenic molecules detected. As allergic sensitization is a transient phenotype and as asthma is a complex disease that changes over time, it would have been interesting to perform a longitudinal analysis. However, EGEA1 questionnaires regarding rhinitis were slightly different to those in EGEA2 and 30% of the participants were children, and no SPT were available at the second-follow-up of EGEA. This disabled the opportunity to perform the longitudinal analysis in EGEA, but the question remains of interest.

Among the 10 studied allergens, the most frequently involved were *D.pteronyssinus*, cat, Timothy grass and Olive tree, and this whatever the group. Participants with As+AR had the highest rate of sensitization to cat and *D.pteronyssinus*, but also to all the allergens related to hay/pollen and *Alternaria*. Participants with AR alone and As+AR were particularly sensitized to allergens related to hay/pollen which bring out the "hay fever" part of allergic rhinitis. Participants with asthma seem to be particularly sensitized to *Alternaria* and *Cladosporium*, which is concordant with the literature (26,27). The 10 allergens tested were chosen for being the most common, but it is possible that participants are sensitized to other allergens such as dog or *Dermatophagoides farinae* (28), and then, considering these other allergens may increase the number of positive SPT. However, it is unlikely that adding more allergens would increase the number of sensitized participants as it has been shown that using from eight to ten allergens allowed the identification of the majority of sensitized subjects (29). Overall, participants of the As+ alone and As+NAR groups had significantly higher rates for *D.pteronyssinus*, cat, Timothy grass and Olive tree than the reference group. This suggests that these allergens are not only related to nasal symptoms or allergic rhinitis, but also to asthma itself.

In the present study, we showed that mono- and polysensitized individuals represent different phenotypes of allergic diseases. This was found for children in the EU-FP7 MeDALL project (5,30) and now also extends to adults. More specifically, we confirmed that asymptomatic subjects are often

monosensitized as shown in Russian and Finnish children for House Dust Mite monosensitization (31). Furthermore, allergic sensitization was lower in asymptomatic subjects than in symptomatic ones as found in a Finnish adult case-control on asthma study (12). We have also found that the polysensitization rate is the highest among participants with both allergic rhinitis and asthma, which is concordant with previous studies among European adults (32,33). Recent studies in genetics, including one using the EGEA study data (34,35), have also shown that genetic variants associated with asthma plus hay fever or asthma plus allergic rhinitis were different from those associated with only asthma or hay fever. This again suggests that asthma plus allergic rhinitis is a very specific phenotype. The As+AR group seems to have a specific phenotype - characterized by a high level of polysensitization, total IgE and eosinophil counts, and severe symptoms. This group is also the one with the youngest age of onset of asthma and rhinitis.

Interestingly, one could note a trend in the number of positive SPTs: being the lowest in asymptomatic and NAR alone participants, the highest in multimorbid diseases (participants with As+AR), and with intermediate levels in participants with AR alone, As+ alone or As+NAR. This trend was also found when looking at each allergen separately. Moreover, nasal symptoms were more severe among participants with As+AR, compared to the other groups with rhinitis. We showed that the As+AR group is the most polysensitized group. This result is concordant with the following studies where polysensitization was associated with more severe symptoms: (i) 9044 children aged 0-18 years in the Netherlands (10), (ii) 2415 young Italian adults with allergic rhinitis (8), (iii) 3225 Spanish and Portuguese patients with allergic rhinitis aged 10-50 years (33), (iv) 130 Korean patients with childhood asthma (36). On the contrary, other studies have shown no change in severity according to polysensitization, neither in the 784 children aged 6-18 years in primary care diagnosed with allergic rhinitis (9), nor in the 523 Finnish adults with asthma from a population-based case-control (12). These discordant results do not seem to be due to the differences in the age of the participants, to the size of the samples, or to geography, as the studies were conducted in both children and adults in America, Europa or Asia. However, the different protocols used to define asthma or rhinitis (by questionnaire, by relevant medication use, by history of symptoms, by lung function test, by a physician or GP, by GINA or by ARIA classification), and allergic sensitization (by SPT or by specific-IgE) may partly explain the between-study discrepancies. Furthermore, we also found that participants with As+NAR had more severe nasal symptoms compared to those with NAR only, meaning that severity is not related only to sensitization, but also to multimorbidity diseases. These results suggest that multimorbidity and polysensitization are two different aspects of allergic disease, probably interacting together.

