

## Letter to the Editor

**Association of childhood allergic asthma with markers flanking the *IL33* gene in Italian families**

To the Editor:

Two recent, independent, large-scale genome-wide association studies described the association of asthma with single nucleotide polymorphisms (SNPs) mapping on chromosome 9 in a region flanking the *IL33* gene.<sup>1,2</sup> No family-based association study has yet been reported to confirm the association that was found in the case-control studies. We have previously reported the preliminary results of a genome-wide linkage study conducted on a sample of Italian asthmatic families that indicated linkage to the microsatellite marker D9S286, which is located in an extended region of chromosome 9p24 that includes the *IL33* gene.<sup>3,4</sup>

We have now investigated whether the SNPs rs1342326 and rs928413, which showed association in the recent study by Moffatt et al.,<sup>2</sup> are associated with childhood asthma or related phenotypes in the abovementioned Italian families. The SNP rs928413 is in absolute linkage disequilibrium (LD; <http://www.hapmap.org>, rel#24) with rs3939286, which was associated to atopic asthma in the study by Gudbjartsson et al.<sup>1</sup> Therefore this last marker was not genotyped because it is tagged by the marker rs928413.

A family-based association study was performed in 137 family trios (father, mother, and asthmatic child) from northeastern Italy ascertained through an allergic asthmatic child. Details of the enrollment criteria were given elsewhere.<sup>5</sup>

Association analysis was conducted by using the transmission disequilibrium test (TDT) with the computer program PLINK.<sup>6,7</sup> The following phenotypes were investigated: doctor-diagnosed asthma according to the Global Initiative for Asthma criteria,<sup>8</sup> increased total serum IgE levels (defined as a qualitative trait with serum concentrations >200 kU/L for adults and >90th percentile in an age-adjusted standard curve value for subjects aged <10 years<sup>5</sup>), bronchial hyperresponsiveness (BHR) to methacholine, positive skin prick test (SPT) responses to common aeroallergens, positive SPT responses to house dust mites, and positive SPT responses to gramineae. Table I reports the phenotype distribution in asthmatic/atopic children and in their parents.

Genotype data were available for all subjects (parents and offspring), and therefore missing parental genotypes did not represent an issue in this study.

The SNPs rs1342326 and rs928413 were in Hardy-Weinberg equilibrium and LD with each other ( $D' = 0.95$ ,  $R^2 = 0.59$ ). The observed minor allele frequency was 23% (minor allele, G; major allele, T) and 31% (minor allele, G; major allele, A) for rs1342326 and rs928413, respectively.

TDT analysis showed a preferential transmission of the rs928413 G allele to subjects with positive SPT responses (transmitted, 65; not transmitted, 43;  $P = .034$ ) and in particular with positive SPT responses to gramineae (transmitted, 54; not transmitted, 33;  $P = .024$ ) but not with positive SPT responses to house dust mite (transmitted, 41; not transmitted, 33;  $P = .353$ ). No significant association was observed between rs928413 and the other phenotypes or between rs1342326 and any of the phenotypes investigated. Even if the study had a limited dimension that might affect statistical power, we emphasize that

the families were enriched for the genetic risk factor because they were in linkage with the 9p24 marker.<sup>3,4</sup>

A 2-loci haplotype TDT was performed. Table II reports the haplotype frequencies observed. Haplotype rs1342326-rs928413/T-A was the most frequent haplotype observed (Table II). Table III shows the result of the haplotype association test. Haplotype rs1342326-rs928413/T-G was preferentially transmitted to children affected by asthma ( $P = .018$ ), BHR to methacholine ( $P = .006$ ), increased IgE levels ( $P = .022$ ), or positive SPT responses ( $P = .015$ ), particularly positive SPT responses to gramineae ( $P = .006$ ). The TDT performed on 2 loci confirms the association found in the rs928413 analysis and adds the information that the rs1342326-rs928413/T-G haplotype (frequency, 8.8%) is associated to allergic phenotypes. This haplotype can therefore be considered the at-risk haplotype, and it might contain the susceptibility allele for the phenotypes investigated. It is worth noting that haplotype analysis showed a significant association for 5 of the 6 phenotypes, and this is far superior to what would be expected by chance ( $P = .00002$  when considering 5 associated phenotypes of the 6 phenotypes or  $P = .001$  for 5 significant results of 18 tests). It is therefore reasonable to conclude that the excess of associations might indicate the presence of a true association.

These results are therefore in agreement with the previous studies because Moffatt et al.<sup>2</sup> indicated association with the rs938413 allele G and Gudbjartsson et al.<sup>1</sup> with the rs3939286 allele A, which is tagged by the rs928413 allele G. It is noteworthy that we observe a significant association of rs928413 with positive SPT responses but not with asthma, and Gudbjartsson et al.<sup>1</sup> reported the association of rs3939286 with atopic asthma but not with nonatopic asthma. This could suggest that rs928413 (and rs3939286) could be mainly associated with the atopic component of the disease. The association to the most common aeroallergens was in particular because of positive SPT response to gramineae, suggesting that rs928413 might be associated with the atopic component of seasonal epidemic asthma. We could not exclude that association with positive SPT responses is independent from asthmatic status because all the children were asthmatic.

The 2 studied SNPs map in an intergenic region, and the closest gene is *IL33*; the 5' side is located in an LD block containing the SNP rs928413 (<http://www.hapmap.org>, rel#24). No significant association was observed between genetic markers and total serum IgE levels by using the family-based association test statistic.

