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Evidence for linkage of a new region (11p14) to eczema and allergic diseases

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Abstract

SUMMARY

Asthma, allergic rhinitis (AR) and atopic dermatitis also called eczema are allergic co-morbidities which are likely to depend on pleiotropic genetic effects as well as on specific genetic factors. After a previous genome-wide linkage screen conducted for asthma and AR in a sample of 295 French EGEA families ascertained through asthmatic subjects, the aim here was to search for genetic factors involved in eczema and more particularly those ones shared by the three allergic diseases using the same EGEA data. In this sake, eczema and phenotypes of 'allergic disease' accounting for the joint information on the presence/absence of the three diseases were examined by linkage analyses using the Maximum Likelihood Binomial (MLB) method. A fine mapping was carried out in regions detected for potential linkage, followed by association studies using the Family Based Association Test (FBAT). Evidence for linkage to 11p14 region was shown for 'allergic disease' and eczema. Linkage was also indicated between eczema and 5q13 and between 'allergic disease' and both 5p15 and 17q21 regions. Fine mapping supported the evidence of linkage to 11p14 and FBAT analyses showed association between 'allergic disease' and a marker located at the linkage peak on 11p14. Further investigations in this region will allow identifying genetic factor(s) which could have pleiotropic effect in the three allergic diseases.

MESH Keywords Adolescent ; Adult ; Child ; Chromosomes ; Human ; Pair 11 ; Eczema ; genetics ; Female ; Genetic Markers ; Genetic Screening ; Humans ; Hypersensitivity ; genetics ; Linkage (Genetics) ; Lod Score ; Male ; Nuclear Family ; Questionnaires

Author Keywords atopic dermatitis ; linkage analysis ; genome screen ; fine mapping ; association study

Introduction

Pleiotropy is likely to play an important role in the genetic determinism of multifactorial diseases as shown by genome-wide linkage screens (Kullo et al. 2005; Marlow et al. 2003; Williams et al. 1999) and recently genome-wide association studies of complex disorders (the WTCCC, 2007). Asthma, allergic rhinitis (AR) and atopic dermatitis (AD) also called eczema, are allergic co-morbidities which may share genetic determinants as suggested by their strong associations at both the individual and familial levels (Dold et al. 1992). Genes with pleiotropic effects underlying these three diseases, may be involved in allergic processes or in the barrier mechanisms of the epithelium (of either the skin, the nose or the lung). However there may also be genes specific to two of the three diseases, e.g. specific to the airway organs as nose and lung, or specific to each of the three diseases.

Few genome-wide searches have been conducted for AR (Haagerup et al. 2001; Yokouchi et al. 2002, Dizier et al. 2005; Bu et al. 2005) and AD (Lee et al. 2000; Cookson et al. 2001; Bradley et al. 2002; Haagerup et al. 2004) contrasting with the numerous genome screens performed for asthma and asthma-associated phenotypes (Wills-Karp and Ewart 2004; Bouzigon et al. 2005). These scans have revealed a small number of regions potentially linked to 2 or 3 of those diseases, but none of them as yet examined jointly these diseases. Candidate gene studies have reported a few genes associated with at least two of these diseases including genes involved in the allergic response such as TLR2 (4q31.3) (Ahmad-Nejad et al. 2004), IL-13 (5q31) (Tsunemi et al. 2002; He et al. 2003; Liu et al. 2000), FCER1B (11q12) (Folster-Holst et al. 1998; Cox et al. 1998), TGFBI (19q13) (Arkwright et al. 2001) and CCL5 (17q11) (Nickel et al. 2000), indicating pleiotropic effect of these genes.

The EGEA study (Epidemiological study on the Genetics and Environment of Asthma) was designed to characterize genes and environmental factors underlying asthma related phenotypes in families ascertained through asthmatic subjects. This study includes extensive information on various biological and physiological phenotypes as well as asthma-associated diseases as AR and eczema. Genome scans were recently carried out in the 295 EGEA family sample for seven asthma/atopy associated phenotypes (Bouzigon et al. 2004) and for asthma and AR (Dizier et al. 2005). Joint analysis of asthma and AR showed strong evidence for a genetic component on the 1p31 region underlying the co-morbidity of asthma plus AR (Dizier et al. 2007). Our present aim was to complete these scans by searching for genetic regions linked to eczema and more precisely to characterize the regions shared by eczema and the two other allergic diseases, asthma and AR. For this purpose, we considered an 'allergic disease' phenotype which was defined in two ways: 1/by the presence of at least one of the allergic diseases (asthma, AR and eczema), 2/using an ordered categorical trait varying from 0 to 3 depending on the number of allergic diseases presented by a subject. The genome-wide linkage analyses for eczema and 'allergic disease' phenotypes were conducted in the sample of 295 EGEA families using the original panel of 378 microsatellites. In regions showing indication of linkage, we conducted fine mapping and association studies in the vicinity of the linkage peaks. The present study is the first genome linkage scan examining jointly asthma, AR and eczema to search for pleiotropic genes involved in these three diseases.

Material and Methods

Family data

The main EGEA sample included 348 nuclear families selected through one asthmatic proband. An additional sample of 40 nuclear families was selected through two asthmatic sibs in order to increase the number of informative families for linkage analyses of asthma. Inclusion criteria met by probands were described elsewhere (Kauffmann et al. 1999). The present scan was applied to 307 EGEA families having at least two sibs with DNA available (Bouzigon et al. 2004). After excluding families with insufficient DNA available or showing Non-Mendelian transmission, the analyzed sample included 295 families (n = 1317 individuals). Ethical committee of the hospital group 'Cochin Saint Vincent de Paul-Sainte Anne' approved the protocol and subjects signed informed consent forms.

