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## Effect of 17q21 Variants and Smoking Exposure in Early-Onset Asthma

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### ABSTRACT

#### BACKGROUND

A genomewide association study has shown an association between variants at chromosome 17q21 and an increased risk of asthma. To elucidate the relationship between this locus and disease, we examined a large, family-based data set that included extensive phenotypic and environmental data from the Epidemiological Study on the Genetics and Environment of Asthma.

#### METHODS

We tested 36 single-nucleotide polymorphisms (SNPs) in the 17q21 region in 1511 subjects from 372 families for an association with asthma. We also tested for genetic heterogeneity according to the age at the onset of asthma and exposure to environmental tobacco smoke in early life.

#### RESULTS

Eleven SNPs were significantly associated with asthma ( $P < 0.01$ ), of which three (rs8069176, rs2305480, and rs4795400) were strongly associated ( $P < 0.001$ ). Ordered-subset regression analysis led us to select an onset at 4 years of age or younger to classify patients as having early-onset asthma. Association with early-onset asthma was highly significant ( $P < 10^{-5}$  for four SNPs), whereas no association was found with late-onset asthma. With respect to exposure to environmental tobacco smoke in early life, we observed a significant association with early-onset asthma only in exposed subjects ( $P < 5 \times 10^{-5}$  for six SNPs). Under the best-fitting recessive model, homozygous status (GG) at the most strongly associated SNP (rs8069176) conferred an increase in risk by a factor of 2.9, as compared with other genotypes (AG and AA) in the group exposed to environmental tobacco smoke ( $P = 2.8 \times 10^{-6}$ ;  $P = 0.006$  for the test for heterogeneity of the SNP effect on early-onset asthma between groups with tobacco exposure and those without such exposure).

#### CONCLUSIONS

This study shows that the increased risk of asthma conferred by 17q21 genetic variants is restricted to early-onset asthma and that the risk is further increased by early-life exposure to environmental tobacco smoke. These findings provide a greater understanding of the functional role of the 17q21 variants in the pathophysiology of asthma.

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A CONSENSUS IS EMERGING THAT ASTHMA is not a single disease but rather a collection of separate entities with variable expression over the life span. Although various asthma phenotypes have been identified with the use of clinical criteria,<sup>1</sup> little is known about their cause. The identification of their determinants is an important step toward understanding the pathophysiology of asthma. One of the simplest criteria that can be used to differentiate asthma phenotypes is the age at onset. Longitudinal studies have shown that phenotypic features correlate with the age at the onset of asthma in children and adults<sup>2-5</sup> and that asthma-associated phenotypes vary over time.

A genomewide association study of asthma in children identified genetic variants, called single-nucleotide polymorphisms (SNPs), on chromosome 17q21 that are associated with the risk of disease and that regulate the expression of at least one nearby gene, *ORMDL3*.<sup>6</sup> This disease association has been replicated in several studies conducted mainly among children,<sup>7-10</sup> but it is not known whether these variants influence the occurrence of asthma in an age-specific manner. More recently, these genetic variants have been shown to be associated with the expression of *GSDML* (also called *GSDMB*), a second gene from the region (Cookson W: personal communication), and with an increased risk of Crohn's disease.<sup>11</sup>

Environmental factors play a substantial role in the development of asthma, in some instances with the greatest effect at a specific developmental stage. Among these factors, environmental exposure to tobacco smoke in early life has been shown to interact with genetic susceptibility to asthma.<sup>12-17</sup> In a genomewide linkage analysis conducted as part of the Epidemiological Study on the Genetics and Environment of Asthma (EGEA),<sup>14</sup> we showed that markers in the 17q21 region were linked to asthma susceptibility in the presence of exposure to environmental tobacco smoke.<sup>14</sup>

The large number of subjects in the EGEA family-based data set, which includes clinically ascertained cases of asthma in adults and children, makes it possible to test for associations with disease onset at a wide range of ages. The study also provides extensive data on environmental factors and asthma-related phenotypes. We used the EGEA data set to determine the association between 17q21 variants and the age at the onset of asthma and interaction between this association

and exposure to environmental tobacco smoke in early life. We also tested for an association between these variants and major asthma-associated phenotypes related to atopy, inflammation, and lung function.

