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**Evidence for a pleiotropic QTL on chromosome 5q13 influencing both time to asthma onset and asthma score in French EGEA families**

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## Abstract

Although many genome screens have been conducted for asthma as a binary trait, there is limited information regarding the genetic factors underlying variation of asthma expression. Phenotypes related to variable disease expression include time to asthma onset and variation in clinical expression as measured by an asthma score built from EGEA data. A recent genome scan conducted for this score led to detection of a new region (18p11) not revealed by analysis of dichotomous asthma. Our goal was to characterize chromosomal regions harbouring genes underlying time to asthma onset and to search for pleiotropic QTL influencing both time to asthma onset and the asthma score. We conducted a genome-wide linkage screen for time to asthma onset, modelled by martingale residuals from Cox survival model, in EGEA families with at least two asthmatic sibs. This was followed by a bivariate linkage scan of these residuals and asthma score. Univariate linkage analysis was performed using the Maximum Likelihood Binomial method that we extended to bivariate analysis.

This screen revealed two regions potentially linked to time to asthma onset, 1p31 (LOD=1.70,  $p=0.003$ ) and 5q13 (LOD=1.87,  $p=0.002$ ). Bivariate linkage analysis led to a substantial improvement of the linkage signal on 5q13 ( $p=0.00007$ ), providing evidence for a pleiotropic QTL influencing both variation of time to asthma onset and of clinical expression.

Use of quantitative phenotypes of variable disease expression and suitable statistical methodology can improve the power to detect new regions harbouring genes which may play an important role in onset and course of disease.

## INTRODUCTION

Asthma is a complex disease with variable clinical expression and resulting from many genetic and environmental risk factors (Ober and Hoffjan 2006). Asthma is unlikely to be a single disease but rather a collection of different phenotypes which may represent different manifestations of a common underlying pathological process or may be separate disease entities, as recently reviewed by Wenzel (Wenzel 2006). These phenotypes can be characterized from various criteria including clinical and physiological features such as severity of clinical symptoms, type of asthma treatment, and age of onset of asthma.

It has been shown that use of quantitative scores based on symptoms of asthma rather than simple dichotomous definitions can improve the identification of risk factors (Pekkanen et al. 2005). We have recently conducted a genome-wide screen for a categorical asthma score, which was built from clinical symptoms and type of asthma treatment and represented the whole spectrum of disease expression (from unaffecteds to severely affecteds), in families from the Epidemiological Study on the Genetics and Environment of Asthma (EGEA). This scan led to detection of linkage of this asthma score to 18p11 (Bouzigon et al. 2006), a new region that was not revealed by a previous screen for asthma using a classical dichotomous definition (Bouzigon et al. 2004).

Asthma age of onset is another interesting feature that may be worth considering to characterize the underlying determinants of this disease which displays different characteristics according to the lifetime period at which it occurs. Patients with early age at onset (i.e. before puberty) were reported to have more frequently a family history of asthma, allergy sensitisation and clinical response to triggers than patients with late-onset

disease (Hsu et al. 2004; Miranda et al. 2004; Oryszczyn et al. 2006). Despite a potential longer duration of disease, subjects with early-onset asthma were shown to have marginally better lung function than those with late-onset disease. However, the severity of asthma in early childhood substantially determines the severity of the symptoms in later years (de Marco et al. 2006).

Despite the considerable efforts made over the past ten years to map the chromosomal location of genes that may be involved in the development of asthma (Bouzigon et al. 2005; Ober and Hoffjan 2006), none of the 16 genome scans conducted to date have examined the variability of asthma age-of-onset using survival analysis concepts. Such approach can take into account age at onset for individuals who developed the disease and age at time of examination for those who were free of disease upon examination but were still at risk for the disease. One interesting feature of published genome screens is that a given chromosomal region is often linked to various asthma-associated phenotypes across studies, suggesting that these phenotypes may share genetic determinants. Multivariate genetic linkage analyses of correlated phenotypes have been shown to improve power of detecting genes with small effects where these genes may be missed with univariate analyses (Allison et al. 1998; Marlow et al. 2003). However, the formal characterization of pleiotropic effects of genes underlying two or more asthma-related phenotypes has received little attention.