Phenotypes analyzed

Information on respiratory and allergic symptoms, medical history and environmental factors was collected using a standardized questionnaire (Kauffmann et al. 2002). Various physiological tests were performed, which have been already described (Kauffmann et al. 1999) and included bronchial responsiveness and skin prick tests to common aeroallergens. Eczema was defined by answers to a questionnaire in adults and children. For children, the questions asked to the child's mother (or father) were: 1a/Have your child had eczema before two years of age? 2a/After two years of age? and for adults: 1b/Have you had eczema during childhood? The precise time of eczema onset (before/after 2 years of age) was not asked to adults in order to avoid a large proportion of wrong or missing responses.

Two definitions of eczema were possible: 1) a positive response to 1a for children and to 1b for adults, 2) a positive response to 1a or 2a for children and to 1b for adults. We only used here the second definition since it has the advantage to be homogenous in children and adults. The high frequency of atopy defined by a positive skin test response to at least one allergen in both children and adults reporting eczema

strengthened the validity of our eczema definition. Definitions of asthma and AR used in linkage analyses of the 'allergic disease' phenotype have been previously described (Dizier et al. 2005, 2007). Asthma (AST) was defined using the following criteria: a positive response to at least one of the two questions (1/Have you ever had attacks of breathlessness at rest with wheezing? 2/Have you ever had an asthma attack?), associated with either the presence of BHR (defined as a fall in baseline FEV₁, the forced expiratory volume at one second, 20% at 4mg/ml methacholine), an increase of baseline FEV₁ 12% after bronchodilator use, hospitalization for asthma in life or asthma therapy. The AR trait was defined by the presence of at least one of the following symptoms: sneezing or runny nose to hay/flower or animals or dust.

The 'allergic disease' phenotype was defined in two ways 1/by the presence of at least one of the 3 allergic diseases (ECZ±AST±AR), and that, whatever the disease(s) was, 2/using an ordered categorical trait varying from 0 to 3 depending on the number of allergic diseases presented by a subject.

Genotypes

A total of 1317 subjects were genotyped with a panel of 396 microsatellites (378 autosomal markers). The genotyping protocol was described elsewhere (Bouzigon et al. 2004; Dizier et al. 2005). The Linkage Marker Set MD 10 (Applied Biosystems, Foster City, CA, USA) formed the core marker set for the genome-wide screen. The microsatellite markers, labelled with fluorescent dyes (FAMTM, HEXTM, NEDTM), are distributed at an average marker density of 10 centimorgan (cM roughly every 10 million bases in the genome) and have an average heterozygosity of 75%.

Before statistical analysis, rigorous genotype quality assurance was performed to ensure accurate binding of alleles. In the four regions detected potentially linked to eczema and/or to the 'allergic disease' phenotype (s), fine mapping was carried out by genotyping additional markers in an interval spanning about 10 cM on each side of the linkage peaks: 2 markers on 5p15, 3 markers on 5q13, 8 markers on 11p14-q13 and 4 markers on 17q21.

Consistency of the data with Mendelian inheritance and presence of double recombination between loci was evaluated using Pedcheck (O'Connell and Weeks 1998) and Mendel (Lange et al. 2001). Hardy-Weinberg equilibrium was also checked for all markers.

Linkage analysis methods

Linkage analyses were performed by a model-free approach, the Maximum Likelihood Binomial (MLB) method which is likelihood-based and can be applied to the whole sibship of affected subjects (Majumder and Pal 1987; Satsangi et al. 1996; Abel et al. 1998). In brief, the principle of this method is based on the binomial distribution of the number of affected sibs receiving a given parental allele from heterozygous parents. The probability α for an affected sib to receive from his/her parent the marker allele transmitted with the disease allele is equal to 0.5 under the null hypothesis of no linkage and α is greater than 0.5 under the hypothesis of linkage. Test for linkage was performed using a likelihood ratio test statistic, $=2\text{Ln}[L(\alpha)/L(\alpha=0.5)]$ with the statistic being distributed asymptotically as a mixture distribution of 0.5^2 0df and 0.5^2 1df. The test can also be expressed either as a one-sided standard normal deviate denoted as $Z_{\text{MLB}} = \frac{1}{2}$ or as a lod score criterion, $\text{lod MLB} = \frac{1}{2} \ln(10)$. This method has been extended to categorical traits by introducing a latent binary variable ($Y=\{0,1\}$) which captures the linkage information between the observed categorical phenotype (Z) and the marker (M). This method requires to assign the probability of the latent variable (i.e. being affected or not) according to each observed category of the phenotype. The likelihood of the observations is then written by use of binomial distributions of parental marker alleles among offspring according to the value of the unobserved binary variable. Multipoint linkage analyses of binary and polychotomous phenotypes were conducted with MLBGH (Abel and Müller-Myshok 1998) which incorporates the MLB method into the multipoint approach of Genehunter (Kruglyak et al. 1996).

