

Letter to the Editor

Interaction between atopy and blood eosinophils in the development of childhood wheeze

To the Editor:

Very recently, a synergistic interaction between atopy and blood eosinophil levels in the prevalence of current childhood asthma was described.¹ This departure of additivity of effects suggests a joint action of both risk factors through at least 1 common causal pathway.¹ However, these cross-sectional data precluded investigating temporality, which is desirable because the involvement of eosinophils in pathogenesis rather than as effector cells in asthma as well as the role of atopy in the development of asthma is not fully understood.^{2,3}

The aim of this analysis was to investigate the interaction between atopy and blood eosinophil levels in childhood wheeze incidence in a prospective, population-based cohort study.

Details of the methods are published in this article's Online Repository at www.jacionline.org. In brief, the baseline assessment was conducted in rural areas around Ulm, southern Germany, among 6- to 10-year-old school children in 2006 as part of the GABRIELA study.⁴ Of the 2248 children with baseline parental questionnaires, 92% completed at least 1 follow-up in the period 2010 to 2012. The ethics committee of Ulm University approved the study.

Atopy was defined as allergen-specific serum IgE against 1 or more of *Dermatophagoides pteronyssinus*, cat dander, rye, timothy grass, mugwort, or common silver birch. Analyses were performed with the cutoffs 0.35 kU/L and 3.5 kU/L. Eosinophil levels were measured with an automatic whole blood cell count and expressed as percentage of leukocyte count. Analyses were performed with levels dichotomized at the median of 2.88% and at the third quartile of 4.84%. To analyze wheeze incidence, the study population was restricted to children without any reports of wheeze or asthma diagnosis ever in life at baseline, also precluding eosinophil levels to be affected by the intake of asthma medication. Incident wheeze was defined as parental reports of wheeze or asthma medication (minimizing underreporting of symptoms due to effective treatment) in the past 12 months in at least 1 follow-up.

Three measures of interaction were calculated by using stratified weighted logistic regression models: (1) the excess risk due to interaction relative to the risk without exposure (RERI), (2) the attributable proportion of disease that is due to interaction among persons with both exposures (AP), and (3) the synergy index (S), which is the excess risk from exposure (to both exposures) when there is interaction relative to the excess risk from exposure (to both exposures) without interaction. In the absence of interaction, RERI and AP are 0 and S is 1; a value greater than this reference indicates synergy, whereas a lower value indicates antagonism. Odds ratios were adjusted for age, sex, familial allergies, parental smoking, parental education, time of blood sampling, and duration of storage until whole blood cell count.

In total, data on atopy, eosinophil levels, and incident wheeze were available for 1096 children whose characteristics are shown in Table I. During the follow-up period, the incidence of wheeze

TABLE I. Characteristics of the study population (n = 1096)*

Baseline variable	n (%)
Age (y), mean (min; max)	8.4 (5.7; 11.5)
Sex (male)	518 (46.8)
Familial allergies	446 (48.0)
Parental smoking	555 (55.8)
High parental education	298 (31.7)
Atopy $\geq$ 0.35 kU/L	311 (32.8)
Atopy $\geq$ 3.5 kU/L	149 (18.0)
Relative blood eosinophil levels (%), median (first quartile; third quartile),	2.88 (1.72; 4.84)
Incident wheeze	78 (8.3)

*In unweighted n (weighted %) unless noted otherwise.

was 8.3%. Atopy as well as eosinophil levels were associated with wheeze incidence: adjusted odds ratio (95% CI) for atopy at 0.35 kU/L and at 3.5 kU/L: 3.91 (2.24-6.84) and 5.34 (2.95-9.65), respectively; for eosinophil levels more than or equal to median and more than or equal to third quartile: 1.74 (0.95-3.19) and 3.07 (1.72-5.48), respectively.