The MeDALL study in birth cohorts showed that multimorbid-polysensitized participants have a more persistent disease, and the authors suggested that a recurrence of a Th2 pathway may partly explain the results (5). The current study confirms the findings of the MeDALL study in adults, with

a multimorbid-polysensitized phenotype associated with an earlier onset and a greater severity compared to other phenotypes. Therefore, the same hypothesis may be proposed to explain, at least in part, our results. Our results suggest that this multimorbid-polysensitized phenotype could constitute a specific phenotype. A key unanswered question is the extent to which a particular phenotype (pattern) profile may identify “treatable" traits. Further researches is required to explore this possibility. Overall, this study emphasized the importance of taking into account multimorbidity between asthma and rhinitis and showed that allergic sensitization should not be used as a dichotomic variable. This result may lead to a different classification of allergic phenotypes in future epidemiological studies.

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392 **Supporting Information**

393 Additional Supporting Information may be found in the online version of this article:

394 Table E1: OR with different adjustments of the association between polysensitization (*versus* no or
395 monosensitized) and the 6 groups

396 Table E2: adjusted OR of the association between allergic sensitization to each of the 10 allergen and
397 the 6 groups

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Table 1: Characteristics of the participants

		No asthma, no rhinitis	NAR alone	AR alone	Asthma alone (As+)	Asthma +NAR	Asthma + AR	p crude, overall
N		362	169	167	65	78	358	
Age, mean±sd		46.8±16.3	47.2±16.3	45.2±14.8	40.8±17.1	40.2±17.9	38.4±16.0	<0.0001
Sex, % women		50.0	60.9	57.5	47.7	43.6	48.0	0.02
Tobacco status, %	Non-smoker	49.7	50.3	52.7	41.5	50.0	51.1	0.51
	Ex-smoker	29.2	26.0	26.9	35.4	23.1	22.9	
	Smoker	21.1	23.7	20.4	23.1	26.9	26.0	
BMI, mean±sd		24.6±3.8	23.9±3.8	24.1±3.5	24.8±3.7	25.0±4.4	23.7±3.9	
Educational level, %	Low	30.9	27.8	21.0	21.5	29.5	16.3	0.0008
	Medium	23.8	25.4	22.8	24.6	21.8	32.6	
	High	45.3	46.7	56.3	53.8	48.7	51.1	
Current nasal symptoms, %			84.4	87.3		85.5	90.7	0.17
Eyes symptoms associated, %			32.1	76.6		47.4	80.4	<0.0001
Persistence of nasal symptoms	Rare		50.7	42.5		53.6	30.4	<0.0001
%	Intermittent		17.8	26.7		17.4	31.0	
	Persistent		31.5	30.8		29.0	38.7	
Severity of nasal	No		76.7	50.7		64.7	40.4	<0.0001
symptoms (disturbance), %	Low		17.1	33.6		22.1	32.4	
	Medium		4.8	13.0		5.9	18.3	
	High		1.4	2.7		7.4	9.0	
Age of onset of nasal symptoms, mean±sd			32.7±18.8	25.1±15.0		23.2±17.7	14.2±12.2	<0.0001
Eczema, %		22.7	25.6	35.3	38.5	38.5	52.7	<0.0001
Conjunctivitis, %		13.8	22.3	46.7	26.6	25.7	55.5	<0.0001
Sinusitis, %		34.9	47.6	59.3	47.7	50.0	58.0	<0.0001

