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A single-nucleotide polymorphism in intelectin 1 is associated with increased asthma risk

To the Editor:

The susceptibility of individuals to developing asthma is the result of a complex interplay between genetics and environmental trigger factors. The advent of high-throughput genomic technologies has aided the elucidation of polymorphisms in more than 30 genes that have been associated with increased risk of developing asthma (see review¹). Tackling the problem from a different perspective, transcriptomic studies in mouse model systems² have elucidated a number of signature genes associated with pathological responses in asthma and allergy, which can then be studied in more detail as potential "asthma genes."

Kuperman et al² showed that intelectin (Itln)-1 was highly upregulated in murine airways overexpressing IL-13. They also demonstrated that mRNA transcripts for human Itln1 were significantly upregulated in the bronchial brushings of patients with asthma compared with control volunteers.² We have previously demonstrated Itln expression in intestinal goblet cells and shown that Itln1 is highly upregulated in a model human goblet cell line in response to IL-4 or IL-13.³ Intelectins are also strongly expressed in goblet cells in sheep airways,⁴ and therefore we reasoned that human Itln1 might play a role in airway mucus during asthmatic exacerbations. To support this idea, we looked for evidence of baseline intelectin expression in the airways, using induced sputum from asymptomatic patients with asthma (n = 10) compared with control volunteers (n = 10). The samples used were those collected and processed as described in the recently published study by Gray et al.⁵ Using a chicken anti-Itln antibody,⁴ intelectin expression in these samples was investigated by Western blot. A 37-kD intelectin band was detected in 6 of 10 asthmatic sputum samples and in 2 of 10 controls (Fig 1). These bands were absent when the primary antibody was preincubated with excess of the antigenic peptide⁴ (not shown).

Having demonstrated Itln protein expression in airway secretions, we then investigated whether a known single nucleotide missense polymorphism in human *ITLN1* was associated with susceptibility to asthma. The Val109Asp polymorphism (rs2274907) in *ITLN1* exon 4 was genotyped in a large white United Kingdom family cohort (N = 1523, accounting for 342 families^{6,7}) by using KASPar competitive allele-specific PCR (Kbiosciences, Herts, United Kingdom). Association with asthma diagnosis was performed by using the family-based association test (version 1.5.1) under the additive and biallelic model. A *P* ≤ .05 was considered significant. The results showed that the

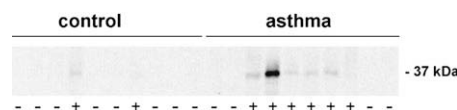


FIG 1. Intelectin detection in induced sputum by Western blotting. Processed sputum (7.5 μ L) from control and asthmatic subjects was separated by reducing SDS-PAGE, blotted to polyvinylidene fluoride membrane, and probed with a chicken anti-intelectin antibody.⁴ Detection was with rabbit antichick peroxidase (Sigma, Poole, United Kingdom) and chemiluminescent peroxidase substrate (Sigma). Lanes are marked negative (–) or positive (+) for Itln detection.

more common T (Val) allele (allele frequency 0.68) was significantly overrepresented in individuals with asthma (*P* = .05).

The Val109Asp single-nucleotide polymorphism (SNP; rs2274907) in *ITLN1* exon 4 was previously investigated by Schaffler et al,⁸ who determined that this SNP was not associated with disease susceptibility in a small study of patients with type 2 diabetes mellitus (n = 114), ulcerative colitis (n = 59), or Crohn disease (n = 131) compared with control subjects (n = 276). However, a much larger meta-analysis (3230 cases and 4829 controls) has recently identified that another SNP (rs2274910), located 220 bp distant and in complete linkage disequilibrium with rs2274907 (*r*² = 1 in CEU HapMap data; <http://www.hapmap.org>) in *ITLN1* intron 3, is associated with risk of Crohn disease.⁹

To conclude, we have found evidence of a possible association between a SNP in *ITLN1* and asthma susceptibility that requires to be confirmed by independent studies. If confirmed, polymorphisms in *ITLN1* would be linked to the increased incidence of both asthma and Crohn disease, as recently described for the gene *ORMDL3*.⁹ Further focused studies are required to investigate this link.

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Allergen challenge dose-response slope

To the Editor:

The dose-response curve for methacholine using doubling doses at 5-minute intervals is, on average, approximately linear; that is, double the dose causes double (or slightly more) the response. We have observed that the dose response to doubling doses of allergen administered at 12-minute intervals is frequently steep. This retrospective review was done to compare the dose-response slopes of the last 2 doses of methacholine and allergen in the same individuals.

