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ABSTRACT

Single nucleotide polymorphisms (SNPs) at chromosome 17q21 confer an increased risk of early-onset asthma. The objective was to study whether 17q21 SNPs modify associations between early respiratory infections and asthma.

Association analysis was conducted in 499 children (268 with asthma, median age 11y) from the Epidemiological Study on the Genetics and Environment of Asthma (EGEA). The 12-year follow-up data were used to assess persistent or remittent asthma in young adulthood. Respiratory infection before 2y of age was assessed retrospectively.

For the twelve 17q21 SNPs studied, the odds ratios (ORs) for association between infection and early-onset asthma (age at onset ≤4y) were higher in carriers of risk genotypes (ORs between 3.42 and 6.36) than in non-carriers (ORs 1.84-2.44; p interaction=0.02-0.04 for five SNPs). Risk genotypes also increased the association between infection and childhood asthma that remits in adulthood (ORs 4.84-7.16 in carriers and 1.74-2.25 in non-carriers; p interaction=0.008-0.05 for ten SNPs). In children with 17q21 risk genotypes and early-life environmental tobacco smoke (ETS) exposure, associations between infection and asthma were further enhanced.

17q21 genetic variants and early ETS exposure enhance the association between early respiratory infections and early-onset asthma and childhood asthma that remits in adulthood.

Keywords: environmental tobacco smoke exposure; epidemiology; gene-environment interaction; *GSDML*; *ORMDL3*; respiratory infection

INTRODUCTION

Infants with early lower respiratory tract infections, which are mainly caused by viruses such as respiratory syncytial virus (RSV) or rhinovirus (RV), are at increased risk of recurrent wheezing and childhood asthma [1-4]. It is not clear whether respiratory infection is a marker identifying infants with a predisposition to develop asthma, or whether infection is causally related to the inception of asthma [5, 6]. Early life is a crucial period for lung development, and it has been argued that viral infection itself may actually increase the risk of asthma by influencing the development of immune response or by altering lung function [4, 7, 8]. However, a recent Danish twin pair study found no evidence to support a causal role of infection [6].

Only a minority of infants with respiratory viral infections such as bronchiolitis develop recurrent wheezing and asthma, suggesting that genetic and environmental factors may influence the association between asthma and infection [9]. For instance, exposure to environmental tobacco smoke (ETS) may play such a role [10]. Single nucleotide polymorphisms (SNPs) in innate immunity and other immune response genes, have been shown to be associated with severity of RSV bronchiolitis [11, 12], however there is a lack of studies investigating genetic factors that influence the association between bronchiolitis and asthma [9].

A genome-wide association study (GWAS) has shown associations between SNPs on chromosome 17q21 and childhood asthma [13], which has been replicated by several studies [14-17]. In the Epidemiological Study on the Genetics and Environment of Asthma (EGEA) it has been shown that the increased risk conferred by 17q21 variants was restricted to early-onset asthma, and was further enhanced by early-life environmental tobacco smoke (ETS) exposure [16]. The latter finding was recently replicated in white subjects [18]. It is still unclear how these genetic variants influence the risk of asthma. Interestingly, the expression of one of the 17q21 genes, *ORMDL3*, was found to be strongly induced by stimulation with polyinosine-polycytidylic acid in lung fibroblasts, suggesting that *ORMDL3* might play a role in viral respiratory infections [14]. Thus, interactions between 17q21 variants and early-life events appear to play an important role in the development of early-onset asthma. It is, however, not known whether these effects persist after childhood.

The objective of the present study was to test the following two hypotheses: 1) the association between early respiratory infections and asthma, particularly early-onset asthma, is influenced by genetic variants in the 17q21 region, and 2) the modifying effect of 17q21 variants on the association between infections and early-onset asthma is further increased by ETS exposure in early life. Furthermore, we tested the same hypotheses for two additional asthma phenotypes: childhood asthma that was either persistent or remittent in young adulthood.

METHODS

Study population and design

EGEA combines a case-control study and a family study. The design and protocol have been reported in detail elsewhere [19]. Probands (asthmatic patients aged 7 to 70 years) were recruited from six chest clinics in five French cities between 1991 and 1995 (EGEA1). Family members of asthmatic probands were included, either by including the proband's parents and siblings, or by including the proband's spouse and children. In addition, population-based controls were recruited.