Family based association test

Association studies were performed using the FBAT method (Horvath et al. 2001), which is based on the following score S_i for n_i offspring in the i th family: $S_i = \sum_{j=1, n_i} X_{ij} T_{ij}$, where T_{ij} is a function of j th offspring phenotype and X_{ij} a function of j th offspring genotype in family i . The standard choice, as classically used by the TDT (Spielman et Ewens, 1998), is to let $T_{ij}=1$ for affecteds and $T_{ij}=0$ for unaffecteds and X_{ij} be the number of A alleles (additive coding). We used the standard coding for T_{ij} and both the multi-allelic and additive codings for X_{ij} . When considering the additive coding (i.e. testing alternatively one allele against all other 2 ones), $[S-E(S)]/\text{Var}(S)$ follows a χ^2 with 1 degree of freedom while for the multi-allelic coding, the statistic $(S-E(S))\text{Var}(S)^{-1} (S-E(S))$ has an approximate χ^2 distribution with degrees of freedom equal to the number of alleles minus one. This method was applied to test association in presence of linkage. We also used the approach using the empirical variance-covariance estimator which is robust to the correlation among sibling marker genotypes (Lake et al. 2000). The Family-Based Association Test was conducted for all genotyped microsatellites in all regions detected by the MLB approach.

Results

Distribution of eczema

Among the 726 genotyped siblings of the 295 EGEA families selected through one asthmatic subject, frequencies of sibs with eczema and 'allergic disease' (ECZ ± AST ± AR) were respectively equal to 33% and 72% (Table 1). These frequencies did not differ according to gender for eczema ($p=0.77$) but were significantly higher in males than in females for 'allergic disease' ($p=0.01$). Mean ages among siblings with eczema and with 'allergic disease' were 14.3 and 15.8 years respectively. The proportion of sibs with asthma and AR among those having eczema and the distribution of asthma, eczema and AR among sibs with 'allergic disease' are also given in Table 1. We also examined % of atopy or SPT (defined by a positive skin test response to at least one allergen) in siblings with eczema and 'allergic disease'. Frequencies of asthma, AR and SPT among sibs with eczema were respectively equal to 71%, 52% and 77%. Among siblings having 'allergic disease', frequencies of eczema, asthma, AR and SPT were equal respectively to 46%, 77%, 62% and 78%. The proportion of siblings with 'allergic disease' having all three diseases (ECZ+AST+AR) is as high as 20%, demonstrating the strong relationship among these diseases. However, these frequencies may be partly explained by the ascertainment of our sample through asthmatic subjects. Considering the 'allergic disease' phenotype defined by the ordered categorical trait, the numbers of siblings with (0, 1, 2 or 3) disease(s) were (207, 197, 218 or 104). The distributions of the 295 EGEA families according to the number of genotyped sibs available for linkage analysis of eczema and 'allergic disease' phenotypes are shown in Table 2. To provide a summary indicator of the amount of information available for analysis, the numbers of independent sib-pairs computed from sibship sizes (a sibship of size n corresponds to $(n-1)$ independent sib-pairs) are also given in Table 2.

Linkage analysis

Linkage peaks (expressed in terms of LOD-MLB scores) detected at $p \leq 0.005$ are presented in tables 3 (eczema) and 4 (ECZ ± AST ± AR). LOD-MLB curves for chromosomes harbouring these peaks are shown in Figures 1 (eczema) and 2 (ECZ ± AST ± AR). The Information Content (IC), which measures the informativity of the data for linkage analysis, was also given at each marker position on these figures. For eczema, one region, 5q13, showed a linkage peak with $p=0.002$ and another one, 11p14, contained a peak with $p=0.0007$.

Interestingly, the 'allergic disease' phenotype (ECZ ± AST ± AR) was also found to be linked to the 11p14 region ($p=0.0005$). Two other regions were revealed: 5p15 ($p = 0.002$) and 17q21 ($p=0.003$). When analyzing the 'allergic disease' phenotype defined by the ordered categorical trait, a linkage peak was also detected in the 11p14 region, but with a smaller intensity ($p=0.01$). No other region was detected (results not shown).

As shown in figure 1, fine mapping, conducted in the two regions potentially linked to eczema, led to an increase in evidence of linkage for eczema on both 5q13 ($p = 0.0007$) and 11p14 ($p = 0.00005$). However, the fine mapping did not change much the linkage results on 5p15, 11p14 and 17q21 for the 'allergic disease' phenotype (ECZ ± AST ± AR) (table 4).

Family-based association analyses

FBAT analysis did not indicate any linkage/association to eczema (i.e. no result leading to a p value ≤ 0.005) in any region detected by the MLB method. In contrast, evidence for linkage and association was found between 'allergic disease' (ECZ ± AST ± AR) and D11S4152 marker located on 11p14 region ($p=0.004$ and 0.05 using FBAT without and with the empirical variance/covariance estimator respectively) at the same position as the MLB linkage peak.

Discussion

The present genome-wide scan is the first one searching the existence of genetic determinants predisposing to any of the three diseases, asthma, AR and eczema. It also allowed making comparisons of the linkage signals detected here for eczema to those previously reported for asthma and AR in the same families (Dizier et al. 2005). Concerning eczema, there is a strong linkage signal with the 11p14 region. Evidence for linkage to this region was further supported by the fine mapping with an improvement of the linkage signal (p -value decreasing from $p=0.0007$ to 5.10^{-5}). Indication of linkage is also showed for another region 5q13. While 5q13 was not previously detected for asthma or AR in the EGEA study, there was in the 11p14 region a small linkage signal for the two diseases. No linkage signal was detected for eczema to regions reported in our previous scans conducted for AR and/or asthma (1p31, 2q32, 3p24-p14, 9p22 and 9q22-q34) (Dizier et al. 2005). A strong linkage signal of the 11p14 region was also observed with the 'allergic disease' phenotype defined by the presence of at least one allergic disease. Moreover, an association found between this phenotype and a marker on 11p14 region, located at the linkage peak, strengthened this linkage result. Two additional regions, 5p15 and 17q21, were also detected for linkage to 'allergic disease', but with a smaller intensity.