We reviewed allergen and methacholine inhalation tests performed in our laboratory over the past 25 years. Allergen inhalation is done by administering doubling concentrations (ie, doses) for 2 minutes of tidal breathing at 12-minute intervals between the start of one and the start of the next concentration.¹ Methacholine is inhaled in doubling concentrations for 2 minutes of tidal breathing at 5-minute intervals.² We selected allergen challenge tests in which the FEV₁ decrease 10 minutes after the last concentration was 19% or more. We examined screening or

placebo (ie, unmedicated) allergen challenges, and for subjects with multiple studies, we selected the first study meeting the criterion to avoid any selection bias. We also examined an unmedicated methacholine challenge done within a 1-week period and also the first we identified with a 19% or more FEV₁ decrease at the last concentration. The primary comparison was the percentage FEV₁ decrease at the second-to-last concentrations of allergen and methacholine and the ratio between the last and second-to-last response; these were compared by using the paired *t* test.³

We identified 54 subjects (32 female subjects; 26.1 ± 7.9 [SD] years old) whose challenges met the selection criteria. Mean FEV₁ was 3.46 ± 0.77 L or $93.7\% \pm 12.8\%$ predicted. The mean FEV₁ decrease at the last concentration was $25.3\% \pm 5.0\%$ and $25.4\% \pm 4.4\%$ for allergen and methacholine, respectively (Fig 1, panels 1 and 2, respectively; $P = .87$). The mean FEV₁ decrease at the second-to-last last concentration of allergen was $8.9\% \pm 3.1\%$, which was significantly lower than that seen for the second-to-last concentration of methacholine (ie, $12.2\% \pm 3.2\%$; Fig 1; $P < .0001$). The ratio of the last to second-to-last response was 3.2 ± 1.5 for allergen compared with 2.3 ± 0.8 for methacholine (Fig 1, $P < .0001$).

These data indicate that the dose-response curve for doubling doses of allergen administered at 12-minute intervals is steeper than that for doubling doses of methacholine administered at 5-minute intervals. The response at the threshold ($\geq 19\%$) was more than 3-fold that of the response at the second-to-last (ie, half) concentration for allergen compared with slightly more than 2-fold for methacholine. The most plausible explanation would be differences in the duration of effect and corresponding differences in the cumulative effect of allergen and methacholine. Methacholine has only a slight cumulative effect when doubling doses/concentrations are administered at 5-minute intervals.⁴ Although we are not aware of comparative data for allergen challenges, the maximal response occurs, on average, at 20 or 30 minutes after

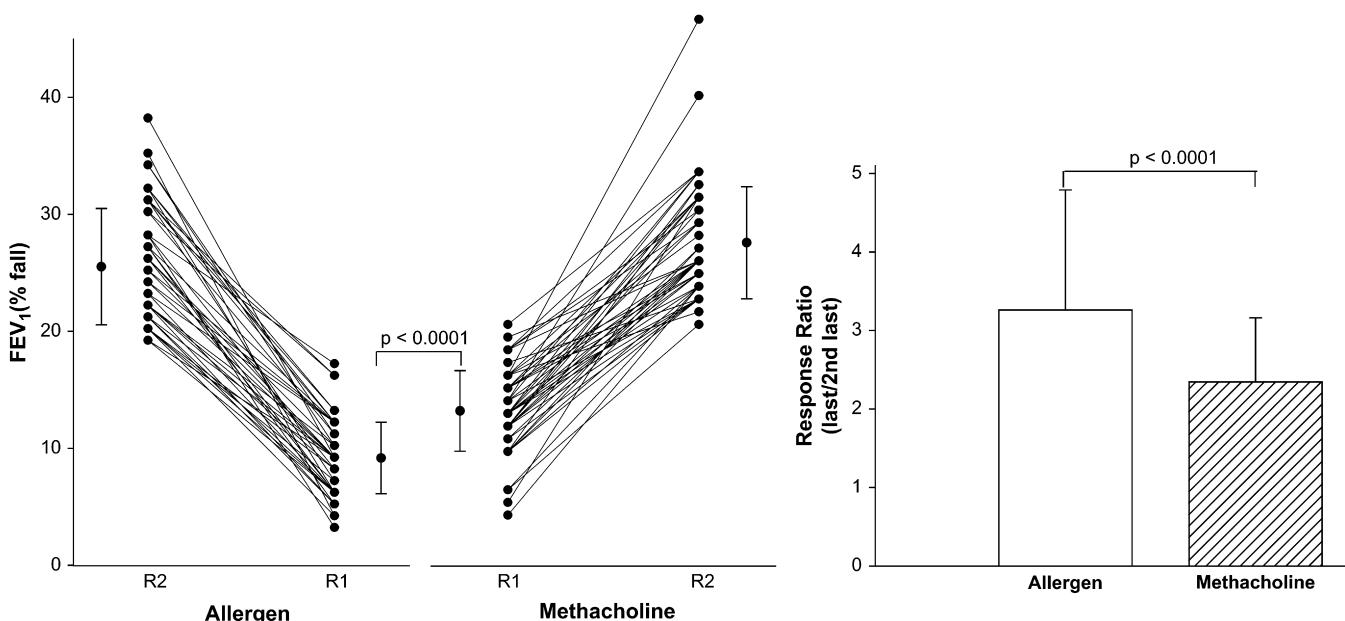


FIG 1. Individual terminal dose-response slopes (with mean and SD) to allergen (right) and methacholine (center). The left panel shows the mean ratios defined as the response to the last concentration (mean approximately 25%) divided by the response to the second-to-last concentration of allergen and methacholine, respectively.