In the present study, association analyses were conducted in children (age ≤16 yrs) from the EGEA families and control sample. Of the 604 children enrolled at EGEA1, we used data of 499 children (268 with asthma, 231 without asthma) who had complete information on 17q21 genotypes, age at onset of asthma, and respiratory infections (Figure E1 in the online supplement shows a flowchart). A twelve-year follow-up of EGEA1 has been conducted recently (EGEA2; 2003-2007) [20]. Activity of asthma at EGEA2 was obtained in 236 of 268 children with asthma at EGEA1 (88%).

All participants or their parents gave written informed consent.

Genotyping

Genotyping was described before [16]. In the present study, we examined the 11 SNPs located between 35.23 and 35.34 Mb on chromosome 17q21 that were significantly associated with asthma ($p<0.01$) in EGEA families [16]. We also included an additional SNP, rs7216389, since it was the most studied 17q21 variant in replication studies subsequent to the initial GWAS [13]. The 12 SNPs were in strong linkage disequilibrium (LD) with one another, with pairwise LD coefficient D' between 0.87 and 1.0 [16].

Health outcomes and exposure variables

Inclusion criteria used at EGEA1 to define asthma in probands, and the definition of asthma in relatives or population-based controls have been described before [19, 21]. Early-onset asthma was defined as being 4 years old or younger at the onset of asthma [16]. A self-completed questionnaire was used to assess whether children with asthma at the first survey still had active asthma at EGEA2, which was defined as having had an asthma attack or used asthma medication during the last 12 months. In subjects with asthma at EGEA1, persistent and remittent asthma in young adulthood were defined according to activity of asthma (yes/no) at EGEA2. Subjects without asthma at EGEA1 were the reference group in all analyses.

Respiratory infection in early life was based on parental report at EGEA1, and defined as a positive answer to the question "Did your child have bronchitis or bronchiolitis before the age of 2?". ETS exposure in early life (≤ 2y) was defined as described before [16].

Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) for association between infection and asthma were estimated by using the GEE (generalized estimating equations) approach with the logit link function to take into account family dependence among siblings . The sibship was defined as the cluster unit. ORs were adjusted for age and sex. To test whether the association of infection with asthma was influenced by 17q21 genetic variants, we introduced the infection effect, the SNP effect, and a SNP x infection term in the model while adjusting for age and sex. For 17q21 SNPs, we assumed a recessive genetic model (homozygous risk allele genotype versus other genotypes) which provided the best fit for association between the 17q21 locus and early-onset asthma as found previously [16]. The SNP x infection interaction was tested by using the generalized score test which follows a chi-square distribution with 1 degree of freedom. We used the delta-beta statistic to identify influential observations and we assessed the effect of excluding these observations on the results. Empirical p-values for tests of interaction between SNP and infection were also computed by simulations (i.e. marker genotypes were simulated under the null hypothesis of no SNP x infection interaction by using the observed marker allele frequencies and LD pattern among SNPs and keeping the observed phenotypic data in the EGEA children). For each SNP, the p-value was estimated by the proportion of 1,000 simulations leading to a test statistic for interaction as large or larger than the one observed. Finally, we also tested whether the relationship between infection and asthma was modified by both 17q21 variants and exposure to ETS in early life, using a similar strategy.

RESULTS

Descriptive characteristics

Characteristics of study subjects with and without asthma at recruitment (EGEA1) are given in Table 1. As expected, objectively measured markers of asthma such as atopy, total IgE, peripheral blood eosinophils, FEV_1, and bronchial hyperresponsiveness were significantly associated with diagnosed asthma ($p<0.05$). The median age of the study subjects at the second survey (EGEA2) was 22.4 y (interquartile range 20.0-24.2 y). Of 167 children with early-onset asthma, 84 had persisting asthma, 62 had remittent asthma, and 21 had missing data at the second survey. Of 101 children with late-onset asthma, 44 had persistent asthma, 46 had remittent asthma, and 11 had missing data at the second survey.

Early infections

Early respiratory infection was significantly associated with all asthma phenotypes, but most strongly with early-onset asthma and asthma remittent in young adulthood (Table 2). Odds ratios changed only marginally after adjusting for age and sex. Further adjustment for ETS or atopy did not change the associations. Associations between respiratory infection and asthma appeared stronger in boys than in girls: the OR for the association between infection and early-onset asthma was 3.70 (2.17-6.32) in boys and 2.29 (1.14-4.59) in girls, but these ORs were not significantly different ($p=0.43$). There was no association between the 17q21 SNPs and early respiratory infection ($p>0.1$, data not shown). There were also no associations between 17q21 SNPs and infection when stratified analyses were performed in children with asthma (ORs between 0.85 and 1.57, $p>0.1$) or without asthma (ORs between 0.59 and 0.84, $p>0.1$).