None of the regions detected by the previous genome scans conducted for AD (Lee et al. 2000, Cookson et al. 2001, Bradley et al. 2002, Haagerup et al. 2004) was found by the present analysis. However, reported results were already heterogeneous across the four previous published AD/eczema genome scans. Heterogeneity of results could be due to different modes of family ascertainment, phenotype definitions and linkage analysis statistics. There was indeed heterogeneity regarding the mode of ascertainment, since eczema family samples in previous scans were recruited through one or two affected siblings while the present sample consisted of families with at least two affected siblings, selected from the primary sample of 295 EGEA families ascertained through asthmatic subjects. Interestingly, the Swedish and Danish studies (Bradley et al. 2002; Haagerup et al. 2004) showing the most similar results (3p and 18q detected by both scans) used the most similar phenotype definitions (clinical characteristics associated with elevated specific IgE levels to at least one allergen). Note also, that we used, here, responses to diagnosis questions on eczema and not presence of clinical symptoms or presence of atopy. However, most siblings with eczema in our sample were atopic (77%). We repeated linkage analyses by examining eczema associated with a positive skin test response to at least one allergen, as also recommended by the Nomenclature review committee of the World Allergy Organisation (2003) (Johansson et al. 2004). This did not lead to detect any of the regions found in the Swedish and Danish scans (Bradley et al. 2002; Haagerup et al. 2004), nor regions reported by any other scans (data not shown). However the 11p14 region was still detected although in a slightly lesser extent ($p=0.001$), probably because of the important decrease in number of affected sibs. Some siblings may have been discarded since the tested allergens did not include food allergens. It has indeed been shown that, early in life, eczema is marked by a much higher frequency of food sensitization than aeroallergen sensitization (Guillet and Guillet 1992). Moreover, a transient form of eczema without any detectable IgE sensitization has been described in children, which develop a sensitization to aeroallergens and to food allergens later in life (Novembre et al. 2001). We also repeated linkage analyses by considering as additional criterion for eczema, an age at onset before two years of age for the children. That is considered as a good indicator of the presence of atopic eczema. This information being not available for adults, the criterion was thus unchanged for adults, i.e. an onset during childhood. These analyses led to an evidence of linkage as strong as the one with the eczema phenotype used here and allowed us to check our findings with the 11p14 region. One main topic of our study was to search for pleiotropic effect between the allergic diseases. In this sake, we previously examined eczema associated with either asthma or AR (as already done for asthma associated with AR), but there was no improvement of any linkage signal (result not shown). Moreover, it was not possible to analyze the most stringent phenotype of eczema associated with both asthma and AR because of the too small number of affected sibs.

In the same spirit, we then examined the 'allergic disease' phenotypes. Linkage analysis of the 'allergic disease' phenotype defined by the presence of any of the three diseases, revealed a strong linkage signal to 11p14. This latter result might be partly explained by the strong evidence of linkage of this region to eczema and by the linkage signals to AR ($p=0.01$) and asthma ($p=0.02$). When using the other 'allergic disease' phenotype defined by an ordered categorical trait, a linkage signal although of small magnitude, was also found in the 11p14 region. These different results could suggest that a genetic factor in the 11p14 region is involved more likely in the presence/absence of the allergic disease than in the intensity of the disease measured by the ordered categorical trait, i.e. the number of allergic disease(s) presented by the subject. Finally the family sampling through asthmatic subjects might have affected the conclusions of our analyses by leading to detect preferentially genes involved in both eczema and asthma (i.e. with pleiotropic effect). Indeed most sibpairs with eczema were also asthmatic. That could explain why the 11p14 region was founded here linked to both eczema and 'allergic disease'.

Regions found in our study linked to eczema and/or to 'allergic disease' have been previously reported by EGEA scans for other phenotypes and/or by other genome scans for asthma and asthma-related phenotypes. The 5q13 region potentially linked here to eczema and to 'allergic disease', was reported by a Danish scan (Haagerup et al. 2001) that showed a small linkage signal to AR. The 11p14 region detected here to be linked to eczema and 'allergic disease' was reported for atopy and asthma related phenotypes in some studies (Wjst et al. 1999, Altmuller et al. 2005, Ober et al. 2000, Postma et al. 2005, Ferreira et al. 2006). Note that there was no indication of linkage of this region, neither to IgE nor to SPT, but only an indication of linkage to AR and asthma in the whole EGEA sample (Dizier et al. 2005) and to IgE in a subset of families with at least 2 two asthmatic sibs (Dizier et al. 2000). The 17q21 region, found here potentially linked to 'allergic disease', indicated linkage to atopy in the EGEA families (Bouzigon et al. 2004) and was reported linked to atopy and asthma-related phenotypes by other scans (CSGA 1997; Koppelman et al. 2002). This region contains a few candidate genes shown to be associated to AD and which are involved in the immune response as SCYA5 and RANTES (Nickel et al. 2000). Finally, the 5p15 region suggesting linkage to 'allergic disease' indicated linkage to atopy in EGEA families (Bouzigon et al. 2004), to BHR in the Hutterites (Ober et al. 2000), to asthma in Afro-American CSGA families (1997) and to AD in the Swedish families (Bradley et al. 2002).