Allergic rhinitis, %		5.5	0	73.7	0	0	81.3	<0.0001
Hay fever, %		10.8	0	77.8	0	0	78.2	<0.0001
Current asthma, %		0	0	0	91.5	96.6	97.1	0.17
Asthma Symptom score, %	**0**	77.6	66.2	62.5	27.7	22.4	17.6	<0.0001
	1	19.9	25.4	31.2	36.2	36.2	27.6	
	2	2	7	3.8	25.5	13.8	22.8	
	3	0.5	1.4	2.5	6.4	19	20.2	
	4	0	0	0	4.3	6.9	9.9	
	5	0	0	0	0	1.7	1.8	
BHR, % of yes		23.7	28.4	29.8	55.8	69.8	67.8	<0.0001
FEV1, % predicted ±sd		107	106	109	94.9	95.5	98.2	0.0006
Age of onset of asthma, mean±sd					15.8±15.5	19.9±16.3	13.9±14.3	0.0015
Eosinophils, *, mean±sd		149 ±106	178±145	191±123	196±129	249±198	253±192	<0.0001
Total IgE, *, IU/mL, geometric mean ±sd		33.9±3.7	47.9±4.6	79.4±3.6	72.4±5.1	100.0±5.6	166.0±3.6	<0.0001
Number of positive SPT, mean±sd		1.4±0.9	1.5±1.1	2.7±1.7	2.6±1.6	2.3±1.5	3.5±1.8	<0.0001

NAR: Non-allergic rhinitis, AR: Allergic rhinitis, sd: standard deviation
FEV1: Forced Expiratory Volume in one second, BHR: Bronchial Hyper Responsiveness (Methacholine test, PD20≤4 mg, Methacholine challenge test was not performed if baseline FEV1 <80% predicted, PD= Provocative Dose), IgE: Immunoglobulin E, SPT: skin prick test

Table 2: Odds Ratio of the association between polysensitization (*versus* no or monosensitized) and the 6 groups

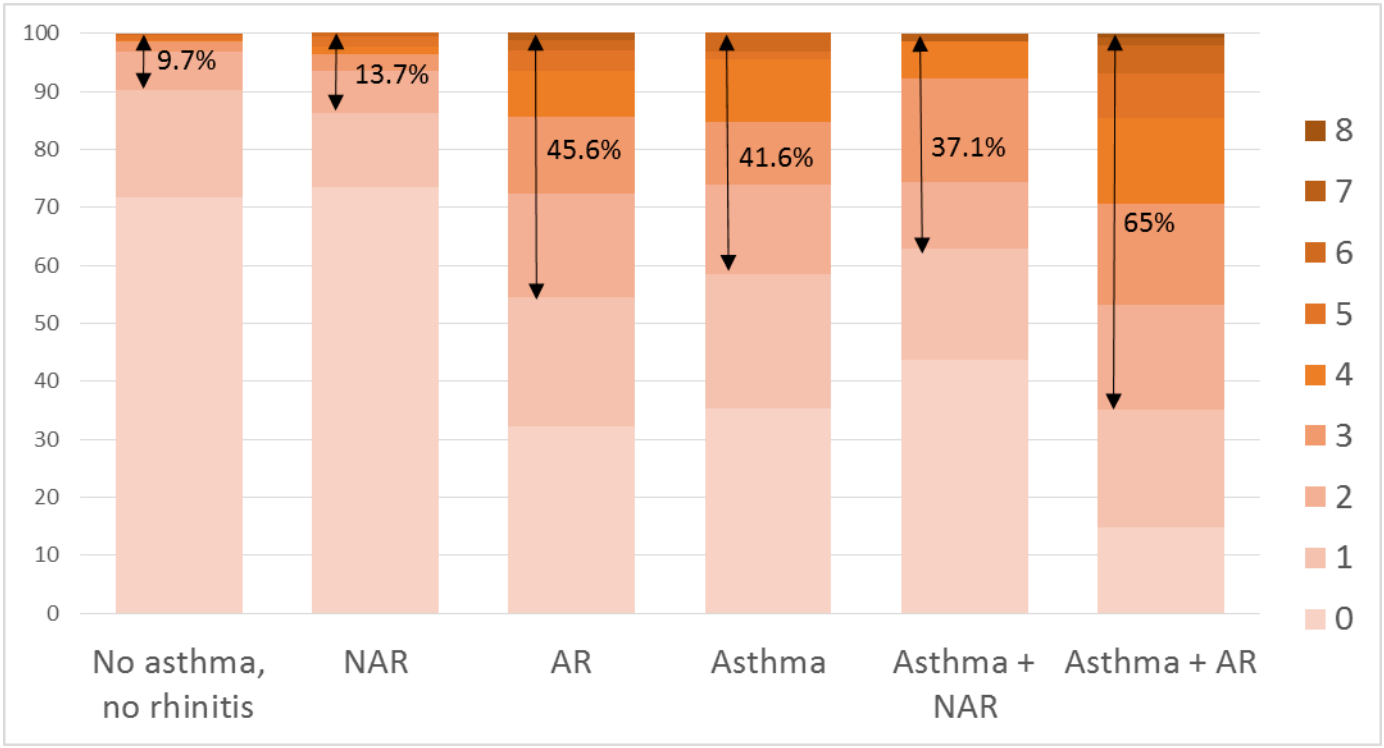
OR [95% CI]	No asthma, no rhinitis	NAR alone	AR alone	Asthma alone (As+)	Asthma +NAR	Asthma + AR	p-value
crude OR	1 (ref)	1.47[0.84-2.58]	7.8[4.91-12.40]	6.64[3.63-12.14]	5.53[3.11-9.84]	17.34[11.50-26.15]	
aOR (on age, sex and education)	1 (ref)	1.59 [0.89-2.84]	8.62[5.30-14.02]	6.01[3.20-11.31]	4.79[2.62-8.75]	15.24[9.95-23.34]	