We can speculate that SNP rs928413 might be in LD with variants affecting gene expression. For its characteristics and functions, *IL33* represents an attractive candidate gene for the association with allergic asthma-related traits. The *IL33* gene, a member of the IL-1 family, is an inducer of  $T_H2$  cytokine immunity and systemic inflammation through a complex including the membrane-bound ST2 protein. Binding of IL-33 to its receptor, which is present on mast cells, basophils, eosinophils, natural killer cells, and natural killer T cells, triggers the release of several proinflammatory mediators, induces systemic  $T_H2$ -type inflammation *in vivo*, and contributes to allergen-induced airway inflammation and hyperresponsiveness.<sup>9</sup>

In conclusion, the results of this family-based association study in an Italian population extends the previously described asthma

**TABLE I.** Phenotype distribution in asthmatic/atopic children and their parents

| Phenotypes            | Subjects |                | Parents |                | Children |                |
|-----------------------|----------|----------------|---------|----------------|----------|----------------|
|                       | No.      | Prevalence (%) | No.     | Prevalence (%) | No.      | Prevalence (%) |
| Asthma                | 409      | 43             | 272     | 14.3           | 137      | 100            |
| BHR <sup>+</sup>      | 391      | 36.6           | 266     | 18.2           | 125      | 81.6           |
| IgE <sup>+</sup>      | 347      | 58.5           | 232     | 41.8           | 115      | 92.2           |
| SPT <sup>+</sup>      | 408      | 59.8           | 272     | 40.8           | 136      | 97.8           |
| SPT <sup>+</sup> -GMN | 408      | 41.2           | 272     | 22.4           | 136      | 78.7           |
| SPT <sup>+</sup> -HDM | 408      | 37.3           | 272     | 21.3           | 136      | 69.1           |

Clinical data were not available for all the participants.

BHR<sup>+</sup>, Presence of bronchial hyperresponsiveness to methacholine; IgE<sup>+</sup>, increased total IgE levels; Parents, subjects without any parent in the family structure;

Prevalence, number of subjects with the phenotype on the total number of subjects characterized for the phenotype; SPT<sup>+</sup>, positive SPT response; SPT<sup>+</sup>-GMN, positive SPT response to gramineae; SPT<sup>+</sup>-HDM, positive SPT response to house dust mite; Subjects, total number of subjects phenotyped in the sample.

**TABLE II.** Haplotype frequencies reconstructed on chromosomes of parents

| rs1342326-rs928413<br>haplotype | Frequency expected<br>under linkage<br>equilibrium | Observed<br>frequencies |
|---------------------------------|----------------------------------------------------|-------------------------|
| T-A                             | 0.547                                              | 0.693                   |
| G-G                             | 0.066                                              | 0.211                   |
| T-G                             | 0.234                                              | 0.088                   |
| G-A                             | 0.153                                              | 0.008                   |

association with 9p24 markers to allergic childhood asthma traits, which is in agreement with the suggested function of *IL33* “as an endogenous alarmin...to alert the immune system of tissue injury or infection.”<sup>10</sup>

Future studies will be necessary to define the role of this region in the regulation of expression of the *IL33* gene or to identify as yet unknown allergic asthma susceptibility genes.

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Supported by the Italian Ministry of University and Research.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

**TABLE III.** Haplotype association with allergic phenotypes

| Phenotype             | Haplotype<br>rs1342326rs928413 | T/NT             | $\chi^2$ Test | P value     |
|-----------------------|--------------------------------|------------------|---------------|-------------|
| Asthma                | G-G                            | 48.5/43.5        | 0.172         | .678        |
|                       | <b>T-G</b>                     | <b>28.5/12.5</b> | <b>5.625</b>  | <b>.018</b> |
|                       | T-A                            | 46.5/62.5        | 2.867         | .091        |
| BHR <sup>+</sup>      | G-G                            | 33.5/35.5        | 0.058         | .810        |
|                       | <b>T-G</b>                     | <b>22.5/7.5</b>  | <b>7.500</b>  | <b>.006</b> |
|                       | T-A                            | 35.5/47.5        | 1.735         | .188        |
| IgE <sup>+</sup>      | G-G                            | 28.5/31.5        | 0.150         | .699        |
|                       | <b>T-G</b>                     | <b>22.5/9.5</b>  | <b>5.281</b>  | <b>.022</b> |
|                       | T-A                            | 35.5/45.5        | 1.235         | .267        |
| SPT <sup>+</sup>      | G-G                            | 47.5/37.5        | 0.405         | .525        |
|                       | <b>T-G</b>                     | <b>26.5/11.5</b> | <b>5.921</b>  | <b>.015</b> |
|                       | T-A                            | 43.5/63.5        | 0.017         | .897        |
| SPT <sup>+</sup> -GMN | G-G                            | 38.5/33.5        | 0.347         | .556        |
|                       | <b>T-G</b>                     | <b>22.5/7.5</b>  | <b>7.500</b>  | <b>.006</b> |
|                       | T-A                            | 33.5/52.5        | 4.198         | .040        |
| SPT <sup>+</sup> -HDM | G-G                            | 29.5/30.5        | 0.172         | .678        |
|                       | T-G                            | 17.5/8.5         | 3.115         | .077        |
|                       | T-A                            | 33.5/41.5        | 0.85          | .356        |

The TDT test was used in the families. Haplotypes showing a significant excess of transmission to affected children are shown in boldface.

NT, Number of times the haplotype was not transmitted; T, number of times the haplotype was transmitted.

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doi:10.1016/j.jaci.2011.05.004