We chose here a quite stringent threshold ($p 0.005$) to select linked regions, because of the increase of type one error due to multiple testing. As in the vast majority of published scans for asthma, none of our results reached the genome-wide significant level ($p 2.10^{-5}$) proposed by (Lander and Kruglyack 1995), although LOD scores in the 11p14 region reached the criterion of suggestive linkage ($p 7.10^{-4}$) (Lander and Kruglyack 1995). Evidence for linkage to 11p14 was further supported by the fine mapping that led to a strong improvement of the

linkage signal (p-value decreasing from $p=0.0007$ to 5.10^{-5}), the former signal being close to the genome-wide significant level (Lander and Kruglyack, 1995).

In conclusion, evidence of linkage of 11p14 to eczema and 'allergic disease' was here well supported. Moreover, the association found between the 'allergic disease' phenotype and a marker located at the 'allergic disease' linkage peak in the 11p14 region, strengthened the linkage results and suggested that a gene with a pleiotropic effect tightly linked to that peak, may be involved in the allergic diseases. However, whether the 11p14 region contains one or more genetic factor(s) require further investigation. Association studies with SNPs covering this region and with candidate genes involved in the allergic response and the skin inflammation response are currently being carried out which can lead to identify the genetic factor(s) on 11p14.

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References:

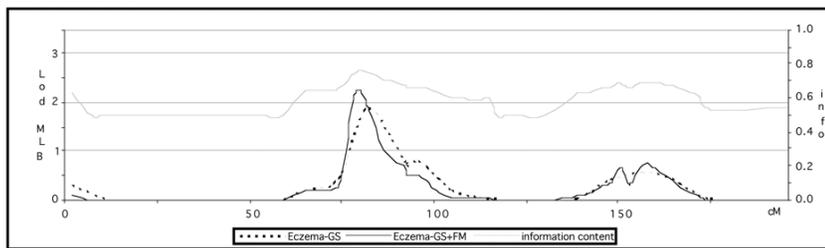
- Abel L , Alcais A , Mallet A 1998; Comparison of four sib-pair linkage methods for analyzing sibships with more than two affecteds: interest of the binomial maximum likelihood approach. *Genet Epidemiol.* 15: (4) 371- 390
- Abel L , Muller-Myhsok B 1998; Robustness and power of the maximum-likelihood-binomial and maximum-likelihood-score methods, in multipoint linkage analysis of affected-sibship data. *Am J Hum Genet.* 63: (2) 638- 647
- Ahmad-Nejad P , Mrabet-Dahbi S , Breuer K , Klotz M , Werfel T , Herz U , Heeg K , Neumaier M , Renz H 2004; The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol.* 113: (3) 565- 567
- Altmuller J , Seidel C , Lee YA , Loesgen S , Bulle D , Friedrichs F , Jellouschek H , Kelber J , Keller A , Schuster A , Silbermann M , Wahlen W , Wolff P , Schlenvoigt G , Ruschendorf F , Nurnberg P , Wjst M 2005; Phenotypic and genetic heterogeneity in a genome-wide linkage study of asthma families. *BMC Pulm Med.* 5: 1-
- Arkwright PD , Chase JM , Babbage S , Pravica V , David TJ , Hutchinson IV 2001; Atopic dermatitis is associated with a low-producer transforming growth factor beta(1) cytokine genotype. *J Allergy Clin Immunol.* 108: (2) 281- 284
- Bouzigon E , Dizier MH , Krähenbühl C , Lemaître A , Annesi-Maesano I , Betard C , Bousquet J , Charpin D , Gormand F , Guilloud-Bataille M , Just J , Le Moual N , Maccario J , Matran R , Neukirch F , Oryszczyn MP , Paty E , Pin I , Rosenberg-Bourgoin M , Vervloet D , Kauffmann F , Lathrop M , Demenais F 2004; Clustering patterns of LOD scores for asthma-related phenotypes revealed by a genome-wide screen in 295 French EGEA families. *Hum Mol Gen.* 13: 3103- 3113
- Bouzigon E , Demenais F , Kauffmann F 2005; Genetics of asthma and atopy: how many genes?. *Bull Acad Natl Med.* 189: (7) 1435- 48
- Bradley M , Söderhäll C , Luthman H , Wahlgren CF , Kockum I , Nordenskjöld M 2002; Susceptibility loci for atopic dermatitis on chromosomes 3, 13, 15, 17 and 18 in a Swedish population. *Hum Mol Genet.* 11: (13) 1539- 1548
- Bu LM , Bradley M , Söderhäll C , Wahlgren CF , Kockum I , Nordenskjöld M 2005; Genome-wide linkage analysis of allergic rhinoconjunctivitis in a Swedish population. *Clin Exp Allergy.* 36: 204- 210
- Collaborative Study on the Genetics of Asthma (CSGA) 1997; A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat Genet.* 15: 389- 392
- Cookson WO , Ubhi B , Lawrence R , Abecasis GR , Walley AJ , Cox HE , Coleman R , Leaves NI , Trembath RC , Moffatt MF , Harper JI 2001; Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nat Genet.* 27: (4) 372- 3
- Cox HE , Moffatt MF , Faux JA , Walley AJ , Coleman R , Trembath RC , Cookson WO , Harper JI 1998; Association of atopic dermatitis to the beta subunit of the high affinity immunoglobulin E receptor. *Br J Dermatol.* 138: (1) 182- 187
- Dizier MH , Besse-Schmittler C , Guilloud-Bataille M , Annesi-Maesano I , Boussaha M , Bousquet J , Charpin D , Degioanni A , Gormand F , Grimfeld A , Hochez J , Hyne G , Lockhart A , Luillier-Lacombe G , Matran R , Meunier F , Neukirch F , Pacheco Y , Parent V , Paty E , Pin I , Pison C , Scheinmann P , Thobie N , Vervloet D , Kauffmann F , Feingold J , Lathrop M , Demenais F 2000; Genome screen for asthma and related phenotypes in the French EGEA study. *Am J Respir Crit Care Med.* 162: 1812- 1818
- Dizier MH , Bouzigon E , Guilloud-Bataille M , Betard C , Bousquet J , Charpin D , Gormand F , Hochez J , Just J , Lemaître A , Le Moual N , Matran R , Neukirch F , Oryszczyn MP , Paty E , Pin I , Vervloet D , Kauffmann F , Lathrop M , Demenais F , Annesi-Maesano I 2005; Genome Screen in the French EGEA Study: detection of linked regions shared or not shared by Allergic Rhinitis and Asthma. *Genes Immun.* 6: 95- 102