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## METHODS

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### STUDY POPULATION

The protocol for data collection in the EGEA has been described in detail previously.<sup>18</sup> The sample included 1621 subjects in 388 nuclear families who were recruited through probands with asthma identified in seven clinical centers in five French cities. The ages of the subjects at the time of the study ranged from 7 to 65 years. Probands and their first-degree relatives responded to a questionnaire that was based on international standardized tools to diagnose asthma and to determine respiratory and allergic symptoms, treatments, and environmental exposures.<sup>18</sup> Information on age at the onset of symptoms of asthma and exposure to tobacco smoke in early life was obtained from adults with asthma and from parents of children with asthma. The sample included 651 patients with asthma, with an age at onset ranging from less than 1 year to 57 years. Information about exposure to environmental tobacco smoke in early life was known for 98% of family members. We also examined four main asthma-related phenotypes — atopy (defined as at least one positive response to 11 allergens on a skin-prick test), total IgE levels, blood eosinophil counts, and the percentage of the predicted forced expiratory volume in 1 second (FEV<sub>1</sub>) — according to age, sex, and height (for details regarding phenotypes, see the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)). We obtained written informed consent from all study subjects or their parents.

### GENOTYPING

DNA was available from 1543 of the 1621 subjects enrolled in the EGEA. We genotyped 38 SNPs located between 35.23 and 35.38 Mb on chromosome 17q21, including the key SNPs previously associated with asthma, along with other SNPs present in the region of strongest association (for details regarding genotyping, see the Supplementary Appendix).<sup>6</sup> Genotyping was performed on an ABI7900HT Sequence Detection System with the use of TaqMan assays (Applied Biosystems).

After applying quality-control procedures, we retained 36 SNPs genotyped in 1511 subjects from 372 families for analysis.

#### STATISTICAL ANALYSIS

We tested for an association between SNPs and asthma with a likelihood-based method,<sup>19,20</sup> implemented in the Linkage and Association Modeling in Pedigrees (LAMP) program for analysis of families of arbitrary size and mode of selection ([www.sph.umich.edu/csg/abecasis/lamp](http://www.sph.umich.edu/csg/abecasis/lamp)). This approach provides a powerful test of association with family structures similar to those of study families.<sup>20</sup> The association was evaluated with the use of a likelihood-ratio test statistic, which compares the null hypothesis of an absence of association and linkage to the alternative of association and linkage. Asymptotically, the likelihood-ratio test statistic is distributed as a chi-square with 2 degrees of freedom under a general model for SNP effect and a chi-square with 1 degree of freedom when a specific genetic model is assumed.<sup>19,20</sup> We evaluated the strength of the association (SNP effect size) under the best-fitting genetic model (recessive), using the ratio of estimates of penetrance for the homozygous risk-allele genotype to penetrance for other genotypes.

To investigate genetic heterogeneity according to the age at disease onset and to identify a cutoff point for classification into an early-onset group and a late-onset group, we performed ordered-subset regression. Subjects with asthma were ranked on the basis of increasing age at the first onset of symptoms, and logistic regression of asthma status on SNPs was performed in each ordered age-specific subset. We identified the subset for which the difference of the likelihood-ratio test statistics for association between the age-specific subsets and the total sample was maximal. The significance of the maximal difference in test statistics for association was assessed by permutation. Since this analysis provided evidence of heterogeneity according to the age at onset, we compared results in the early-onset group and the late-onset group by using LAMP and examining disease in one age-at-onset category, with all subjects in the other category being assigned an unknown disease status. To formally test for heterogeneity of the SNP effect according to the age at onset, we considered two independent samples of families, with all affected subjects in the offspring generation belonging to a single age-at-onset category.

To investigate genetic heterogeneity according to early-life exposure to environmental tobacco smoke, we grouped families according to the exposure status of the offspring. All offspring in a given family were concordant with respect to exposure status in 91.2% of the families, and we assumed that the disease status of the parents and their early-life exposure to environmental tobacco smoke were unknown. The significance of the heterogeneity of association between sibships with tobacco exposure and those without such exposure was assessed with the use of the likelihood-ratio test from LAMP. P values were determined by random permutation of the exposure status of sibships among those having the same number of affected siblings; the distribution of the number of siblings was similar in sibships with exposure to tobacco smoke and those without such exposure (10,000 replicates). In the above analyses, we used phenotype and exposure information from the offspring's generation only. We performed a second test of association between early-onset asthma and 17q21 SNPs in parents who were exposed to environmental tobacco smoke early in life and in those who did not have such exposure, using Fisher's exact test. The association between 17q21 markers and asthma-related phenotypes was investigated by means of regression analysis with the use of Stata software, version 10.0 (see the Methods section in the Supplementary Appendix).