The goal of the present study was to characterize chromosomal regions that may harbour genes underlying time to asthma onset and to search for pleiotropic genetic determinants that may influence both variability in asthma age-of-onset and variation in clinical expression of asthma as measured by the asthma score. We first carried out a

genome-wide linkage screen, in EGEA families with at least to asthmatic sibs, for time to asthma onset modelled by martingale residuals (MR) obtained from Cox proportional hazards model, using the Maximum Likelihood Binomial (MLB) linkage analysis method (Abel and Muller-Myhsok 1998; Alcais and Abel 1999). We then extended the MLB method to bivariate linkage analysis of MR and asthma score.

## **SUBJECTS AND METHODS**

### **EGEA study**

The protocol of the EGEA data collection has been described elsewhere (Kauffmann et al. 2001; Kauffmann et al. 1997). Subjects answered a detailed questionnaire regarding respiratory symptoms and treatment based on international standardized questionnaires. Among the whole EGEA family sample ascertained through one asthmatic proband, 119 nuclear families including at least two asthmatic sibs were selected for the present study. Asthma was defined using the same criteria as in previous EGEA linkage scans (Bouzigon et al. 2004), associating report of asthma attacks or attacks of breathlessness with wheezing ever and either airway hyperresponsiveness or reversible obstruction or hospitalisation for asthma or asthma treatment.

### **Time to asthma onset**

For individuals who developed asthma, information on asthma age at onset was obtained from adult asthmatics or parents of asthmatic children who answered to the following question: “How old were you when you had your first asthma attack?” or “How old was your child when he (or she) had his (her) first asthma attack?”. For individuals who were free of disease upon examination, we considered age at examination; these individuals represented censored observations, as they were still at risk for disease.

## **Asthma score**

The asthma score, we analyzed previously (Bouzigon et al. 2006), was built to represent the whole spectrum of disease expression, encompassing both non-asthmatics and asthmatics. This score ranged from 0 to 4: a score of zero was assigned to non-asthmatics and the scores of 1 to 4 represented various degrees of asthma severity. To briefly summarize, the four classes of asthma severity were defined in two steps by combining clinical items and level of treatment. In step 1, the sum of the following clinical items regarding the last 12 months was computed: frequency of asthma attacks (graded from 0 to 3); symptoms between asthma attacks (graded from 0 to 3); and hospitalisation for asthma (no(0)/yes(1)). This sum of clinical items was grouped into 4 classes: 0, 1, 2 and  $\geq 3$ . In step 2, this 4-class variable built in step 1 was combined with the type of anti-asthmatic treatment in the past 12 months as reported in the EGEA questionnaire (no treatment, treatment without inhaled steroids, treatment with inhaled steroids) following the concept of combining treatment and symptoms according to the 2004 GINA guidelines (National Heart, Lung and Blood Institute 2002) and resulting into an asthma severity score taking the values 1, 2, 3 or 4.

## **Genotyping**

Genotyping of 396 microsatellites was performed in EGEA families with at least two sibs with DNA (Bouzigon et al. 2004). These markers were distributed at an average marker density of 10 centimorgans ([cM]) and had an average heterozygosity of 75%. After rigorous genotype quality control, the final sample included 110 families (508



subjects) with at least two asthmatic sibs, comprising 218 genotyped parents (99% of all parents) and 288 genotyped sibs.

### Statistical analyses

Time to asthma onset was analyzed using survival analysis methods. We considered the martingale residuals from Cox proportional hazards model, as defined by Barlow and Prentice (Barlow and Prentice 1988), using STATA 7.0. The observed times were failure times (age at disease onset) in asthmatics and censoring times (age at examination or death from a competing cause) in non-asthmatics. The martingale residual  $r_{Mi}$  for individual  $i$ , is:

$$r_{Mi} = \delta_i - \hat{\Lambda}_0(t_i) \exp(\beta z_i),$$

where  $\delta$  is a censoring indicator which is equal to one if the failure time is less than the censoring time (affected) or zero otherwise (unaffected),  $\hat{\Lambda}_0(t_i)$  is the estimated cumulative baseline hazard function evaluated at age at asthma diagnosis (age at onset) if  $i$  is asthmatic or at age at examination if  $i$  is unaffected, and  $\beta$  is the estimated regression coefficient associated with covariate  $z$ . The only covariate included in the model was sex.