- Dizier MH, Bouzignon E, Guilloud-Bataille M, Genin E, Oryszczyn MP, Annesi-Maesano I, Demenais F 2007; Evidence for a Locus in 1p31 Region Specifically Linked to the Co-Morbidity of Asthma and Allergic Rhinitis in the EGEEA Study. *Hum Hered.* 63: (3-4) 162- 167
- Dold S, Wjst M, von Mutius E, Reitmeir P, Stiepel E 1992; Genetic risk for asthma, rhinitis, and atopic dermatitis. *Arch Dis Child.* 67: (8) 1018- 1022
- Ferreira MA, Visscher PM, Martin NG, Duffy DL 2006; A simple method to localise pleiotropic susceptibility loci using univariate linkage analyses of correlated traits. *Eur J Hum Genet.* 14: (8) 953- 62
- Folster-Holst R, Moises HW, Yang L, Fritsch W, Weissenbach J, Christophers E 1998; Linkage between atopy and the IgE high-affinity receptor gene at 11q13 in atopic dermatitis families. *Hum Genet.* 102: (2) 236- 239
- Guillet G, Guillet MH 1992; Natural history of sensitizations in atopic dermatitis. A 3-year follow-up in 250 children: food allergy and high risk of respiratory symptoms. *Arch Dermatol.* 128: (2) 187- 192
- Haagerup A, Bjerke T, Schiote PO, Binderup HG, Dahl R, Kruse TA 2001; Rhinitis-a total genome-scan for susceptibility genes suggests a locus on chromosome 4q24-q27. *Eur J Hum Genet.* 9: (12) 945- 952
- Haagerup A, Bjerke T, Schiote PO, Dahl R, Binderup HG, Tan Q, Kruse TA 2004; Atopic dermatitis -- a total genome-scan for susceptibility genes. *Acta Derm Venereo.* 84: (5) 346- 352
- He JQ, Chan-Yeung M, Becker AB, Dimich-Ward H, Ferguson AC, Manfreda J, Watson WT, Sandford AJ 2003; Genetic variants of the IL13 and IL4 genes and atopic diseases in at-risk children. *Genes Immun.* 4: (5) 385- 389
- Horvath S, Xu X, Laird NM 2001; The family based association test method: strategies for studying general genotype-phenotype associations. *Eur J Hum Genet.* 9: (4) 301- 306
- Johansson SGO, Bieber T, Dahl R, Friedmann PS, Lanier Q, Lockey RF, Motala C, Ortega Martell JAO, Platts-Mills TAE, Ring J, Thien F, Van Cauwenberge P, Williams HC 2004; Revised nomenclature of allergy for global use: Report of the nomenclature review committee of the world allergy organization, October 2003. *J Allergy Clin Immunol.* 113: 832- 836
- Kauffmann F, Dizier MH, Annesi-Maesano I, Bousquet J, Charpin D, Demenais F, Ecochard D, Feingold J, Gormand F, Grimfeld A, Lathrop M, Matran R, Neukirch F, Paty E, Pin I, Pison C, Scheinmann P, Vervloet D, Lockhart A 1999; EGEEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)-- descriptive characteristics. *Clin Exp Allergy.* 29: (Suppl 4) 17- 21
- Kauffmann F, Annesi-Maesano I, Liard R, Paty E, Faraldo B, Neukirch F, Dizier MH 2002; Construction et validation d'un questionnaire en épidémiologie respiratoire. L'exemple du questionnaire de l'étude épidémiologique des facteurs génétiques et environnementaux de l'asthme, l'hyperréactivité bronchique et de l'atopie (EGEEA). *Rev Mal Resp.* 19: (3) 323- 333
- Koppelman GH, Stine OC, Xu J, Howard TD, Zheng SL, Kauffman HF, Bleecker ER, Meyers DA, Postma DS 2002; Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J Allergy Clin Immunol.* 109: (3) 498- 506
- Kullo IJ, de Andrade M, Boerwinkle E, McConnell JP, Kardia SL, Turner ST 2005; Pleiotropic genetic effects contribute to the correlation between HDL cholesterol, triglycerides, and LDL particle size in hypertensive sibships. *Am J Hypertens.* 18: 99- 103
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES 1996; Parametric and nonparametric linkage analysis: A unified multipoint approach. *Am J Hum Genet.* 58: 1347- 1363
- Lake SL, Blacker D, Laird NM 2000; Family-based tests of association in the presence of linkage. *Am J Hum Genet.* 67: (6) 1515- 1525