Influence of 17q21 genotype on the association of infection with asthma

The association between respiratory infection and early-onset asthma was stronger in carriers of risk genotypes for each of the twelve 17q21 genetic variants (ORs ranged between 3.42 and 6.36, according to the genetic variant) than in non-carriers (ORs between 1.84 and 2.44, Table 3). Statistically significant interaction between infection and 17q21 genotype was observed for five SNPs ($p=0.02$ to 0.04, Table 3). In contrast, associations between infection and asthma with onset at 5 years of age or older did not differ significantly according to genotype: ORs observed in carriers of risk genotypes were 0.7-fold to 1.7-fold the ORs observed in non-carriers (p-values for interaction ranging between 0.29 and 0.93, data not shown).

We then examined children with asthma in childhood with active or non-active asthma in young adulthood (persistent or remittent asthma, *versus* no asthma in childhood). ORs for the association between infection and childhood asthma that remits in adulthood were 2.1 to 3.7 times higher in children with risk genotypes than in children with non-risk genotypes (ORs ranged between 4.84 and 7.16 in carriers and between 1.74 and 2.25 in non-carriers; p interaction 0.008 to 0.05 for ten SNPs, Table 4). The association between infection and childhood asthma persisting into young adulthood was not significantly modified by 17q21 SNPs (ORs differed by a factor 0.9 to 1.9, p interaction ranging from 0.18 to 0.91; data not shown).

The delta-beta statistic did not identify individuals who had a strong influence on the effect estimates shown in Table 3 and 4. We estimated the parameters of the model again without the ten most influential observations. P-values for interaction that were less than 0.05 in Table 3 still reached that significance level, except for rs8076131 ($p=0.07$). P-values for interaction that were less than 0.05 in Table 4 still reached that significance level, except for rs903281 ($p=0.08$), rs4795405 ($p=0.07$), and rs4794820 ($p=0.11$).

ETS exposure

We further studied the influence of both 17q21 genotypes and ETS exposure in early life on the associations between respiratory infection and asthma. Figure 1 shows association between infection and either early-onset asthma or remittent asthma according to ETS exposure and risk genotype at rs8069176, the SNP showing the most significant gene-environment interactions. The association between infection and early-onset asthma and remittent asthma was very strong among ETS exposed subjects with the rs8069176 GG genotype (early-onset asthma OR 9.98 [3.56-27.97],

asthma remittent in adulthood OR 10.68 [3.58-31.82]). ORs for the association between infection and early-onset asthma were significantly different between children exposed to ETS and children not exposed to ETS, but only in carriers of the risk genotype (interaction infection x ETS; p=0.03 in children carrying rs8069176 GG). The other 11 SNPs showed similar patterns, with a very strong association between infection and early-onset asthma among children exposed to ETS and carrying the risk genotype (ORs ranged between 4.65 [1.62-13.34] for rs9303277 CC and 10.04 [3.63-27.69] for rs2305480 GG), and weaker associations in the other children (data not shown).
Estimation of the parameters of the model without the ten most influential observations led to a slight decrease in evidence for associations between infection and early-onset asthma: among ETS exposed subjects with the rs8069176 GG genotype the OR was 9.18 [3.06-27.6].

DISCUSSION

This study shows that 17q21 risk genotypes increased the positive association between early respiratory infection and asthma, and this was restricted to early-onset asthma and asthma that remits in young adulthood. The association between infection and early-onset asthma (or remittent asthma) was further enhanced when children with 17q21 risk variants were exposed to ETS in early life. The present study supports earlier findings showing the importance of early life events in the association between 17q21 variants and asthma [16].

Infections and asthma

Lower respiratory tract infections may identify children with preexisting physiological or immunological abnormalities, or may provide a first trigger to start wheezing in infants who are prone to develop asthma. Otherwise, it has been suggested that viral respiratory infections and asthma may be causally related. There is some evidence of a causal link, including recent findings from an experimental mouse model showing that innate immune activation by viral respiratory infection can lead to the development of chronic inflammatory airway disease [7, 8, 22]. Infants with 17q21 risk variants may respond differently to lung injury caused by respiratory viral infections, which may increase their susceptibility to develop recurrent wheezing or asthma. However, we cannot draw conclusions regarding the causality of association between early respiratory infection and asthma, and if we assume a non-causal association between infection and asthma, an explanation for our findings might be that 17q21 variants increase the risk to develop asthma by interacting with risk factors underlying early virus-induced wheezing. Gene-gene or gene-environment interaction between 17q21 variants and common risk factors for respiratory infection and asthma (such as innate immune gene variants) could thus influence the risk of asthma.