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## RESULTS

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#### 17q21 VARIANTS AND ASTHMA

The characteristics of the 1511 genotyped subjects belonging to the 372 EGEA families are presented in Table 1. When we analyzed the data without stratification according to the age at the onset of asthma, we found that 11 of the 17q21 SNPs were significantly associated with asthma ( $P < 0.01$ ). Of these SNPs, three (rs8069176, rs2305480, and rs4795400) were strongly associated ( $P < 0.001$ ) (Table 2, and Table 2 in the Supplementary Appendix). These three SNPs were among those with the most significant association ( $P \leq 2.5 \times 10^{-8}$ ) in the initial genomewide association study.<sup>6</sup>

#### 17q21 VARIANTS AND AGE AT ONSET OF ASTHMA

Ordered-subset regression analysis, performed for the 11 SNPs significantly associated with asthma, showed that the maximal difference in test statistics for association between the ordered age-spe-

**Table 1. Characteristics of 1511 Genotyped Subjects from 372 Families.\***

| Variable  | All Subjects<br>(N=1511) | Parents<br>(N=708) | Offspring<br>(N=803) |
|---|--------------------------|--------------------|----------------------|
| Male sex — no. (%)  | 776 (51.4)               | 349 (49.3)         | 427 (53.2)           |
| Age — yr  |                          |                    |                      |
| Median  | 31.1                     | 44.5               | 14.5                 |
| Interquartile range   | 13.7–43.8                | 39.7–50.5          | 10.4–21.8            |
| Asthma — no. (%)  | 651 (43.1)               | 224 (31.6)         | 427 (53.2)           |
| Age at onset of asthma — yr                                     |                          |                    |                      |
| Median  | 7                        | 25                 | 4                    |
| Interquartile range   | 3–18                     | 7–38               | 2–9                  |
| Exposure to environmental tobacco smoke in early life — no. (%) | 941 (62.3)               | 491 (69.4)         | 450 (56.0)           |
| Atopy — no. (%)   | 812 (53.7)               | 298 (42.1)         | 514 (64.0)           |
| Log <sub>10</sub> total IgE — IU/ml                             |                          |                    |                      |
| Median  | 2.1                      | 1.8                | 2.3                  |
| Interquartile range   | 1.5–2.5                  | 1.3–2.3            | 1.7–2.7              |
| Log <sub>10</sub> eosinophil count — cells/mm <sup>3</sup>      |                          |                    |                      |
| Median  | 2.3                      | 2.2                | 2.4                  |
| Interquartile range   | 2.0–2.5                  | 2.0–2.4            | 2.1–2.7              |
| FEV <sub>1</sub> — % of predicted value                         |                          |                    |                      |
| Median  | 99.7                     | 103.9              | 95.6                 |
| Interquartile range   | 89.8–109.6               | 91.5–113.4         | 88.8–105.1           |

\* FEV<sub>1</sub> denotes forced expiratory volume in 1 second.