Eosinophil levels were positively associated with atopy ($P_{\text{F test}} < .001$; see Fig E1 in this article's Online Repository at www.jacionline.org). Stratification by atopy and eosinophil levels showed the highest wheeze incidence among atopic children with high eosinophil levels (Fig 1). The interaction (measured by RERI, AP, and S) was not statistically significant at low cutoffs for atopy and eosinophils (Fig 1). Among atopic children, increasing the cutoffs led to slightly decreased wheeze incidence among children with low eosinophil levels but a marked increase among children with high eosinophil levels (see Figs E1 and E3 in the Online Repository at www.jacionline.org). This resulted in statistically significant interaction indices.

In this study, atopy (ie, increased specific serum IgE levels) was clearly associated with increased relative blood eosinophil levels, which most likely reflects the role of both biomarkers in the T_H2 pathway.² On one hand, wheeze occurred in subjects with atopy or high eosinophil levels alone. On the other hand, among nonatopic children, the incidence of wheeze was similar among those with high and low eosinophil levels, suggesting that high eosinophil levels alone cannot drive wheeze development.

There are several reasons why the interaction between atopy and eosinophils was not statistically significant at lower cutoffs of both factors. Cases of incident wheeze were too few to investigate stronger case definitions. The sample size led in particular to uncertain estimates in the low prevalence group of atopic children without high eosinophil levels. This uncertainty was overcome by the marked increase in wheeze incidence among atopic children with high eosinophil levels at higher cutoffs. Furthermore, although blood eosinophil levels are suggested to be useful biomarkers contributing to the characterization of subphenotypes of asthma,³ they poorly predict sputum eosinophil levels.⁵ Similarly, the more sensitive but less specific cutoff of 0.35 kU/L for atopy may result in children without active allergic inflammation to be classified as atopic. In addition, atopy may not be causally related to wheeze even if temporality

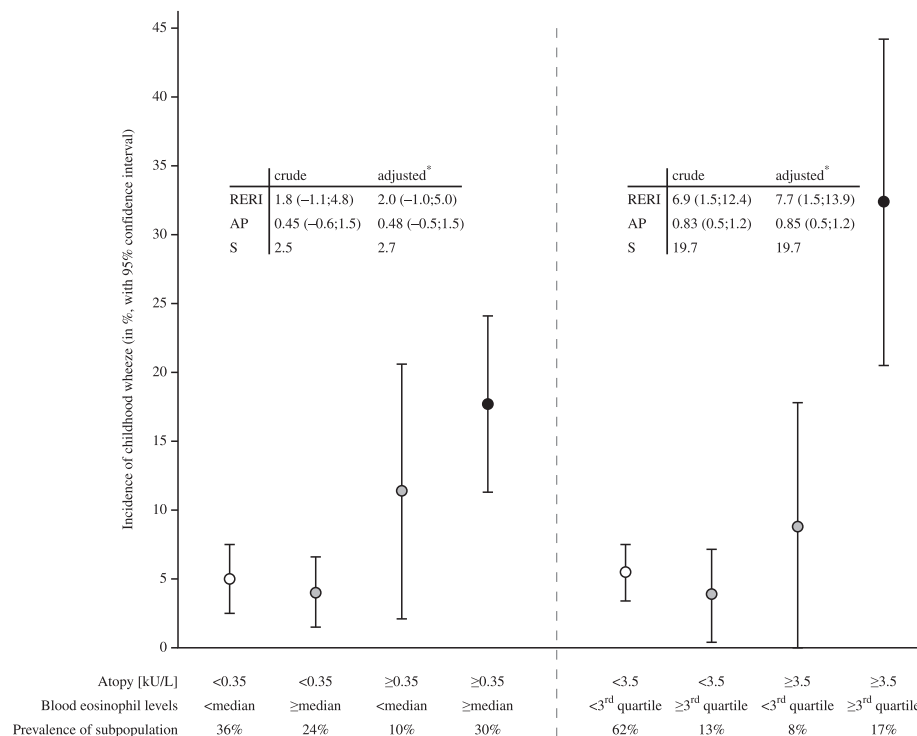


FIG 1. Incidence of wheeze in subpopulations defined by strata of atopy and blood eosinophil levels with measures of interaction. Symbol color indicates low/high atopy and eosinophil indices: *white*, both low; *gray*, either low, the other high; *black*, both high. *Adjusted for baseline age, sex, familial allergies, parental smoking, parental education, time of blood sampling, and duration of storage until whole blood cell count.