A large genomic region of interest

The function of the studied 17q21 genes is largely unknown, especially with respect to asthma. A relationship between *ORMDL3*, viral infection, and asthma was suggested by Hirota et al. who found that *ORMDL3* expression was strongly induced in an experimental model of viral stimulation [14]. A recent study reported that ORMDL3 facilitated endoplasmic reticulum mediated inflammatory responses [23]. Among the 12 SNPs studied, one belongs to *ORMDL3* while the other ones are intergenic (three SNPs) or belong to other genes, *IKZF3* (one SNP), involved in the regulation of lymphocyte development, *ZPBP2*, or zona pellucida-binding protein 2 (one SNP), and *GSDML* (six SNPs), encoding one of the gasdermin proteins implicated in epithelial barrier function and skin differentiation [24]. These 17q21 SNPs are strongly associated with transcript levels of *ORMDL3* [13], and, as found more recently, with transcript levels of *GSDML*, indicating that both *ORMDL3* and *GSDML* are coregulated by *cis*-acting genetic variants [25]. Therefore, our epidemiologic observations are consistent with biological findings which suggest that genetic variants act over a large genomic region, playing a role in transcriptional activity of at least three genes (*ZPBP2*, *GSDML*, and *ORMDL3*) [25].

Lung function and atopy

Association between 17q21 SNPs and FEV_1 was not found in EGEA and other populations, but in these populations lung function was not measured in early childhood [15, 16]. There is ample evidence that diminished airway size is a risk factor for wheezing during viral infections in early life [4]. It could, therefore, also be speculated that 17q21 variants modify association between a reduced pulmonary function in infancy and early-onset asthma. A reduced maximal flow at functional residual capacity ($V_{max}FRC$) during the first year of life was associated with transient, but not persistent, early wheezing, a phenotype which is strongly related to viral infections, in the Tucson Children's Respiratory Study [26]. However, it has also been shown that diminished values of $V_{max}FRC$ at 1 month are associated with persistent wheeze at 11 years of age in children from the Perth study [27]. EGEA probands were recruited at the age of 7 years or older, and therefore, our study cannot be directly compared with birth cohort studies such as the Tucson and Perth studies [26-28], in as much as children with asthma at school age were included more frequently in EGEA.
Both atopy and early-life respiratory viral infections are independently associated with subsequent asthma, and the combination of atopy and respiratory infections has been associated with a strongly increased risk to develop asthma [29]. Most studies show that 17q21 SNPs are not associated with atopy or total IgE as such, but possibly with atopic asthma [15-17]. However, because of the strong association between atopy and asthma in EGEA children (almost 90% of the children with asthma were atopic), we could not perform separate analyses for atopic and non-atopic asthma.

Persistent and remittent asthma in young adulthood

The longitudinal design of EGEA provides a unique opportunity to study the persistence of childhood asthma into young adulthood. The study did not have sufficient statistical power to perform separate analyses according to the age at onset among subjects with persistent or remittent asthma, but the majority of children with asthma (62%) had a first asthma attack at the age of 4 years or younger. The modifying effect of 17q21 variants on the association between

infection and asthma was most pronounced for asthma that remitted in young adulthood, suggesting that the functional role of 17q21 variants may be restricted to early-life events, which may be outgrown at a later age.

ETS, early events, and epigenetics

We studied whether ETS exposure in early life further modified associations between respiratory infection and asthma, although this resulted in relatively small subgroups. The additional modifying effect of early-life ETS exposure is, however, plausible, since ETS exposure has been shown to influence the child's lung function and immune system. Children exposed to ETS *in utero* or in early life exhibit small but clear deficits in lung function, and there has been clear evidence of a dose-dependent relationship between the amount smoked by mothers and lung function in children [30]. It has also been shown that exposure to ETS in early life can influence signalling through innate immune receptors, which may be implicated in the increased predisposition to infection in ETS exposed infants [31]. Moreover, in mouse models, cigarette smoke has been shown to affect antiviral pro-inflammatory processes [10]. Verlaan et al. [25] suggested that environmental triggers and common 17q21 variants may influence epigenetic states by shifting the equilibrium in favour of one of the chromatin conformations resulting in changes in gene expression. The very early window of expression and the interaction with ETS both support the hypothesis of a role of epigenetic changes.

