

Original article

A genome-wide screen on the genetics of atopy in a multiethnic European population reveals a major atopy locus on chromosome 3q21.3

Background: Dissecting complex diseases in underlying distinct traits and studying these for their genetic basis might enhance the power as well as the specificity, of detection of disease genes. These phenotypes are known as intermediate phenotypes.

Objective: We were interested in the atopic basis of asthma, and used the sensitization to mite (*Dermatophagoides pteronyssinus*) allergens as a pathophysiologically important intermediate phenotype.

Methods: This time we performed a genome-wide scan based on the same already used multiethnic European population consisting of 82 nuclear families with at least two affected siblings. We carried out nonparametric as well as parametric MOD-score analyses based on the genotypes of 603 microsatellite markers.

Results: In comparison with our first genome-wide candidate region search three novel regions additionally appeared to be significant. We obtained significant results for the region 2p12 with a MOD score of 3.35 and for the region 16q21 with a MOD score of 4.18. The most significant result was found for the region 3q21.3 with the same microsatellite marker, which showed significant linkage to atopic dermatitis (AD) in another study with a MOD score of 4.51 and an nonparametric linkage analysis (NPL) of 4.00.

Conclusion: Our findings indicate that atopy, allergic asthma, allergic rhinitis and AD on the one hand are distinct traits on both the clinical and genetic basis, but on the other hand, our results also underline that these traits are closely related diseases concerning the atopic basis of the traits.

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Allergy and asthma are closely related complex diseases caused by a combination of both genetic and environmental factors (1). Genome screens for asthma and related phenotypes have been completed in 11 study populations. Details of these genome screens are available in the Asthma Gene Database (2). The results of the genome screens for asthma and related phenotypes identified at least 18 regions of the genome that probably house asthma/atopy genes, which reflects the extensive genetic heterogeneity that characterizes these phenotypes (3).

Asthma is most prevalent in children and young adults, and in 90% of cases associated with immunoglobulin E (IgE)-mediated reactions to common inhaled

allergens. Typical allergen sources include house dust mites (HDM), grass pollens, and animal danders. Studies have demonstrated that there is a strong and consistent association between sensitization to HDM allergens and both childhood and adult asthma (4, 5), which HDM exposure precedes the development of asthmatic symptoms (6), and that avoidance reverses the effect (7). *Dermatophagoides pteronyssinus* represents the predominant HDM worldwide, and multiple IgE-binding components have been identified in extracts of this species. Der p 1 and Der p 2 are the major Der p allergens. More than 80% of asthmatic individuals from different populations are sensitized to one of these allergens (8).

A lot of time has been spent defining the phenotype bronchial asthma in terms of an equal study definition, although asthma represents a rather polymorphic clinical picture. An alternative might be the use of distinct aspects of the phenotype asthma such as inflammation, bronchial hyperresponsiveness, or sensitization to certain allergens. Recent studies (9) have shown that the use of intermediate phenotypes might be a powerful tool in asthma genetics. One genome-wide linkage study used the phenotype total IgE-concentration (10), and two further studies focused on the phenotype mite sensitization as intermediate phenotype (11, 12). The resulting LOD scores were rather high and none of those regions previously linked to the inflammatory phenotype in general rather than asthma in particular showed up in these studies.

As our first genome-wide candidate-region search (12) covers until then known potential asthma and atopy candidate regions we conducted a genome-wide scan for asthma and atopy to find new chromosomal regions. For this purpose, we used the Weber 9 marker set to cover the whole genome. The typing was undergone in the same European population as described in Kurz et al. (12).

Material and methods

Subjects

The study population used in this genome-wide screen was based on the already used population (12) excluding 17 families because for lack of DNA. All families presented in this study were recruited in the course of the study 'Immunogenetics and Allergy: Towards Prevention and Care (IGA)', founded by the European Science Foundation (ESF). Families with at least two siblings sensitized to HDM allergens were ascertained for this study. The composition of families was as follows: total population: 82 families with 366 individuals. German population: 37 families with 159 individuals. British population: 19 families with 102 individuals. Portuguese population: 26 families with 105 individuals. As our population was selected without regard to clinical symptoms of bronchial asthma, 47% of all children studied were asthmatic according to the definition stated below.

Our population was furthermore selected without regard to other clinical symptoms of atopy which could influence the total IgE level. There was no substantial difference in the level of total IgE (in kU/l) among the subpopulations according to geographic origin (British population: 25% with 1–99 kU/l IgE, 71% with 100–1000 kU/l IgE, and 4% with > 1000 kU/l IgE; Portuguese population: 12% with 1–99 kU/l IgE, 75% with 100–1000 kU/l IgE, and 13% with > 1000 kU/l IgE; Italian population: 40% with 1–99 kU/l IgE, 44% with 100–1000 kU/l IgE, and 16% with > 1000 kU/l IgE; German population: 18% with 1–99 kU/l IgE, 72% with 100–1000 kU/l IgE, and 10% with > 1000 kU/l IgE).