is established, which is reflected in population-attributable risk proportions around 60%.⁶

Moreover, there is evidence that suggests that different sensitization patterns drive different phenotypes of wheeze or asthma⁷ and differential effects of eosinophil levels on asthma over time during childhood have been described.⁸ However, atopy and eosinophil levels were assessed only once and for atopy a limited number of allergens were tested, both precluding the exploration of sophisticated patterns. Likewise, because of the exclusion of children who had wheeze or asthma at baseline, analyses were restricted to late-onset wheeze, which has a stronger association with atopy than does early-onset persistent wheeze.⁹ Nonetheless, increased wheeze incidence in both atopic groups—low and high eosinophil levels—would not explain the discrepancy in the interaction measures between asthma at baseline and during follow-up.

In conclusion, the detected synergistic interaction provides evidence that atopy and eosinophils act through a common causal pathway in the pathogenesis of childhood wheeze. Both biomarkers may be useful for predicting and phenotyping childhood wheeze in clinical routine, but special care should be devoted to patterns and levels of atopic sensitization and blood eosinophils.

The baseline assessment was conducted in close collaboration with Prof Erika von Mutius (LMU, Munich, Germany), Prof Charlotte Braun-Fahrlander (Swiss Tropical and Public Health Institute and University of Basel, Switzerland), Prof Elisabeth Horak (Innsbruck Medical University, Austria), and Prof Andrzej Boznanski (Wrocław Medical University, Poland).

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METHODS

Study design and population

A prospective, population-based cohort study was conducted in rural areas around Ulm, southern Germany, with baseline assessment among 6- to 10-year-old school children in 2006 as part of the GABRIELA study. Details of the baseline assessment have been described elsewhere.^{E1}

In brief, the baseline assessment was conducted as a 2-phase population-based survey with a first “screening” phase among 23,040 children (60% participation rate). In this phase, exposure to farming environments and the prevalence of asthma and allergic disease were assessed by using a parental questionnaire. From the 11,169 children with preliminary consent for the in-depth assessment in the second phase, 2,497 children were randomly sampled stratified by categories of exposure to farming environments. Of these, 2,248 (90%) contributed to the study, with a baseline parental questionnaire further assessing the prevalence of asthma and allergic disease. Yearly follow-ups were conducted among these 2,248 children in the period 2010 to 2012, and 92% completed at least 1 follow-up. The ethics committee of Ulm University approved the study.

Exposure definition

At baseline, blood was withdrawn, transported at 4°C to 8°C, and handled in a central laboratory at Ulm University on the same day as fieldwork was conducted.

Serum IgE antibodies against individually tested allergens (*D pteronyssinus*, cat dander, rye, timothy, mugwort, and common silver birch) were measured in one laboratory at the Robert-Koch-Institute, Berlin, Germany (UNICAP 1000, Phadia AB, Uppsala, Sweden; $n = 1988$). Atopy was defined as allergen-specific serum IgE against 1 or more of *D pteronyssinus*, cat dander, rye, timothy, mugwort, or common silver birch. Analyses were performed with the cutoffs 0.35 kU/L and 3.5 kU/L.

An automatic whole blood cell count was performed with ethylenediaminetetraacetic acid (EDTA) blood (Coulter LH750; Beckman Coulter, Inc, Fullerton, Calif; $n = 1897$) on the day of blood sampling. Relative blood eosinophil levels were measured with an automatic whole blood cell count and expressed as percentage of leukocyte count. Analyses were performed with levels dichotomized at the median of 2.88% and at the third quartile of 4.84%.