Descriptive statistics of age at asthma onset, age at examination and martingale residuals (MR) were computed in asthmatics and non-asthmatics and by gender. The medians of MR across asthma score classes were compared using non-parametric Kruskal-Wallis Rank Test. The relationship between MR and asthma score was estimated by Spearman rank correlation. All computations were done using STATA 7.0.

Linkage analysis of the martingale residuals was performed using the Maximum Likelihood Binomial method (Abel and Muller-Myhsok 1998; Alcais and Abel 1999), as implemented in MLB-GENEHUNTER (Abel and Muller-Myhsok 1998). As opposed to the Variance Components (VC) method, this method does not require the assumption of normality of the distribution of the quantitative phenotype (Alcais and Abel 1999) and is therefore well suited to the analysis of martingale residuals which usually display skewness and kurtosis. The principle of this approach is to introduce a latent (unobserved) binary variable ( $Y=\{0;1\}$ ) which captures the linkage information between the observed quantitative phenotype and the marker. For each observed phenotypic value, the latent (unobserved) variable  $Y$  can take the value of 1 (being affected) with probability  $P_i$  and the value of 0 (being unaffected) with probability  $1-P_i$ . The idea is that the probability for the latent variable  $Y$  to be equal to 1 increases as the observed phenotypic values increase. Linkage is then investigated for all possible sets of  $Y$  values within sibships weighted by their probabilities. The link function between the observed phenotype and latent variable was based on the empirical trait distribution. When analysing MR, we divided the trait values into 10 consecutive subclasses according to its decile distribution. If  $d(z_i)$  is the decile corresponding to the value of the phenotype  $z_i$ ,  $d(z_i) = \{1;2; \dots ;10\}$ , the corresponding probabilities were  $P(y_i = 1 | z_i) = 0.1[d(z_i)] - 0.05$ , and  $P(y_i = 0 | z_i) = 1 - P(y_i = 1 | z_i)$ . Siblings belonging to classes 1-4 had negative martingale residuals while sibs in classes 5-10 had positive residuals. Given a set of values of the latent variable  $Y$  in a sibship, the distribution of marker alleles from heterozygous parents among siblings is written by use of a binomial distribution which depends on one parameter,  $\alpha$  (probability that sibs with  $Y=1$  receive

one of the two marker alleles with disease allele from his/her heterozygous parent,  $1-\alpha$  being the corresponding probability among sibs with  $Y=0$ ). Test for linkage (null hypothesis of no linkage ( $\alpha = 0.5$ ) vs the alternative hypothesis of linkage ( $\alpha > 0.5$ )) is performed using a likelihood-ratio (LR) test,  $LR=2\text{Ln}[L(\alpha)/L(\alpha=0.5)]$  with LR being asymptotically distributed as a mixture of  $0.5 \chi^2_0$  and  $0.5 \chi^2_1$ . This statistic divided by  $2\text{ln}10$  is a LOD score.

We extended the MLB method to bivariate linkage analysis of the martingale residuals and asthma score, following an approach proposed by Mangin *et al.* (Mangin *et al.* 1998). These authors showed that the likelihood-ratio test used to test for the presence of a pleiotropic QTL in multivariate variance components (VC) analysis is asymptotically equivalent to the sum of likelihood-ratio tests of univariate VC analyses applied to the principal components of the traits. In a first step, a principal components (PC) analysis was applied to the two phenotypes, MR and asthma score, to obtain two uncorrelated principal components, using STATA 7.0. These components were then subjected to independent univariate linkage analyses based on the MLB method. In the MLB analysis, we used the decile distribution for each PC, as described above. In a second step a combined test statistic, T, was constructed by summing the univariate MLB likelihood-ratio (LR) test statistics obtained for each of the two PCs:  $T = \sum_{i=1}^2 LR_i$ , where the distribution of each  $LR_i$  test, under the null hypothesis of no linkage, is a mixture of  $\frac{1}{2} \chi^2_0 + \frac{1}{2} \chi^2_1$ . Since the two principal components are independent, the asymptotic distribution of the combined test statistic T is a mixture of  $\frac{1}{4} \chi^2_0 + \frac{1}{2} \chi^2_1 + \frac{1}{4} \chi^2_2$ .