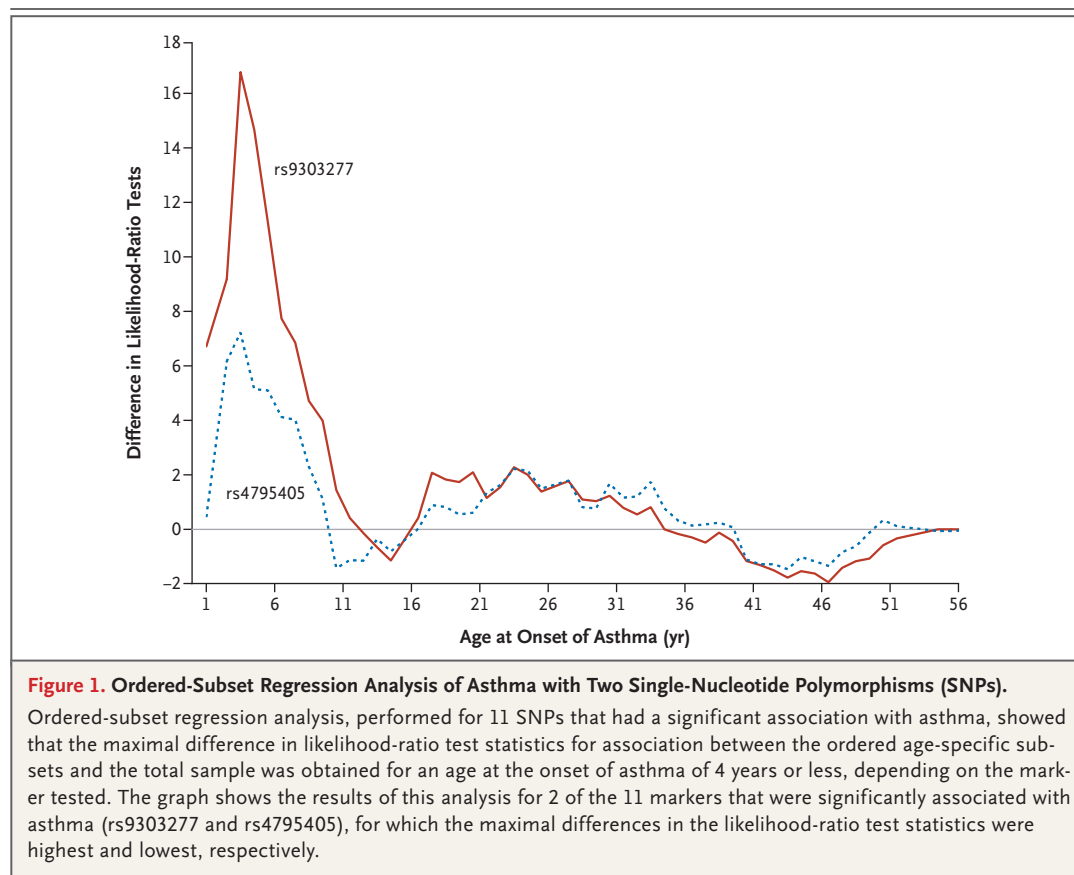
cific subsets and the total sample was obtained at an age at onset of 4 years or younger, depending on the marker tested (Fig. 1). On the basis of 10,000 permutations, the maximal difference in test statistics for association was found to be significant for all 11 markers (empirical P values ranging from  $1 \times 10^{-4}$  for rs9303277 to 0.02 for rs4795405). We then used 4 years of age as the cutoff point to assign subjects to early-onset and late-onset subgroups.

The early-onset subgroup included 235 subjects with asthma among 1270 subjects, and the late-onset subgroup included 395 subjects with asthma among 1282 subjects. The association between the SNPs and early-onset asthma was found to be much stronger than that for asthma as a whole (Table 2). We obtained  $P \leq 7 \times 10^{-4}$  for the 11 SNPs and  $P < 1 \times 10^{-5}$  for 4 of the SNPs, with the strongest associations at rs9303277 ( $P = 3.1 \times 10^{-6}$ ) and rs8069176 ( $P = 5.8 \times 10^{-6}$ ). These results remained significant ( $P < 1 \times 10^{-3}$  for the four most strongly associated SNPs) after a conservative adjustment with the Bonferroni correction for the 108 tests

conducted (36 SNPs tested in the early-onset subgroup, the late-onset subgroup, and the total group). In contrast, we found no significant association with asthma when the onset was after 4 years of age (Table 2). Tests for heterogeneity of the association with asthma between two independent family samples (152 families with early-onset asthma and 175 families with late-onset asthma) were significant for 10 of the 11 asthma-associated SNPs (Table 2).

#### EARLY EXPOSURE TO TOBACCO SMOKE

We next examined the influence of exposure to environmental tobacco smoke in early life. Initially, we used phenotype and exposure data for offspring only. There were 179 families in which all offspring had exposure to environmental tobacco smoke and 130 families in which all offspring did not have such exposure. Evidence for an association between early-onset asthma and all SNPs that previously showed an association with disease was highly significant in the smoke-exposed families (Table 3). The strongest asso-



ciations were observed at rs8069176 ( $P=2.8 \times 10^{-6}$ ) and rs2305480 ( $P=8.7 \times 10^{-6}$ ). Conversely, the associations were not significant in the unexposed families ( $P \geq 0.02$ ) (Table 3). The patterns of heterogeneity of association according to exposure status were similar for all 11 SNPs and were significant on the basis of permutation tests for 8 SNPs (Table 3).