Outcome definition

To analyze wheeze incidence, the study population was restricted to children without any reports of wheeze or asthma diagnosis ever in life at baseline, also precluding eosinophil levels to be affected by the intake of asthma medication. Incident wheeze was defined as parental reports of wheeze or asthma medication (minimizing underreporting of symptoms due to effective treatment) in the past 12 months in at least 1 follow-up.

Putative confounding variables

All putative confounding variables were determined at baseline. In addition to age and sex, the following variables were considered: Familial allergies were present if either asthma or hay fever or atopic eczema was reported for either parent or a sibling and absent otherwise. Parental education was high if either parent was qualified for university entrance according to the country-specific schooling systems and low otherwise. Parental smoking was present if either parent reported current smoking and absent otherwise.

At baseline, blood withdrawal was conducted between 8:25 AM and 2:36 PM (median at 10:25 AM; first quartile, 9:35 AM; third quartile, 11:20 AM). Duration of storage until the whole blood cell count ranged between 0.7 hours and 10.1 hours, with a median of 3.7 hours (first quartile, 2.8 hours; third quartile, 4.7 hours). Time of blood withdrawal was correlated with the duration of storage: later time of the day led to decreased storage time ($R^2 = 0.29$, $P < .001$). Blood eosinophil levels decreased with increasing time of day at blood withdrawal and increased with increasing time until the whole blood cell count was performed (Fig E2). After mutual adjustment, the effect of storage time was

no longer significant ($P_{\text{F test}} = .72$) whereas the effect of time of day was somewhat attenuated and was marginally significant ($P_{\text{F test}} = .066$). Time of blood withdrawal and duration of storage until whole blood cell count were also considered as confounding variables.

Statistical analyses

At baseline, 13% of the children did neither provide a valid serum sample nor an EDTA sample for whole blood cell count. A further 4% were excluded because of a missing EDTA sample although they had provided a serum sample. In total, data on atopy and blood eosinophil levels were available for 1860 children. After restriction to children without any reports of wheeze or asthma diagnosis ever in life at baseline, 1096 children remained for the analyses of incident wheeze. Subjects who had missing data for all variables used in the respective definition of exposure, outcome, or confounding variables were excluded from the reference group.

To account for the complex survey design with stratified random sampling, the data were analyzed with stratified weighted statistical methods by using the Taylor series method to estimate variances. Percentages were calculated with 95% Wald confidence limits. Differences in blood eosinophil levels across levels of atopy, sampling time, and duration of storage were tested with an F test by using log-transformed data of eosinophil levels. Models were adjusted for all putative confounding variables mentioned above. Further adjustment for exposure to farming environments did not change the observed associations and interactions. Statistical significance was assumed at P values of less than .05 or when 95% CIs excluded the null value.

Three measures of interaction were calculated: (1) the relative excess risk due to interaction (RERI, also called interaction contrast ratio), which is the excess risk due to interaction (also called interaction contrast) relative to the risk without exposure; (2) the attributable proportion of disease that is due to interaction among persons with both exposures (AP); and (3) the synergy index (S), which is the excess risk from exposure (to both exposures) when there is interaction relative to the excess risk from exposure (to both exposures) without interaction. In the absence of interaction, RERI and AP are 0 and S is 1; a value greater than this reference indicates synergy, whereas a lower value indicates antagonism.