## RESULTS

### Descriptive statistics of the phenotypes

As seen in Table 1, the 288 genotyped siblings included 82% of asthmatics. The median (25-75 quartiles) of age at examination of all sibs was 12 years (10-18); it was 12 years (10-18) in asthmatics and 12 years (8-18) in non-asthmatics. In asthmatics, the median (25-75 quartiles) of age at asthma onset was 4 years (2-8) and the median of duration of disease between age at onset and age at examination was 8 years with 25-75 quartiles being (5– 11).

Table 1

The proportion of males did not differ between asthmatic and non-asthmatic sibs (59.5% and 53% respectively,  $p = 0.40$ ). However, while the median of age at examination in non-asthmatics did not differ between males and females (15.1 years of age *versus* 12.2 years of age,  $p = 0.39$ ), the median of age at asthma onset in asthmatics was higher in females than in males (6 years of age *versus* 4 years of age,  $p = 0.01$ ). Thus, sex was included as a covariate in the Cox proportional hazards model from which martingale residuals were obtained. The distribution of these martingale residuals showed a median equal to 0.23, the 25-75 quartiles being (-0.47 - 0.65). This phenotype displayed significant skewness ( $p < 10^{-4}$ ) and kurtosis ( $p = 0.01$ ).

Regarding the asthma score, 18% of sibs had a score of zero, 38% a score of 1, 19% a score of 2, 12% of score 3 and 12% a score of 4 (Table 1). Figure 1 shows the distribution of martingale residuals according to the five classes of the asthma score. As expected, the median of MR was much lower in non-asthmatics (median = -1.16) than in asthmatics (median  $\geq 0.40$ ). In asthmatics, the medians of MR did not differ among the four classes of the asthma score, these medians being 0.40 for scores of 1, 2 and 4 and

Figure 1

0.53 for a score of 3 ( $p = 0.64$ ). However, the range of the 25-75% quartiles increased as the asthma score increased, this range being (0.15-0.70) for a score equal to 1 and (-0.26-0.74) for a score equal to 4. Interestingly, the duration of disease had a similar distribution across the four asthma score classes ( $p = 0.82$ ), with medians of 8 for scores of 1 and 4 and medians of 7 for scores of 2 and 3. The Spearman rank correlation between the martingales residuals and the asthma score was 0.41 ( $p \leq 10^{-4}$ ).

### Genome-wide linkage screen

The genome-wide linkage results for the univariate analysis of martingale residuals and bivariate analysis of MR and asthma score are presented in Figure 2. The regions detected at a  $p$ -value  $\leq 0.005$  are shown in Table 2. For purpose of comparing the results of bivariate and univariate analyses, the results previously obtained from univariate linkage analysis of the asthma score are also shown in Table 2.

There was suggestive evidence for linkage of martingale residuals to 1p31 (LOD=1.70 at D1S2797,  $p=0.003$ ) and 5q13 (LOD=1.87 at D5S424,  $p=0.002$ ). An additional linkage signal was observed in the 14q13 region (LOD = 1.21 at D14S70,  $p = 0.009$ ). As seen from table 2, the 5q13 and 14q13 regions included also linkage signals for the asthma score (LOD =1.22 at 14 cM telomeric from D5S424,  $p = 0.009$  and LOD = 1.20 at D14S70,  $p = 0.009$ ) while the 18p11 region that led to the highest evidence for linkage to this score (LOD = 2.40,  $p = 0.0004$ ) did not show any linkage signal to MR.

Prior to the bivariate genome-wide linkage analysis of the martingale residuals and asthma score, principal component analysis was applied to these two phenotypes and showed that the first and second principal components contributed respectively to 71%

and 29% of the overall phenotypic variance. For the first component, the coefficients for MR and asthma score were each equal to 0.71 while, for the second component, the coefficients were 0.71 for MR and -0.71 for asthma score.

The bivariate linkage analysis of martingale residuals and asthma score led to a substantial improvement of the linkage signal on 5q13 (combined test statistic T being equal to 16.9,  $p = 0.00007$ ) at the same marker position (D5S424) found linked to MR by univariate analysis (Figure 3 and Table 2). This result provides significant evidence for a pleiotropic QTL influencing both variability in time to asthma onset and variation in asthma clinical expression score in this region. There was also indication for potential pleiotropic effect of 14q13 region on these two phenotypes ( $p = 0.006$ ), although the increase in evidence for linkage as compared to the univariate linkage analyses of each phenotype was relatively small (the p-values associated with the univariate linkage signals being each equal to 0.009). Alternatively, there was no evidence for shared determinants by MR and asthma score on either 1p31 potentially linked to time to asthma onset or 18p11 found linked to the asthma score.