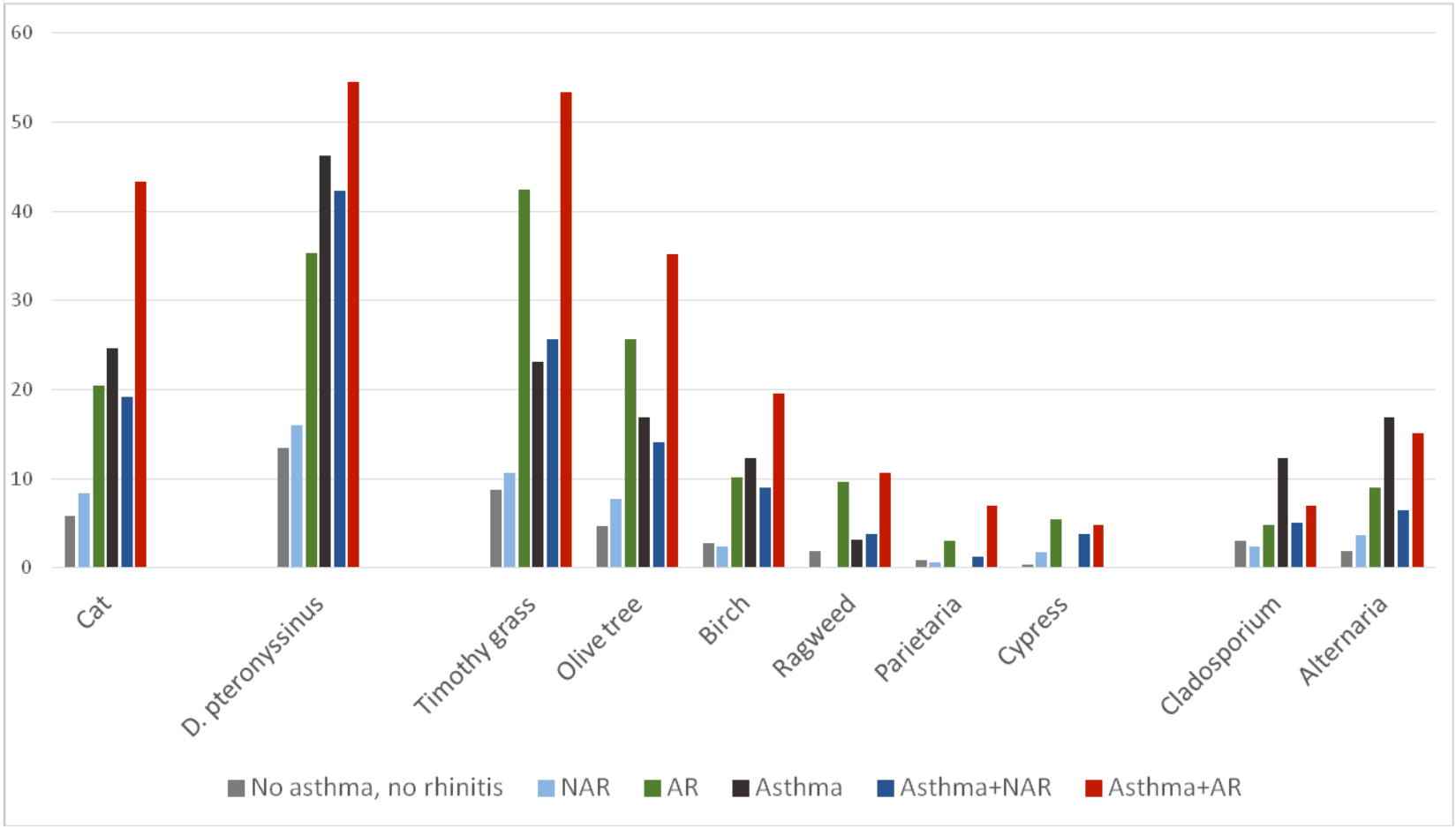
aOR: adjusted Odd Ratio, NAR: Non-allergic rhinitis, AR: Allergic rhinitis.