When smoking exposure was not taken into account, the alleles associated with an increased risk of asthma were identical to those initially reported.<sup>6</sup> Under the best-fitting recessive model (Table 3 in the Supplementary Appendix), the overall risk of early-onset asthma when smoking exposure was not taken into account was increased by a factor of 1.7 or more for subjects who were homozygous for the asthma-associated alleles, as compared with those with other genotypes, whereas the risk was increased by a factor of 2.3 or more for homozygous subjects with early-life exposure to environmental tobacco smoke. Moreover, for SNPs showing significant heterogeneity ( $P \leq 0.01$ ) with respect to exposure to environmental tobacco

smoke in early life, the ratio of the SNP effect on early-onset asthma was 1.9 or more in subjects with early exposure, as compared with those who did not have such exposure (Table 3).

We sought confirmation that the association between 17q21 variants and early-onset asthma was largely restricted to subjects who were exposed to environmental tobacco smoke by examining data from the parental generation (Table 4 in the Supplementary Appendix). The EGEA families included 475 parents with known asthma status who were exposed to tobacco smoke in early childhood (24 with early-onset asthma, 321 without asthma, and 130 with late-onset asthma) and 195 parents without early exposure (13 with early-onset asthma, 131 without asthma, and 51 with late-onset asthma). Among parents who were exposed to environmental tobacco smoke, early-onset asthma was significantly associated with four SNPs ( $P \leq 0.01$ ). In contrast, there was no evidence of an association among parents without such exposure ( $P \geq 0.11$ ).

The SNPs that were most strongly associated

**Table 2.** Association between 11 Single-Nucleotide Polymorphisms on Chromosome 17q21 and Asthma, Early-Onset Asthma, and Late-Onset Asthma.\*

| Gene and SNP            | Position | P Value for Total Subjects† |                             |                            | P Value for Independent Samples for Test of Heterogeneity‡ |                           | P Value for Test for Heterogeneity of SNP Effect, According to Age at Onset§ |
|-------------------------|----------|-----------------------------|-----------------------------|----------------------------|--|---------------------------|--|
|                         |          | Asthma (N=1511)             | Early-Onset Asthma (N=1270) | Late-Onset Asthma (N=1282) | Early-Onset Asthma (N=584)                                 | Late-Onset Asthma (N=668) |  |
| <i>Mb</i>               |          |                             |                             |                            |  |                           |  |
| <i>IKZF3</i>            |          |                             |                             |                            |  |                           |  |
| rs9303277               | 35.230   | 0.002                       | 3.1×10 <sup>-6</sup>        | 0.06                       | 1.0×10 <sup>-4</sup>                                       | 0.34                      | 1.9×10 <sup>-4</sup>   |
| <i>ZBP2</i>             |          |                             |                             |                            |  |                           |  |
| rs11557467              | 35.282   | 0.004                       | 2.0×10 <sup>-5</sup>        | 0.12                       | 6.5×10 <sup>-4</sup>                                       | 0.30                      | 0.001  |
| Intergenic region       |          |                             |                             |                            |  |                           |  |
| rs8069176               | 35.311   | 2.1×10 <sup>-4</sup>        | 5.8×10 <sup>-6</sup>        | 0.07                       | 1.6×10 <sup>-4</sup>                                       | 0.25                      | 0.002  |
| <i>GSDML (or GSDMB)</i> |          |                             |                             |                            |  |                           |  |
| rs2305480               | 35.316   | 1.4×10 <sup>-4</sup>        | 9.3×10 <sup>-6</sup>        | 0.02                       | 2.4×10 <sup>-4</sup>                                       | 0.08                      | 0.001  |
| rs2305479               | 35.316   | 0.005                       | 6.6×10 <sup>-6</sup>        | 0.22                       | 1.3×10 <sup>-4</sup>                                       | 0.38                      | 3.4×10 <sup>-4</sup>   |
| rs4795400               | 35.321   | 9.4×10 <sup>-4</sup>        | 3.0×10 <sup>-4</sup>        | 0.06                       | 0.004  | 0.20                      | 0.02   |
| rs9303281               | 35.328   | 0.005                       | 4.4×10 <sup>-5</sup>        | 0.18                       | 0.001  | 0.23                      | 0.004  |
| rs7219923               | 35.328   | 0.009                       | 9.7×10 <sup>-5</sup>        | 0.17                       | 0.003  | 0.30                      | 0.006  |
| <i>ORMDL3</i>           |          |                             |                             |                            |  |                           |  |
| rs8076131               | 35.334   | 0.003                       | 1.0×10 <sup>-4</sup>        | 0.03                       | 0.002  | 0.18                      | 0.003  |
| Intergenic region       |          |                             |                             |                            |  |                           |  |
| rs4795405               | 35.342   | 0.002                       | 3.2×10 <sup>-4</sup>        | 0.05                       | 0.02   | 0.21                      | 0.05   |
| rs4794820               | 35.343   | 0.002                       | 7.4×10 <sup>-4</sup>        | 0.08                       | 0.03   | 0.19                      | 0.11   |