Figure 3

## DISCUSSION

Currently, there is a debate on the disease phenotypes that need to be considered to progress in the understanding of the mechanisms underlying asthma (Anonymous 2006). Up to now, genetic studies have used dichotomous phenotypes, either for the disease status itself (asthma/non asthma) or for subtypes of asthma (eg asthma with early onset, severe asthma...). The present genome-wide scan is the first one that has examined time to asthma onset. It is also the first bivariate genome-wide linkage screen ever conducted for asthma phenotypes and which examined two phenotypes related to variability of disease expression, time to asthma onset and a score measuring variation in clinical expression. The screen for time to asthma onset revealed two potential linkage regions on 1p31 and 5q13. The bivariate linkage scan led to significant evidence for a pleiotropic QTL on 5q13 influencing both the variation in time to asthma onset and the asthma score.

Linkage analysis was based on the MLB method rather than on the more widely used VC method since it does not require normality assumption of the trait distribution. Simulations have shown that the MLB approach does not lead to an increase of type I error even in presence of strong departure from normality (Alcais and Abel 1999). This approach was therefore particularly appropriate for martingale residuals which displayed significant skewness and kurtosis. Moreover, it was the method used for the analysis of the categorical asthma score (Bouzigon et al. 2006). To our knowledge, the formal characterization of pleiotropic effects of genes underlying asthma-related phenotypes has received little attention whereas multivariate linkage analyses have been performed for some time for other complex diseases (Kullo et al. 2005; Marlow et al. 2003; Williams et

al. 1999). To carry out bivariate analysis of MR and asthma score, we extended the approach proposed by Mangin et al. (Mangin et al. 1998) for multivariate variance components analysis to the MLB method. The principle of replacing the likelihood-ratio test statistic of the multivariate linkage analysis by the sum of test statistics obtained from univariate linkage analyses of principal components of correlated phenotypes has been also used in the context of Haseman-Elston (H-E) sib-pair method for quantitative traits (Elston et al. 2000). This PC-based H-E multivariate test was shown to have identical and even higher power than a more complex multivariate H-E test (Gorlova et al. 2002). Although principal components are linear functions of the original phenotypes, simulations have shown that linkage analysis of standard principal components can detect accurately the genetic model underlying complex quantitative phenotypes (Moser et al. 2001). Moreover, this PC-based approach can be easily extended to the analysis of multiple traits without increasing the number of parameters to be estimated which raises major numerical problems when using the full multivariate approach.

Among the two linkage regions that were detected for time to asthma onset, 1p31 was previously revealed by a linkage screen for dichotomous asthma in the same EGEA families ( $p = 0.005$ ) (Bouzigon et al. 2004). Further analysis showed that the 1p31 region was likely to contain a gene specific of the co-morbidity defined by the presence of both asthma and allergic rhinitis (Dizier et al. 2007). Interestingly, the frequency of allergic sensitisation is known to differ in early-onset and late-onset asthmatic patients, which may partly account for the linkage signal detected here for asthma age at onset. The 5q13 region potentially linked to time to onset for asthma was also harbouring a linkage signal



for the asthma score, although smaller and 14 cM apart from the former signal. Bivariate analysis of these phenotypes provided strong evidence for the presence of a pleiotropic QTL on 5q13 influencing time to asthma onset and asthma score. Computation of the ratio of each PC-likelihood-ratio test statistic to the combined test statistic showed a higher contribution from PC<sub>2</sub> (62%) than PC<sub>1</sub> (38%). The first principal component appeared to represent the whole variation of both the asthma score and time to onset, the lowest PC<sub>1</sub> values being observed in non-asthmatics with oldest age at examination (43 years of age) and the highest PC<sub>1</sub> values being observed in asthmatics with the most severe form of disease and youngest age at onset (< 1 year of age). For the second principal component, the lowest values were observed in most severely affected asthmatics with oldest age at onset (30 years of age) while the highest values were obtained in asthmatics with the mildest form of disease and youngest age at onset (< 1 year of age). Note this 5q13 region was not detected by our previous screen of dichotomous asthma and seven asthma-associated phenotypes involved in atopy, inflammation and lung function (Bouzigon et al. 2004). This demonstrates that together considering more refined phenotypes representing variation in disease expression and use of multivariate analysis can increase power to detect new asthma genes. Alternatively, the 14q13 region included potential linkage signals to both time to asthma onset and asthma score at the same marker position but pleiotropic effect of this region was not highly supported, although this latter result would deserve to be confirmed by further genetic studies. Finally, the 18p11 region detected for the asthma score did not appear to influence variation in age at asthma onset. Previous investigation of heterogeneity of linkage to dichotomous asthma according to age at onset in the same EGEA sample