Figure legends:

Figure 1: Number of allergic sensitization –Number of positive SPT- according to the group and percentage of polysensitization

Figure 2: Rate of allergic sensitization to the 10 allergens according to the group


100
90
80
70
60
50
40
30
20
10
0
9.7%
13.7%
45.6%
41.6%
37.1%
65%
8
7
6
5
4
3
2
1
0
No asthma, no rhinitis
NAR
AR
Asthma
Asthma + NAR
Asthma + AR


60
50
40
30
20
10
0
Cat
D. pteronyssinus
Timothy grass
Olive tree
Birch
Ragweed
Parietaria
Cypress
Cladosporium
Alternaria
No asthma, no rhinitis
NAR
AR
Asthma
Asthma+NAR
Asthma+AR

The sensitization pattern differs according to rhinitis and asthma multimorbidity in adults: the EGEA study

Emilie Burte[a,b,c], Jean Bousquet, MD, PhD,[a,b,d], Valérie Siroux, PhD, [e,f,g], Jocelyne Just, MD, PhD [h,i], Bénédicte Jacquemin, MD, PhD,[a,b,c,j,k*], Rachel Nadif, PhD,[a,b,*]

***: contributed equally to the work**

a. INSERM, U1168, VIMA: Aging and chronic diseases, Epidemiological and Public health approaches, F-94807, Villejuif, France
b. Univ Versailles St-Quentin-en-Yvelines, UMR-S 1168, F-78180, Montigny le Bretonneux, France
c. Univ Pompeu Fabra (UPF), Barcelona, Spain
d. University hospital, Montpellier, France,
e. INSERM, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, F-38000 Grenoble, France
f. Univ Grenoble Alpes, F-38000 Grenoble, France
g. CHU de Grenoble, F-38000 Grenoble, France
h. Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, Allergology Department, Paris, France
i. Univ Paris 6 Pierre et Marie Curie, Paris, France
j. ISGlobal- CREAL-Centre for Research in Environmental Epidemiology, Barcelona, Spain
k. CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

Corresponding author:
Emilie Burte
INSERM, U1168, VIMA: Aging and chronic diseases, Epidemiological and Public health approaches, F-94807, Villejuif, France. Phone number: +33 (0) 145 59 50 22, Fax number: 33 (0) 145 59 51 69
E-mail: emilie.burte @inserm.fr

Supplementary material

Respiratory phenotypes

A lung function test with methacholine challenge was performed using a standardized protocol with similar equipment across centers according to the ATS/ERS guidelines (E1). Methacholine challenge was performed unless baseline FEV_1 <80% predicted.

E1. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005 Aug;26(2):319–38.