\* The 11 single-nucleotide polymorphisms (SNPs) listed are from the panel of 36 SNPs at the 17q21 locus that had a significant association with asthma ( $P < 0.01$ ) in the total sample of 1511 genotyped subjects. Early-onset asthma and late-onset asthma were defined by using a cutoff point of 4 years of age (determined from preliminary analysis). In the analysis of early-onset asthma, subjects with late-onset asthma were assigned an unknown disease status, and vice versa. P values in this table have not been corrected for multiple testing.

† P values are based on two-sided likelihood-ratio tests with 2 degrees of freedom under a general codominant model for the SNP effect using the Linkage and Association Modeling in Pedigrees (LAMP) program.

‡ Independent samples for the test of heterogeneity included only families in which all affected subjects in the offspring generation belonged to a single age-of-onset category.

§ P values for the test of heterogeneity of the SNP effect between early-onset and late-onset samples are based on likelihood-ratio tests with 3 degrees of freedom.

with early-onset asthma were in strong linkage disequilibrium with one another, with pairwise linkage disequilibrium coefficient  $D'$  between 0.87 and 1.0. We also observed weaker evidence of disease association with other markers from the region (Table 5 in the Supplementary Appendix) that were in moderate linkage disequilibrium with these SNPs (Fig. 1 in the Supplementary Appendix). However, when a forward stepwise regression was applied, only the SNP with the most significant disease association (rs8069176) entered the model, suggesting that linkage disequilibrium between markers accounted for these observations.

#### ASTHMA-RELATED PHENOTYPES

Analysis of atopy, IgE levels, eosinophil counts, and FEV<sub>1</sub> did not reveal any significant association with the SNPs that we investigated. The results of the analysis are shown in Table 6 in the Supplementary Appendix.

#### DISCUSSION

Our study confirms the association between 17q21 markers and asthma and shows that these markers confer susceptibility specifically to early-onset asthma, thus supporting the hypothesis that asth-

**Table 3.** Analysis of the Association between 11 Single-Nucleotide Polymorphisms (SNPs) on Chromosome 17q21 and Early-Onset Asthma, According to Status with Respect to Exposure to Environmental Tobacco Smoke in Early Life.\*