indicated the presence of a genetic factor modifying asthma age at onset in the 7q21 region (Dizier et al. 2001), a region not detected by the present screen. However, this analysis was part of a first screen using a less dense set of markers (254 microsatellites) than the present one (396 microsatellites) and was based on a different statistical methodology that required stratifying affected sib-pairs using an *a priori* cut-off point, which was chosen to be the median of age-at-onset distribution (4 years of age). This 7q21 signal corresponded to a genetic factor underlying asthma with a different effect according to age at onset (before or after 4 years of age) while the analysis conducted here allowed to reveal genetic factors influencing the whole variation in time to asthma onset in both asthmatics and non-asthmatics. Altogether, these results underline the complexity of the mechanism involved and the interest of combining multiple approaches to unravel the genetic determinants.

As in most genome-wide scans conducted for asthma, our univariate linkage signals did not reach the stringent genome-wide significance level ( $p \leq 2.10^{-5}$ ) proposed by Lander and Kruglyack (Lander and Kruglyack 1995). However, this criterion applied to very dense maps of markers and, if the map is less dense, as it is the case here, it becomes conservative. To our knowledge, genome-wide significance thresholds for bivariate linkage analysis have not been derived. Although bivariate and univariate linkage test statistics cannot be directly compared, we computed the 1-df LOD score ( $LOD_{[1]}$ ) required to give the same p value as is given by the true bivariate test statistic (Almasy et al. 1997). On chromosome 5q13, the  $LOD_{[1]}$  was 3.15 ( $p = 0.00007$ ), which could be considered as significant (Morton 1998). Nevertheless, it has been often advocated that

replication of linkage results across studies are more important than a single high linkage peak and can provide support for the actual involvement of linkage regions. We have carried out an exhaustive compilation of linkage results from published genome scans performed to date in 16 different populations (without counting the EGEA study). We considered all previously reported linkage peaks with  $p \leq 0.01$ , located at 20 cM on either side of the three linkage signals revealed by the present screen. The 5q13 region was reported linked to dichotomous asthma in the Hutterites (an isolated population with European ancestry) and to a lung function phenotype ( $FEV_1$ ) in Australian twins (Ferreira et al. 2005; Ober et al. 2000). Interestingly, a linkage signal located at 30 cM of our peak was also detected in German families for an early asthma onset phenotype (Altmuller et al. 2005). This study examined subtypes of asthma which were analyzed as dichotomous phenotypes, early-onset asthma sib-pairs including one asthmatic sib with asthma symptoms occurring before the age of two years and the other sib having asthma onset before the age of four years. However, a genome screen conducted for variation in wheezing age of onset among asthmatics in an initial smaller sample of those German families (as part of an international Genetic Analysis Workshop) did not identify the 5q13 region but reported evidence for linkage to 6q24-25 (Alcais et al. 2001). Several genome screens have reported linkage signals to the 1p31 region for asthma and a few allergic phenotypes (IgE, atopy and atopic dermatitis) (Altmuller et al. 2005; Blumenthal et al. 2004; Bradley et al. 2002; Haagerup et al. 2002; Koppelman et al. 2002; Mathias et al. 2001; Xu et al. 2001). The 14q13 region was reported linked to asthma, atopy and lung function by three genome-wide screens (CSGA 1997; Blumenthal et al. 2004; Postma et al. 2005).