Limitations

Sub-group analyses led to relatively small numbers of subjects, especially when taking ETS exposure into account. We cannot rule out false negative results due to inadequate power. Although results were not modified by removal of influential subjects from the study sample, interpretation should be cautious and replication is needed to confirm our observations. Another main limitation of our study was that viral respiratory infection was assessed retrospectively by questionnaire. We did not have objective data confirming that the self-reported early-life respiratory infections were viral, although it is well-established that the majority of respiratory illnesses in infancy are caused by viral infections [32]. Parents of children with asthma could have been more likely to recall a respiratory infection episode during the first two years of their child's life than parents of children without asthma. Although a recall bias may have resulted in an overestimation of the association between infection and asthma, it cannot explain the modifying effect of 17q21 variants on this association. We could not assess the role of severity, nor of specific viral infections such as rhinovirus (RV) or respiratory syncytial virus (RSV) bronchiolitis. Recently, outpatient wheezing illness with RV infection was shown to be a much stronger predictor of subsequent asthma than wheezing with RSV [1]. It would be of interest not only to replicate our findings in a prospective study, but also to explore whether 17q21 variants interact differently with RV or RSV bronchiolitis. To prevent false positive results due to potential confounders or data stratification, p-values for tests of interaction of 17q21 SNPs with infection were computed by simulations and led to the same results as those shown here. We did not apply the Bonferroni correction to correct for multiple testing since it would be much too conservative given the high level of LD among the 12 SNPs analyzed. Moreover, further haplotype analysis led to the same results as those shown here.

In conclusion, this study shows an important role of 17q21 variants, early respiratory infection and early exposure to ETS in early-onset asthma. Our longitudinal data also suggest that 17q21 variants modified the association of early respiratory infection and an asthma phenotype that does not persist into adulthood. Therefore, our results add to the evidence that the relevant window of expression for 17q21 variants is early in life.

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FIGURE LEGEND

Figure 1 Association of asthma (early-onset asthma and asthma remittent in adulthood) and early respiratory infection, according to ETS exposure in early life (yes: ETS+, no: ETS-) and rs8069176 genotype (SNP+ = GG, SNP- = AG+AA).