| Gene and SNP            | Position | Risk Allele† | SNP Effect§ | Total Family Sample (N=1087)‡ | P Value¶ | SNP Effect§          | Family Sample with Offspring Exposed to Tobacco Smoke (N=627)‡ | P Value¶ | SNP Effect§ | Family Sample with Offspring Not Exposed to Tobacco Smoke (N=460)‡ | P Value¶ | P Value for Heterogeneity of SNP Effect, According to Exposure Status | Ratio of SNP Effect (Offspring with Exposure vs. Those without Exposure) |
|-------------------------|----------|--------------|-------------|-------------------------------|----------|----------------------|--|----------|-------------|--|----------|---|--|
| <i>Mb</i>               |          |              |             |                               |          |                      |  |          |             |  |          |   |  |
| <i>IKZF3</i>            |          |              |             |                               |          |                      |  |          |             |  |          |   |  |
| rs9303277               | 35.230   | C            | 2.03        | 1.8×10 <sup>-5</sup>          | 2.36     | 1.6×10 <sup>-4</sup> | 1.80   | 0.02     | 1.80        | 0.02   | 0.17     | 0.17  | 1.31   |
| <i>ZBP2</i>             |          |              |             |                               |          |                      |  |          |             |  |          |   |  |
| rs11557467              | 35.282   | G            | 1.95        | 4.0×10 <sup>-5</sup>          | 2.53     | 3.9×10 <sup>-5</sup> | 1.56   | 0.07     | 1.56        | 0.07   | 0.05     | 0.05  | 1.62   |
| Intergenic region       |          |              |             |                               |          |                      |  |          |             |  |          |   |  |
| rs8069176               | 35.311   | G            | 2.02        | 2.0×10 <sup>-5</sup>          | 2.94     | 2.8×10 <sup>-6</sup> | 1.40   | 0.15     | 1.40        | 0.15   | 0.006    | 0.006   | 2.10   |
| <i>GSDML (or GSDMB)</i> |          |              |             |                               |          |                      |  |          |             |  |          |   |  |
| rs2305480               | 35.316   | G            | 1.91        | 5.4×10 <sup>-5</sup>          | 2.77     | 8.7×10 <sup>-6</sup> | 1.40   | 0.17     | 1.40        | 0.17   | 0.008    | 0.008   | 1.98   |
| rs2305479               | 35.316   | C            | 2.03        | 2.6×10 <sup>-5</sup>          | 2.64     | 2.4×10 <sup>-5</sup> | 1.56   | 0.07     | 1.56        | 0.07   | 0.04     | 0.04  | 1.69   |
| rs4795400               | 35.321   | C            | 1.81        | 3.4×10 <sup>-4</sup>          | 2.50     | 5.3×10 <sup>-5</sup> | 1.30   | 0.27     | 1.30        | 0.27   | 0.01     | 0.01  | 1.93   |
| rs9303281               | 35.328   | A            | 1.97        | 7.1×10 <sup>-5</sup>          | 2.46     | 1.1×10 <sup>-4</sup> | 1.59   | 0.07     | 1.59        | 0.07   | 0.11     | 0.11  | 1.55   |
| rs7219923               | 35.328   | T            | 1.90        | 1.4×10 <sup>-4</sup>          | 2.29     | 3.3×10 <sup>-4</sup> | 1.59   | 0.06     | 1.59        | 0.06   | 0.17     | 0.17  | 1.45   |
| <i>ORMDL3</i>           |          |              |             |                               |          |                      |  |          |             |  |          |   |  |
| rs8076131               | 35.334   | A            | 1.76        | 5.1×10 <sup>-4</sup>          | 2.36     | 1.7×10 <sup>-4</sup> | 1.38   | 0.21     | 1.38        | 0.21   | 0.04     | 0.04  | 1.72   |
| Intergenic region       |          |              |             |                               |          |                      |  |          |             |  |          |   |  |
| rs4795405               | 35.342   | C            | 1.81        | 3.8×10 <sup>-4</sup>          | 2.58     | 2.9×10 <sup>-5</sup> | 1.23   | 0.38     | 1.23        | 0.38   | 0.01     | 0.01  | 2.11   |
| rs4794820               | 35.343   | G            | 1.78        | 5.4×10 <sup>-4</sup>          | 2.36     | 1.3×10 <sup>-4</sup> | 1.30   | 0.26     | 1.30        | 0.26   | 0.04     | 0.04  | 1.82   |

\* The 11 SNPs listed are from the panel of 36 SNPs at the 17q21 locus that had a significant association with asthma ( $P < 0.01$ ) in the total sample. P values have not been corrected for multiple testing.

† The alleles shown are those associated with an increased risk of early-onset asthma.

‡ Only families with offspring who were concordant with respect to exposure status were included in the analysis. The total numbers shown are the numbers of subjects in the family sample. The genotypes of parents were used, but it was assumed that their disease status was unknown.

§ The SNP effect was estimated by calculating the ratio of the estimated penetrance for the homozygous risk-allele genotype to the estimated penetrance for other genotypes, as obtained from the Linkage and Association Modeling in Pedigrees (LAMP) program under the best-fitting recessive model.

¶ P values for the test of the SNP effect are based on two-sided likelihood-ratio tests with 1 degree of freedom under the best-fitting recessive model for the SNP effect.

|| P values for the test for heterogeneity of the SNP effect between sibships who were exposed to environmental tobacco smoke and those who were not exposed were obtained from 10,000 permutations of exposure status among sibships.

ma with an onset in early life may differ biologically from asthma with a later onset. This study also shows an interaction between 17q21 variants and exposure in early life to environmental tobacco smoke.

The self-reported year of asthma onset by adult subjects was found to have high accuracy in both a Swedish study<sup>21</sup> of incident cases investigated after 10 years and the longitudinal European Community Respiratory Health Survey.<sup>22</sup> In our study, we found high reproducibility (98%) of the reported age at onset with self-administered questionnaires used before enrolling the subjects in the survey and with the study questionnaires administered face-to-face. Hence, we conclude that erroneous recall of the age at the onset of asthma is unlikely to have significantly affected the results.