- Lander E, Kruglyak L 1995; Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet.* 11: 241- 247
- Lee YA, Wahn U, Kehrt R, Tarani L, Businco L, Gustafsson D, Andersson F, Oranje AP, Wolkertstorfer A, van Berg A, Hoffmann U, Kuster W, Wienker T, Ruschendorf F, Reis A 2000; A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. *Nat Genet.* 26: (4) 470- 473
- Lange K, Cantor R, Horvath S, Perola M, Sabatti C, Sinsheimer JS, Sobel E 2001; Mendel version 4.0: A complete package for the exact genetic analysis of discrete traits in pedigree and population data sets. *Am J Hum Genet.* 69: A1886-
- Liu X, Nickel R, Beyer K, Wahn U, Ehrlich E, Freidhoff LR, Bjorksten B, Beaty TH, Huang SK 2000; An IL13 coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German multicenter atopy study (MAS-90). *J Allergy Clin Immunol.* 106: 167- 170
- Majumder PP, Pal N 1987; Nonrandom segregation: uniformly most powerful test and related considerations. *Genet Epidemiol.* 4: (4) 277- 287
- Marlow AJ, Fisher SE, Francks C, MacPhie IL, Cherny SS, Richardson AJ, Talcott JB, Stein JF, Monaco AP, Cardon LR 2003; Use of multivariate linkage analysis for dissection of a complex cognitive trait. *Am J Hum Genet.* 72: 561- 70
- Nickel RG, Casolaro V, Wahn U, Beyer K, Barnes KC, Plunkett BS, Freidhoff LR, Sengler C, Plitt JR, Schleimer RP, Caraballo L, Naidu RP, Levett PN, Beaty TH, Huang SK 2000; Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. *J Immunol.* 164: (3) 1612- 1616
- Novembre E, Cianferoni A, Lombardi E, Bernardini R, Pucci N, Vierucci A 2001; Natural history of 'intrinsic' atopic dermatitis. *Allergy.* 56: 452- 453
- Ober C, Tsalenko A, Parry R, Cox NJ 2000; A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet.* 67: (5) 1154- 1162
- O'Connell J, Weeks DE 1998; PedCheck: A Program for Identification of Genotype Incompatibilities in Linkage Analysis. *Am J Hum Genet.* 63: 259- 266
- Postma DS, Meyers DA, Jongepier H, Howard TD, Koppelman GH, Bleecker ER 2005; Genomewide screen for pulmonary function in 200 families ascertained for asthma. *Am J Respir Crit Care Med.* 172: 446- 452
- Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP 1996; Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet.* 14: (2) 199- 202
- Spielman RS, Ewens WJ 1998; A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. *Am J Hum Genet.* 62: (2) 450- 458
- Tsunemi Y, Saeki H, Nakamura K, Sekiya T, Hirai K, Kakinuma T, Fujita H, Asano N, Tanida Y, Wakugawa M, Torii H, Tamaki K 2002; Interleukin-13 gene polymorphism G4257A is associated with atopic dermatitis in Japanese patients. *J Dermatol Sci.* 30: (2) 100- 107
- The Wellcome Trust Case Control Consortium 2007; Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. *Nature.* 447: 661- 678
- Williams JT, Begleiter H, Porjesz B, Edenberg HJ, Foroud T, Reich T, Goate A, Van Eerdewegh P, Almasy L, Blangero J 1999; Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. II. Alcoholism and event-related potentials. *Am J Hum Genet.* 65: 1148- 60
- Wills-Karp M, Ewart SL 2004; Time to draw breath: asthma-susceptibility genes are identified. *Nat Genet.* 5: 376- 387
- Wjst M for the German asthma genetics group 1999; Specific IgE-one gene fits all?. *Clin Exp Allergy.* 29: S5- S10
- Yokouchi Y, Shibasaki M, Noguchi E, Nakayama J, Ohtsuki T, Kamioka M, Yamakawa-Kobayashi K, Ito S, Takeda K, Ichikawa K, Nukaga Y, Matsui A, Hamaguchi H, Arinami T 2002; A genome-wide linkage analysis of orchard grass-sensitive childhood seasonal rhinitis in Japanese families. *Genes Immun.* 3: 9- 13