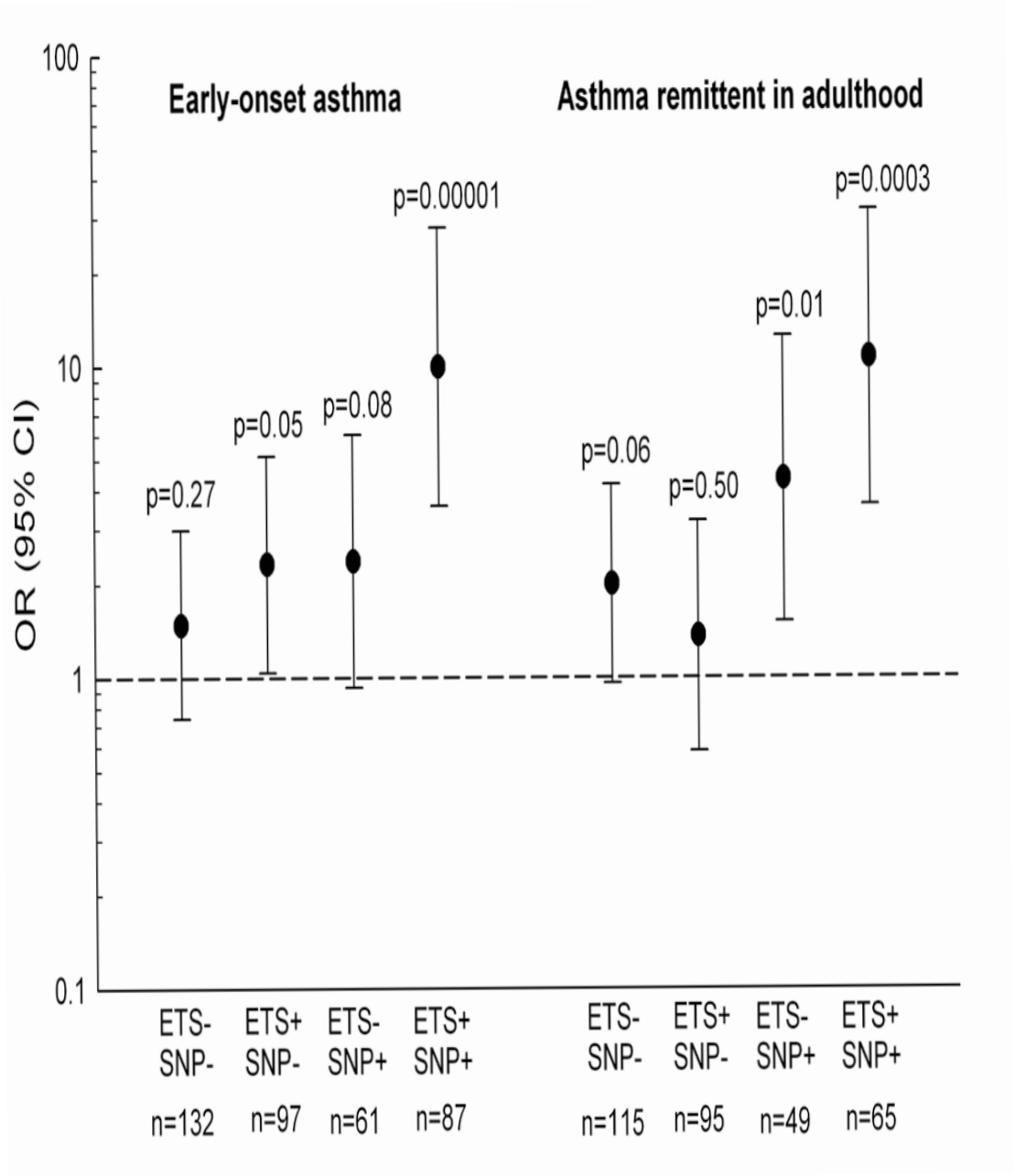

TABLE 1 Characteristics of the study population.

Variable	Data available for n children	Children with asthma	Children without asthma
n	499	268	231
Recruitment category			
Probands, n (%)		164 (61.2)	0 (0)
Relatives, n (%)		96 (35.8)	173 (74.9)
Population-based controls, n (%)		8 (3.0)	58 (25.1)
Male sex, n (%)	499	173 (64.6)	106 (45.9)
Age at recruitment, yr (median (interquartile range))	499	10.8 (9.0 - 12.9)	11.4 (8.7-13.5)
Respiratory infection in early life, n (%)	499	138 (51.5)	66 (28.6)
Exposure to ETS in early life, n (%)	489	122 (46.6)	114 (50.2)
Atopy, n (%)[†]	486	226 (86.6)	91 (40.4)
In sensitized children, ≥2 positive SPT, n (%)		166 (73.5)	47 (51.6)
IgE IU/ml, median (interquartile range) [‡]	489	383 (151-815)	58 (21-177)
Peripheral blood eosinophils, %, median (interquartile range)	484	6.5 (4.0-10.8)	3.1 (2.0-5.0)
Peripheral blood eosinophils, nb/mm^3, median (interquartile range)	484	434 (270-700)	210 (120-360)
FEV_1 % predicted, median (interquartile range)	473	92.9 (84.3-101.3)	94.9 (88.3-102.3)
Bronchial hyperresponsiveness, n (%) [*]	310	127 (92.0)	75 (43.6)
Used inhaled corticosteroids (last 12 months), n (%)	494	102 (38.5)	2 (0.9)
Ever hospitalized for asthma, n (%)	267	72 (27.0)	
Age at onset of asthma, yr (median (interquartile range))	268	4 (2 - 6)	
Early-onset asthma (≤4y), n (%)		167 (62.3)	
Late-onset asthma (>4y), n (%)		101 (37.8)	
Asthma persisting in young adulthood (persistent asthma), n (%)	236	128 (54.2)	
Early-onset in subjects with persistent asthma, n (%)		84 (65.6)	
Late-onset in subjects with persistent asthma, n (%)		44 (34.4)	
Asthma inactive in young adulthood (remittent asthma), n (%)	236	108 (45.8)	
Early-onset in subjects with remittent asthma, n (%)		62 (57.4)	
Late-onset in subjects with remittent asthma, n (%		46 (42.6)	

[†]Atopy was defined as a positive skin prick test (SPT) to at least one of 11 common allergens. [‡]Total serum IgE (in international units (IU) per milliliter) was measured by immunoassay. [*]Bronchial hyperresponsiveness was defined as ≥20% decline of FEV_1 for a methacholine cumulative dose ≤ 4 mg (PD20 ≤ 4 mg).

TABLE 2 Association of asthma phenotypes and early respiratory infection.