Table E1: OR with different adjustments of the association between polysensitization (*versus* no or monosensitized) and the 6 groups

OR [95% CI]	**No asthma, no rhinitis**	**NAR alone**	**AR alone**	**Asthma alone(As+)**	**Asthma +NAR**	**Asthma + AR**
crude OR	1 (ref)	1.5[0.8-2.6]	7.8[4.9-12.4]	6.6[3.6-12.1]	5.5[3.1-9.8]	17.3[11.5-26.2]
aOR (on age, sex and education)	1 (ref)	1.6[0.9-2.8]	8.6[5.3-14.0]	6.0[3.2-11.3]	4.8[2.6-8.8]	15.2[9.9-23.3]
aOR (on age, sex, education, childhood life in farm, parental asthma)	1(ref)	1.7[0.9-3.1]	10.6[6.3-17.8]	6.8[3.5-13.1]	4.8[2.5-9.1]	17.2[10.9-27.1]
aOR (on age, sex, occupation, childhood life in farm, parental asthma)	1(ref)	1.7[0.9-3.2]	10.8[6.4-18.1]	7.2[3.7-13.9]	4.7 [2.4-8.9]	17.5[11.0-27.6]

aOR: adjusted Odd Ratio, NAR: non-allergic rhinitis, AR: allergic rhinitis**.**

Table E2: adjusted OR of the association between allergic sensitization to each of the 10 allergen and the 6 groups

	Group	aOR[95%]
Reference	**No asthma, no rhinitis**	**1.0 (reference)**
Cat	NAR	*1.46 [0.72-2.97]*
(n=255 with positive SPT)	AR	3.98 [2.21-7.17]
	Asthma	4.45 [2.15-9.22]
	Asthma+NAR	3.42 [1.65-7.08]
	Asthma+AR	10.49[6.39-17.22]
Cladosporium herbarum **(n=60 with positive SPT)**	NAR	*0.74 [0.23-2.38]*
	AR	*1.48 [0.58-3.77]*
	Asthma	4.12 [1.57-10.81]
	Asthma+NAR	*1.71 [0.52-5.56]*
	Asthma+AR	2.29 [1.09-4.80]
Olive tree **(n=221 with positive SPT)**	NAR	*1.81 [0.85-3.86]*
	AR	7.19 [3.91-13.22]
	Asthma	3.7[1.62 -8.43]
	Asthma+NAR	2.8[1.24 -6.32]
	Asthma+AR	9.32 [5.42-16.02]
Birch **(n=116 with positive SPT)**	NAR	*0.91 [0.28-2.97]*
	AR	3.92 [1.74-8.86]
	Asthma	4.12 [1.54-11.03]
	Asthma+NAR	2.74 [1 -7.54]
	Asthma+AR	6.8 [3.4 -13.57]
Ragweed **(n=66 with positive SPT)**	NAR	NC
	AR	5.34 [2.14-13.33]
	Asthma	*1.44 [0.29-7.13]*
	Asthma+NAR	*1.8[0.45 -7.19]*
	Asthma+AR	5.77 [2.51-13.26]
Dermatophagoides pteronyssinus **(n=393 with positive SPT)**	NAR	*1.32 [0.78-2.24]*
	AR	3.63 [2.3 -5.72]
	Asthma	4.94 [2.72-9.00]
	Asthma+NAR	4.06 [2.3 -7.15]
	Asthma+AR	6.46 [4.41-9.46]
Alternaria tenuis **(n=98 with positive SPT)**	NAR	*1.87 [0.62-5.68]*
	AR	4.78 [1.9-12.03]
	Asthma	9.14 [3.37-24.83]
	Asthma+NAR	*2.97 [0.91-9.69]*
	Asthma+AR	7.42 [3.3-16.69]
Timothy grass **(n=347 with positive SPT)**	NAR	*1.33 [0.71-2.49]*
	AR	8.48 [5.16-13.96]
	Asthma	2.62 [1.29-5.31]
	Asthma+NAR	2.91 [1.52-5.57]
	Asthma+AR	9.94 [6.45-15.33]

Parieteria judaica **(n=35 with positive SPT)**	NAR	*0.69 [0.07-6.68]*
	AR	*3.39 [0.79-14.44]*
	Asthma	NA
	Asthma+NAR	*1.38[0.14-13.59]*
	Asthma+AR	8.03[2.38-27.17]
Cypress **(n=33 with positive SPT)**	NAR	*6.27[0.65-60.88]*
	AR	19.68[2.47-157.14]
	Asthma	NC
	Asthma+NAR	13.24[1.35-130.21]
	Asthma+AR	16.99[2.23-129.27]
aOR: adjusted OR on age, sex, smoking status and educational level, NAR: non-allergic rhinitis, AR: allergic rhinitis, NC: not calculable (sample too small)		