It has been argued that biologic interaction is departure from additivity of effects.^{E2} The aforementioned 3 interaction indices reflect departure from additivity. Calculations of the interaction indices were performed by using an alternative parameterization within a stratified weighted logistic regression model suggested by Hosmer and Lemeshow.^{E3} This approach is valid under the assumption that the odds ratio approximates the relative risk, also called “rare disease assumption.” This assumption is met in the present analyses with an outcome incidence of about 5% among the unexposed reference group, that is, nonatopic children with low blood eosinophil levels. For RERI and AP, 95% CIs were calculated.^{E3}

In comparison to the recent publication on the synergistic interaction between atopy and eosinophils in the prevalence of asthma,^{E4} only crude estimates of prevalence and incidence of asthma and wheeze were displayed. Adjusted risks are not always readily interpretable because they relate either to an “average person” who usually does not exist (using the conditional marginal approach) or to a standard population (using the predicted marginal approach) that differs across studies.^{E5-E7} Furthermore, the relative (RERI) rather than the absolute excess risk due to interaction was displayed. The absolute excess risk due to interaction can be obtained by multiplying the RERI with the prevalence or incidence among the nonatopic subjects with low eosinophil levels.

The interpretation of the interaction indices calculated from adjusted logistic regression models may be misleading, partly due to difficulties in interpreting adjusted risks as laid out above.^{E8,E9} Thus, the interaction indices based on crude logistic regression models are also provided.

All statistical analyses were performed with SAS 9.3 (SAS Institute, Cary, NC) and STATA SE 10.1 (StataCorp LP, College Station, Tex.).

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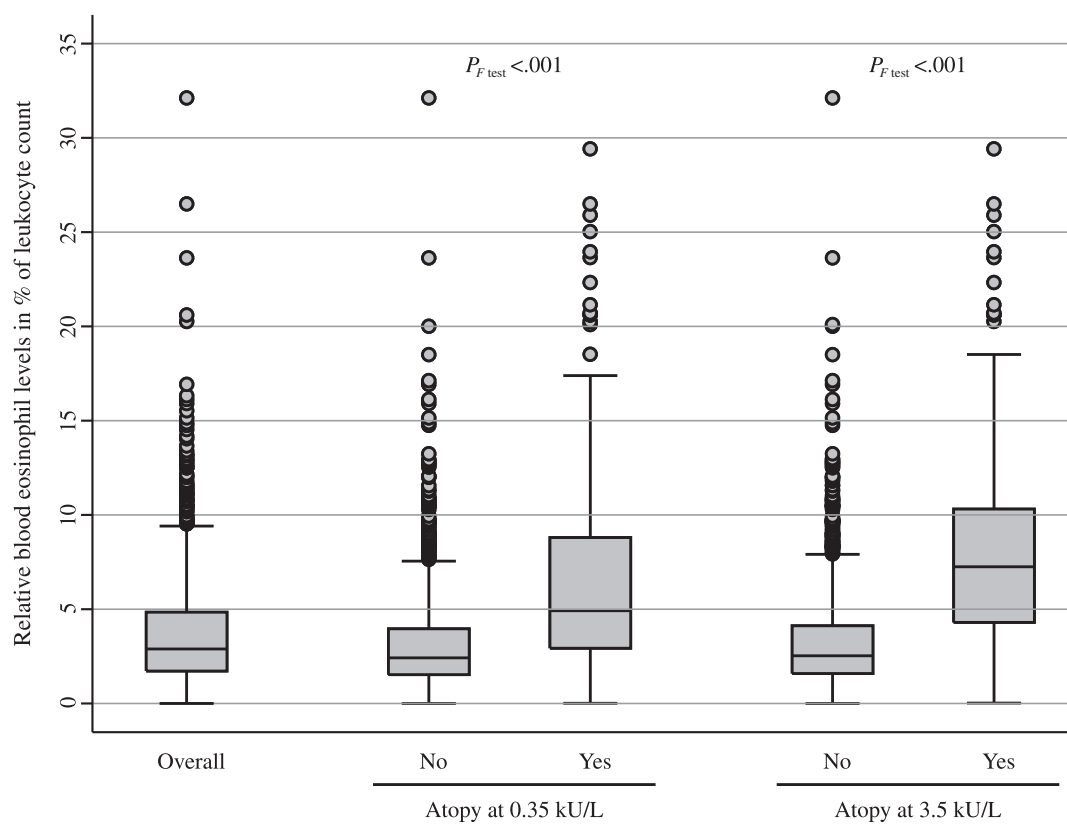


FIG E1. Distribution of relative blood eosinophil levels overall and stratified by atopy.