Phenotype	n	Infection prevalence, n (%)	OR (95% CI)[†]
No asthma[*]	231	66 (28.6)	1.0 (reference)
Asthma	268	138 (51.1)	2.49 (1.73-3.58)
Early-onset asthma	167	93 (55.7)	2.86 (1.88-4.35)
Late-onset asthma	101	45 (44.6)	2.09 (1.29-3.39)
Asthma persistent in adulthood	128	61 (47.7)	2.14 (1.38-3.32)
Asthma remittent in adulthood	108	59 (54.6)	2.93 (1.87-4.59)

[*] Subjects without asthma at EGEA1 were the reference group in all analyses.
[†] Adjusted for age and sex.

TABLE 3 Association of early-onset asthma and early respiratory infection in children, according to 17q21 SNPs.

			Non-carriers of risk genotype		Carriers of risk genotype		
Gene	SNP	Risk genotype	n	OR (95% CI)	n	OR (95% CI)	p interaction
IKZF3	rs9303277	CC	256	2.44 (1.47-4.07)	127	3.42 (1.59-7.39)	0.46
ZPBP2	rs11557467	GG	259	2.35 (1.41-3.90)	129	4.08 (1.91-8.73)	0.22
Intergenic region	rs8069176	GG	234	1.84 (1.06-3.20)	152	5.24 (2.53-10.83)	**0.02**
GSDML (GSDMB)	rs2305480	GG	232	1.89 (1.11-3.22)	151	5.50 (2.66-11.38)	**0.02**
GSDML (GSDMB)	rs2305479	CC	260	2.28 (1.38-3.77)	134	4.15 (1.97-8.76)	0.17
GSDML (GSDMB)	rs4795400	CC	232	1.90 (1.10-3.28)	148	4.73 (2.32-9.65)	**0.04**
GSDML (GSDMB)	rs7216389	TT	264	2.15 (1.30-3.53)	119	6.36 (2.72-14.86)	**0.02**
GSDML (GSDMB)	rs9303281	AA	267	2.24 (1.36-3.68)	125	4.22 (1.97-9.05)	0.16
GSDML (GSDMB)	rs7219923	TT	263	2.15 (1.29-3.57)	120	4.81 (2.13-10.87)	0.08
ORMDL3	rs8076131	AA	244	2.01 (1.20-3.36)	143	4.95 (2.37-10.35)	**0.04**
Intergenic region	rs4795405	CC	239	2.07 (1.21-3.54)	145	4.68 (2.30-9.54)	0.07
Intergenic region	rs4794820	GG	234	2.36 (1.38-4.01)	149	4.49 (2.23-9.04)	0.14

P-values for association of early-onset asthma and infection ranged between 0.03 and $7.5x10^{-4}$ in subjects with the non-risk genotype and between $1.4x10^{-3}$ and $4.6x10^{-6}$ in subjects with the risk genotype. P-values for interaction that were less than 0.05 in this Table still reached that significance level when they were obtained by simulations, except for rs8076131 (p=0.06).

TABLE 4 Association of asthma remittent in adulthood and early respiratory infection in children, according to 17q21 SNPs.

			Non-carriers of risk genotype		Carriers of risk genotype		
Gene	SNP	Risk genotype	n	OR (95% CI)	n	OR (95% CI)	p interaction
IKZF3	rs9303277	CC	234	2.25 (1.35-3.77)	93	4.84 (1.94-12.03)	0.14
ZPBP2	rs11557467	GG	235	2.02 (1.20-3.38)	99	5.66 (2.33-13.76)	**0.04**
Intergenic region	rs8069176	GG	214	1.74 (1.00-3.01)	117	6.37 (2.78-14.58)	**0.008**
GSDML (GSDMB)	rs2305480	GG	209	1.90 (1.11-3.24)	115	6.86 (2.96-15.90)	**0.008**
GSDML (GSDMB)	rs2305479	CC	234	2.21 (1.32-3.71)	101	5.65 (2.36-13.52)	0.06
GSDML (GSDMB)	rs4795400	CC	211	1.85 (1.08-3.17)	114	6.01 (2.59-13.97)	**0.02**
GSDML (GSDMB)	rs7216389	TT	238	2.06 (1.25-3.41)	97	7.16 (2.78-18.44)	**0.02**
GSDML (GSDMB)	rs9303281	AA	236	2.17 (1.31-3.62)	94	6.03 (2.39-15.21)	**0.05**
GSDML (GSDMB)	rs7219923	TT	219	2.09 (1.25-3.51)	110	6.65 (2.52-17.51)	**0.03**
ORMDL3	rs8076131	AA	218	2.01 (1.19-3.38)	111	6.12 (2.58-14.50)	**0.02**
Intergenic region	rs4795405	CC	214	1.99 (1.18-3.38)	118	5.63 (2.39-13.30)	**0.04**
Intergenic region	rs4794820	GG	238	2.24 (1.31-3.80)	92	5.74 (2.54-13.00)	**0.05**

P-values for associations of remittent asthma and infection ranged between 0.05 and $2.2x10^{-3}$ in subjects with the non-risk genotype and between $8.0x10^{-4}$ and $1.3x10^{-5}$ in subjects with the risk genotype. P-values for interaction that were less than 0.05 in this Table still reached that significance level when they were obtained by simulations, except for rs9303281 and rs4794820 (p=0.06).