Ordered-subset regression analysis identified an age at onset of 4 years as the cutoff point for assigning subjects to an early-onset group and a late-onset group. We obtained a similar cutoff point on the basis of disease-status data from both parents and offspring or only offspring. This approach made it possible to avoid prespecification of an arbitrary threshold and may be suited to identifying homogeneous subsets for association studies. A cutoff age of 4 years is consistent with the early-childhood period of wheezing, as defined in longitudinal studies of patients with early-onset persistent wheezing.<sup>2,4,23-26</sup> Early-onset persistent wheezing has been found to have a strong familial component and to be associated with atopy.<sup>25,26</sup> We did not analyze the subphenotype of atopic asthma. Since 83% of offspring with asthma had atopy, the study did not have sufficient power to distinguish whether an association existed between variants at the 17q21 locus and either atopic or nonatopic asthma.

Exposure to environmental tobacco smoke may be subject to recall bias. Although an overall good reproducibility of the report of parental smoking in childhood was found in the EGEA, mothers of children with asthma underreported their smoking habits when questioned on their children's exposure to environmental tobacco smoke.<sup>27</sup> This may have led to the misclassification of a few families as not having been exposed and, consequently, to a conservative test for heterogeneity. Our finding of a significant association between 17q21 variants and early-onset asthma in off-

spring who were exposed to environmental tobacco smoke is in general agreement with the results of our previous study, since the linkage peak was approximately 5 Mb from these variants and only sibling pairs with asthma who had been exposed to environmental tobacco smoke in early life shared a proportion of identical-by-descent marker alleles in excess to that expected under the null hypothesis of no linkage.<sup>14</sup> We observed replication of the association in the parental generation; the association between 17q21 markers and early-onset asthma was significant only among parents who had been exposed to environmental tobacco smoke in early life. Potential underreporting of early-life exposure to environmental tobacco smoke by these parents<sup>27</sup> may have resulted in a conservative test but would not have contributed to a false positive result.

The 17q21 markers associated with early-onset asthma confer an increased risk among homozygotes for the disease-associated allele that is of the same order of magnitude as the risk reported for these markers in the original genomewide association study.<sup>6</sup> However, exposure to environmental tobacco smoke in early life is associated with an even greater risk. The magnitude of this risk is in the upper range of that reported for other early-life risk factors.<sup>28</sup> The single group of disease-associated markers (in strong linkage disequilibrium with one another) at 35.23 to 35.34 Mb on chromosome 17q21 points to a relatively narrow region of interest that includes four genes. These are *IKZF3* (one SNP), involved in the regulation of lymphocyte development<sup>29,30</sup>; *ZBP2*, or zona pellucida-binding protein 2 (one SNP)<sup>31</sup>; *GSDML* (four SNPs), encoding one of the gasdermin proteins implicated in epithelial barrier function and skin differentiation<sup>32</sup>; and *ORMDL3* (one SNP), which encodes transmembrane protein anchored in the endoplasmic reticulum.<sup>33</sup> Three of the SNPs implicating these genes are nonsynonymous variants; the others reside outside of exons. The three nonsynonymous variants are a marker in exon 4 of *ZBP2* (rs11557467, I151S) and two markers in exon 8 of *GSDML* (rs2305480, P298S; and rs2305479, G291R). However, all of the disease-associated markers are strongly associated with transcript levels of *ORMDL3*, as reported previously.<sup>6,34</sup> Subsequent investigations have shown that the same markers are also associated with transcript levels of *GSDML*, indicating that both

ORMDL3 and GSDML are coregulated by *cis*-acting genetic variants (Cookson W: personal communication).

Although the functional role of *ORMDL3* is unknown, recent work has suggested that it may have a role in viral respiratory infections.<sup>8</sup> Passive exposure to tobacco smoke during fetal development and early life is associated with an increased incidence of viral infections in early childhood,<sup>35</sup> and both early environmental exposure to tobacco smoke and early viral infections increase the risk of asthma.<sup>35,36</sup> *GSDML* may be involved in the regulation of the growth and differentiation of epithelial cells.<sup>32</sup> *ADAM33*, variants of which confer a risk of asthma, has a role in the epithelial mesenchymal trophic unit<sup>37</sup> and influences lung function in early life.<sup>38</sup> Our findings provide additional support that early-life events play a critical role in the pathogenesis of asthma.

In conclusion, we have shown that 17q21 variants are associated with the early onset of asthma and interact with exposure to environmental tobacco smoke in early life. These findings provide a greater understanding of the functional role of the 17q21 variants in the pathophysiology of asthma. In addition, the data are consistent with the observation that since early-onset asthma and late-onset asthma have distinct genetic underpinnings, they are likely to result from distinct pathobiologic mechanisms.

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#### APPENDIX

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