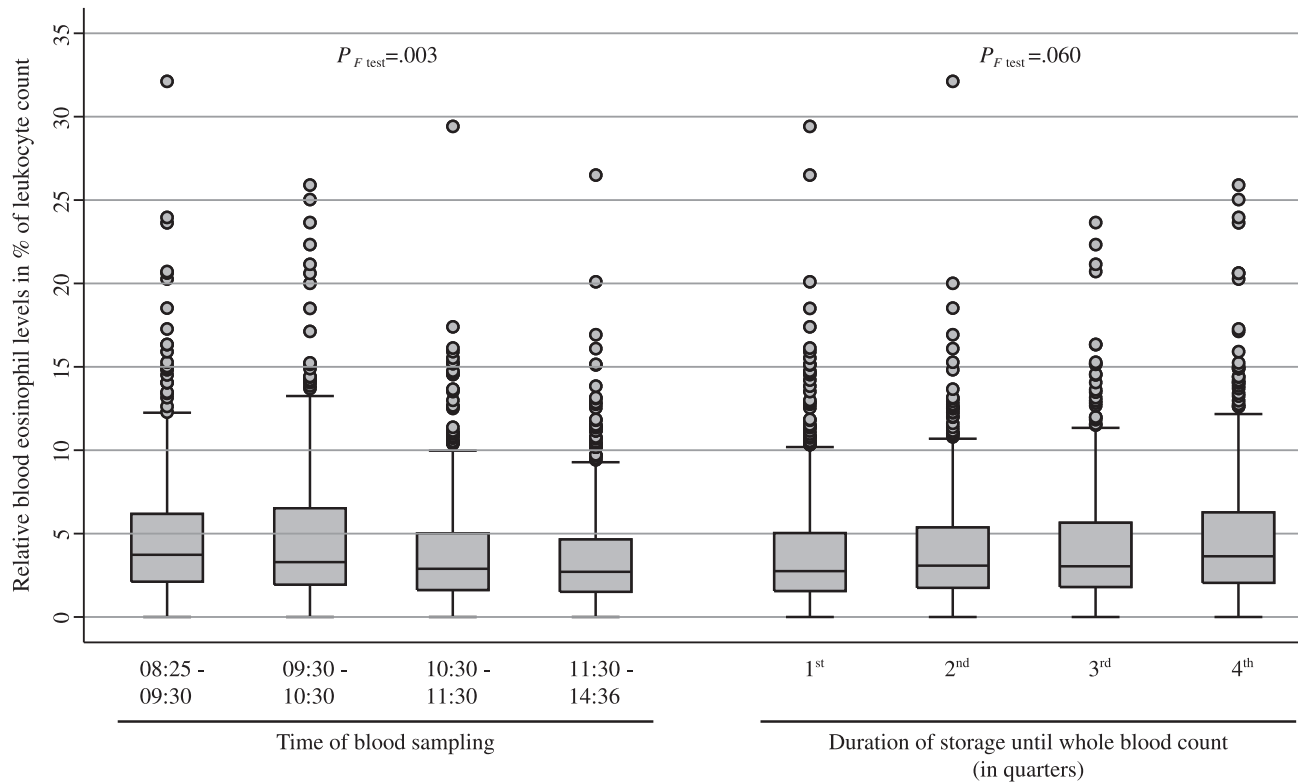


FIG E2. Distribution of relative blood eosinophil levels across time of blood sampling and duration of storage until whole blood cell count.