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The QTL detected on 5q13 is more likely to play a role in childhood asthma and possibly at a young age since 75% of the siblings investigated by the present study were less than 18 years old and 75% of asthmatics had an age of onset of asthma less than or equal to 8 years of age. In an attempt to identify potential candidate genes in the 5q13 region, we found a potential candidate gene, the corticotropin-releasing hormone-binding protein gene (CRHBP). This gene encodes a protein that inactivates the corticotropin-releasing hormone (CRH) activity. This hormone has a central anti-inflammatory action by stimulating the hypothalamic-pituitary-adrenal axis and endogenous glucocorticoid production. In knock-out mice, CRH was shown to enhance allergen-induced airway inflammation and lung dysfunction (Silverman et al. 2004). Further investigation of this gene as possibly influencing asthma expression and age of onset is therefore warranted.

In conclusion, the present study shows that both use of quantitative phenotypes of varying disease expression, time to asthma onset and asthma score, and suitable statistical methodology can improve power to detect new regions harbouring genes that may play an important role in onset and course of disease.

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### **Legend to figure**

Figure 1.

Box-plots of martingale residuals presented for the five values of the asthma score. The black dots indicate the medians.

Figure 2.

Multipoint results of the genome-wide univariate linkage screen for time to asthma onset and asthma score, and of bivariate screen conducted for these two phenotypes in 110 EGEA families with at least two asthmatic sibs.  $\text{Log}_{10}$  p-values are shown on the vertical axis and map distances (in cM) on the horizontal axis.

Figure 3.

Multipoint results of univariate linkage analysis for time to asthma onset and asthma score, and of bivariate analysis conducted for these two phenotypes in EGEA families on chromosome 5.  $\text{Log}_{10}$  p-values are shown on the vertical axis and map distances (in cM) on the horizontal axis.

Table 1. Phenotypic characteristics of 288 genotyped (asthmatics and non-asthmatics) siblings in EGEA families with at least two asthmatic sibs

<b>All siblings</b>			
Asthma, n (%)	237	(82.3)	
Asthma score, n (%)			
0	49	18.6	
1	100	38.0	
2	51	19.4	
3	31	11.8	
4	32	12.2	
<b>Asthmatics</b>			
Sex, n (%male)	141	(59.5)	
Age at examination (years), median (25-75% quartiles)	12.0	(10-18)	
Age at asthma onset (years), median (25-75% quartiles)	4.0	(2-8)	
Duration of asthma disease (years), median (25-75% quartiles)	8.0	(5-11)	
<b>Non-asthmatics</b>			
Sex, n (%male)	26	(53.0)	
Age at examination (years), median (25-75% quartiles)	12.0	(8-18)	

Table 2. Results of univariate and bivariate multipoint linkage analysis for time to asthma onset and asthma score in French EGEA families.

Markers	position*	Time to asthma onset		Asthma score		Bivariate analysis	
		LOD	p	LOD	p	T <sup>†</sup>	p
D1S255	65.5	1.13	0.01	0.00	n.s.	0.98	n.s.
D1S2797	75.7	<b>1.70</b>	<b>0.003</b>	0.10	n.s.	5.89	0.02
D1S2890	85.7	0.63	0.04	0.00	n.s.	4.59	0.04
D5S647	74.1	0.55	0.06	0.02	n.s.	7.95	0.007
D5S424	82.0	<b>1.87</b>	<b>0.002</b>	0.49	0.07	<b>16.85</b>	<b>0.00007</b>
D5S641	92.4	0.55	0.06	0.59	0.05	2.51	0.12
D5S428	95.4	0.35	n.s.	1.22	0.009	3.66	0.07
D14S70	40.1	1.21	0.009	1.20	0.009	<b>8.30</b>	<b>0.006</b>
D14S288	47.5	0.99	0.02	0.75	0.03	5.61	0.02
D14S276	56.4	0.71	0.03	0.62	0.05	5.13	0.03
D18S464	31.2	0.02	n.s.	2.06	0.001	1.40	n.s.
D18S53	41.2	0.02	n.s.	<b>2.40</b>	<b>0.0004</b>	0.87	n.s.
D18S478	52.9	0.01	n.s.	1.37	0.006	0.11	n.s.

\* cM from pter position of linkage peak based on Marshfield map

<sup>†</sup> T: linkage test statistic constructed by summing the resulting univariate test statistics (MLB likelihood-ratio statistics) for the two principal components (see text for detailed information)

n.s.: non-significant p-value