Figure 1

Curves of Lod MLB scores of eczema of genome scan (GS) and fine mapping (FM) for chromosomes with Lod MLB scores ≥ 1.5 in the 295 families.

Chromosome 5



Chromosome 11

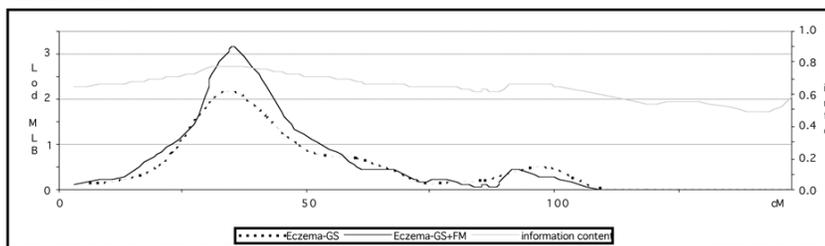
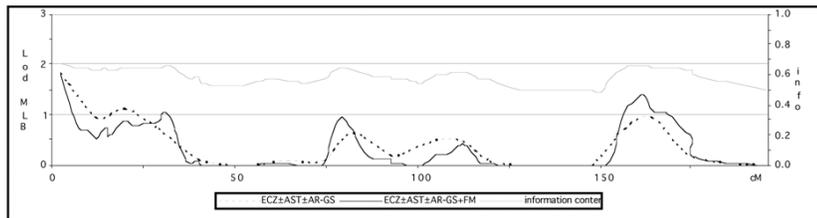


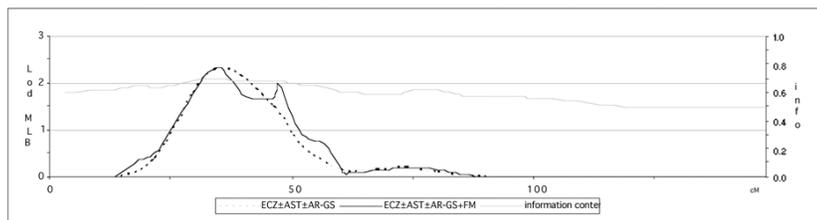
Figure 2

Curves of Lod MLB scores of 'allergic disease' phenotype (ECZ± AST ±AR) of genome scan (GS) and fine mapping (FM) for chromosomes with Lod MLB scores ≥ 1.5 in the 295 families.

Chromosome 5



Chromosome 11



Chromosome 17

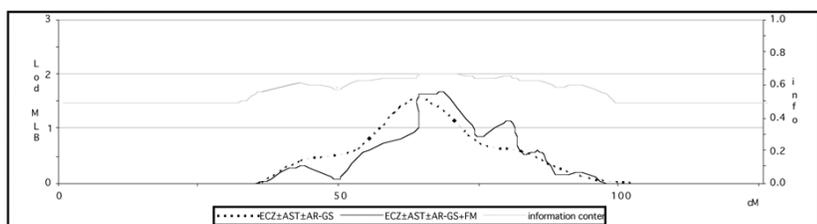


Table 1

Distribution of the eczema and 'allergic disease' phenotype (ECZ± AST ±AR) in the 726 genotyped siblings of the 295 EGEA families

	Frequency (%) in			
	All	Male	Female	
Eczema	33	34	32	
ECZ± AST ±AR	72	76	67	
		Frequency (%) of		
	Eczema	AST	AR	SPT
Among subjects:				
Eczema	100	71	52	77
ECZ± AST ±AR	46	77	62	78

Table 2

Distribution of families according to the number of genotyped sibs available for linkage analyses to eczema and 'allergic disease' phenotypes in the 295 families.

Phenotypes	2 sibs	3 sibs	4 sibs	5 sibs	total
Eczema	47	4	1	1	53 (62)
'allergic disease' ECZ± AST ±AR (1)	154	30	3	2	189(231)
'allergic disease' Categorical trait (2)	174	82	13	25	294 (477)

In parentheses are the corresponding numbers of affected sib-pairs.

(1) 'allergic disease' phenotype defined by the presence of at least one the 3 allergic diseases (asthma, AR, eczema).

(2) 'allergic disease' phenotype defined by the ordered categorical trait varying from 0 to 3 depending on the number of allergic diseases presented by a subject.

Table 3

Results of linkage analyses of eczema in the 295 families using the MLB method

Marker	Position* (cM)	GS	GS+FM
D5S2003	78		2.2 (0.0007)
D5S424	82	1.9 (0.002)	1.9 (0.001)
D5S672	86		1.1 (0.01)
D11S915	31		2.6 (0.0003)
D11S904	34	2.2 (0.0007)	3.0 (0.0001)
D11S4152	35		3.2 (0.00005)

In black are the markers of the genome scan (GS) and the results of linkage (lod MLB and in parentheses, p values) using the multipoint information on the markers of the genome scan only.

In red are the markers of the fine mapping (FM) and the results of linkage (lod MLB and in parentheses, p values) using the multipoint information on markers of the genome scan plus the fine mapping

* the distance from pter retrieved from <http://www.marshmed.org/genetics>.

Table 4

Results of linkage analyses of the 'allergic disease' phenotype (ECZ± AST ±AR) in the 295 families using the MLB method

Marker	Pos* cM ECZ± AST ±AR	GS	GS+FM
D5S1981	2	1.9 (0.002)	1.8 (0.002)
D5S417	7		0.8 (0.03)
D11S915	31		2.1 (0.001)
D11S904	34	2.3 (0.0005)	2.3 (0.0005)
D11S4152	35		2.3 (0.0005)
D17S1868	64	1.6 (0.003)	1.7 (0.003)
D17S806	67		1.6 (0.003)
D17S943	68		1.7 (0.003)

In black are the markers of the genome scan and the results of linkage (lod MLB and in parentheses, p values) using the multipoint information on the markers of the genome scan only (GS).

In red are the markers of the fine mapping (FM) and the results of linkage (lod MLB and in parentheses, p values) using the multipoint information on markers of the genome scan plus the fine mapping (FM).

* the distance from pter retrieved from <http://www.marshmed.org/genetics>.