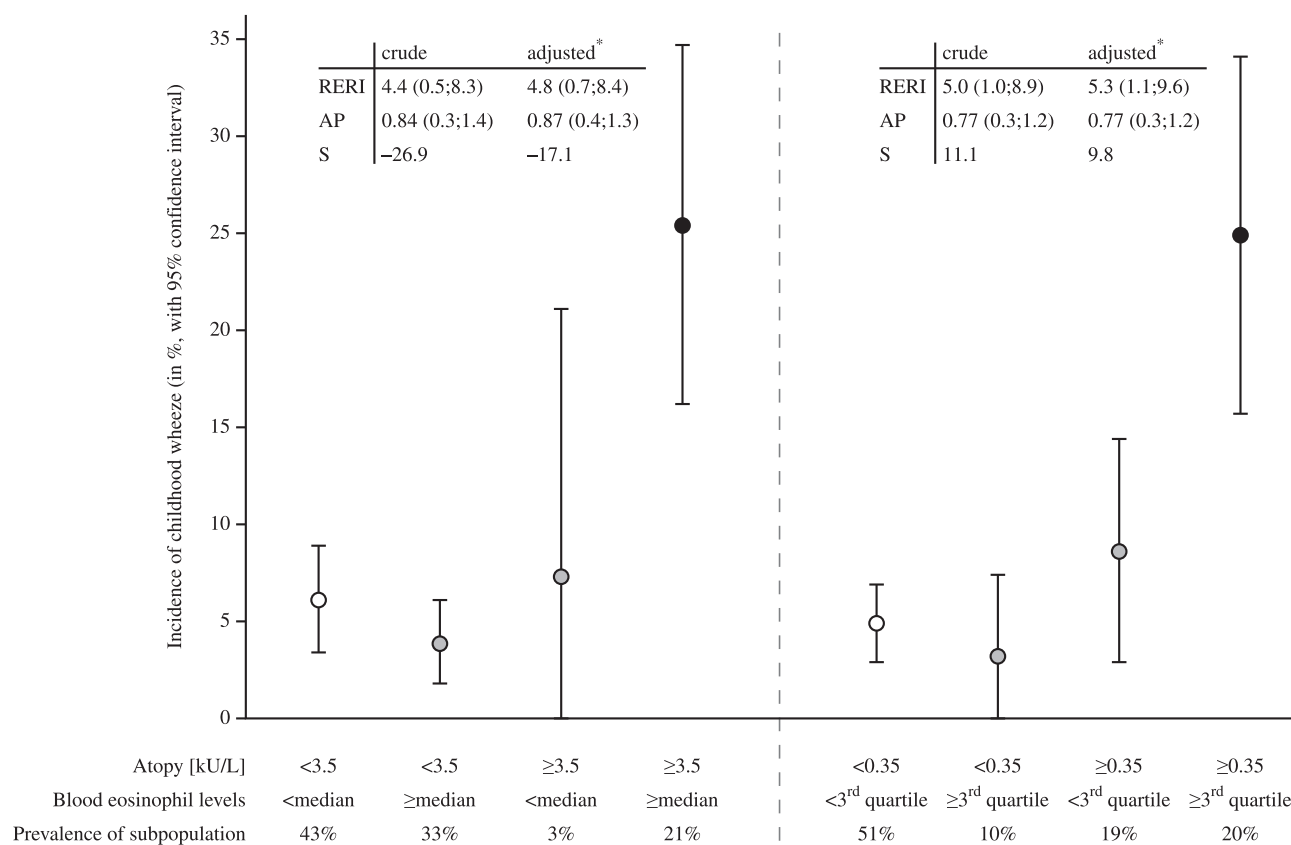


FIG E3. Incidence of wheeze in subpopulations defined by strata of atopy and blood eosinophil levels with measures of interaction. Symbol color indicates low/high atopy and eosinophil indices: *white*, both low; *gray*, either low, the other high; *black*, both high. *Adjusted for baseline age, sex, familial allergies, parental smoking, parental education, time of blood sampling, and duration of storage until whole blood cell count.