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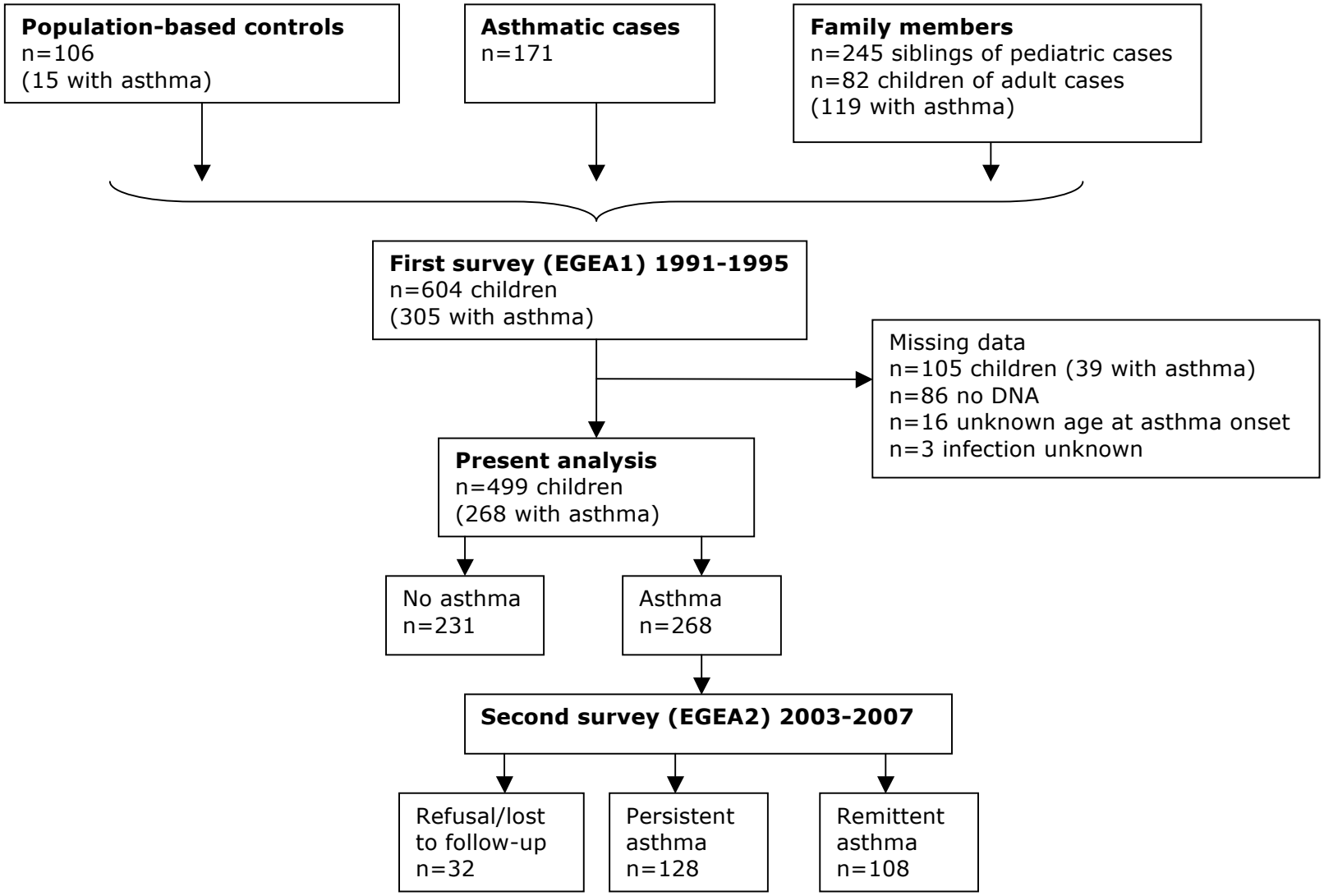

Supplementary Figure E1 Flowchart of EGEA children