Phenotype analysis

Specific IgE-antibodies to highly purified crude extract of *D. pteronyssinus* were detected by an immunochemiluminometric immunosorbent assay [Magic Lite (ML); ALK, Hørsholm, Denmark]. The cut-off point for a positive class 1 titre was > 1.43 ML

units. Asthma was diagnosed on the basis of the patients' history, doctors' diagnosis, and/or recurrent wheezing.

DNA preparation and genotype analysis

The DNA was extracted from peripheral blood leukocytes following standard protocols and was column purified (DNA midi kit; Qiagen, Hilden, Germany) or subjected to phenol/chloroform extraction. We carried out fluorescence-based semiautomated genotyping using the Weber 9 marker set (<http://www.bli.unizh.ch/BLI/Projects/genetics/maps/weber9.html>; accessed 14 July 2004) with 454 microsatellite markers. Marker amplification was performed on microtiter plates, in 96 (Peltier Thermal Cycler PT-225; MJ Research, Waltham, MA) and in 384 (GeneAmp PCR System 9700; Applied Biosystem, Foster City, CA) format. Fragment analysis of PCR pools was conducted on ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) automatic sequencers and genotypes were scored using GeneMapper (ABI) software.

Statistical analysis

For the statistical analysis we used the Weber 9 microsatellite markers and included the marker genotypes from our genome-wide candidate region search (12) so that the statistical calculations are based on the genotypes of 603 microsatellite markers in total.

We performed multipoint linkage analysis using the Marshfield comprehensive human genetic linkage map (slightly modified and supplemented according to the marker order given by Golden Path 13) and transformed genetic distances by the Haldane mapping function. Nonparametric analysis was performed with MERLIN 0.9.3 (13).

In the usual setting of parametric linkage analysis, the trait model has to be specified prior to the analysis. In many cases, the true disease model parameters are unknown; this holds especially for genetically complex traits. Therefore, here, we maximized the LOD scores with respect to the disease model parameters. This procedure, called 'MOD-score analysis' (14) or 'maximizing the maximum LOD score' [MMLS (15)], yields not only information about evidence for linkage and the disease-locus position, but also provides a valid, ascertainment-assumption-free method to estimate the disease model parameters. The program GENEHUNTER-IMPRINTING has been extended to incorporate MOD-score analysis, i.e. automatic maximization of the LOD score with respect to the disease model parameters (disease allele frequency and all penetrances). Practically, the program GENEHUNTER-MODSCORE first calculates the LOD score for a given set of predefined models, with different degrees of dominance, disease allele frequencies, and phenocopy rates. This reduces the probability to fall into a local maximum. Then, the model, which yields the maximum LOD score, is used as a starting point for fine maximization, which is carried out until the MOD score has converged to its maximum. Like with the former version GENEHUNTER-IMPRINTING (16), it is also possible with GENEHUNTER-MODSCORE to take into account imprinting, which is also known as parent-of-origin effect. This is done by distinguishing individuals who are heterozygous at the trait locus by the parent who transmitted the mutation. Therefore, there are two heterozygote penetrances, and four instead of three penetrance parameters altogether. Here, we performed MOD-score analyses using GENEHUNTER-MODSCORE under disease models assuming no imprinting, i.e. with standard three-penetrance models, as well as under trait models, which allow for imprinting, i.e. with four penetrances. This was undergone in order to identify possibly imprinted disease susceptibility genes, which might not be detected if imprinting is not adequately taken into account.

Results

A genome-wide screen for asthma and atopy susceptibility loci was conducted using the Weber 9 marker set (<http://www.bli.unizh.ch/BLI/Projects/genetics/maps/weber9.html>) with 454 microsatellite markers, in 82 nuclear families, comprising 366 individuals of European origin. We were interested in the atopic basis of asthma, and used the sensitization to mite (*D. pteronyssinus*) allergens as a pathophysiologically important intermediate phenotype. The statistical evaluation was based on the Weber 9 microsatellite marker genotypes and the included marker genotypes from our genome-wide candidate region search (12) so that the evaluation was based on 603 microsatellite markers in total. As the mode of inheritance is unknown, we conducted a nonparametric multipoint linkage analysis to search for chromosomal segments in which excess allele sharing of affected relatives might be observed. In order to cover potential differences among the individual populations, all calculations were performed in the total population as well as the subpopulations given by the geographic origin.

Furthermore, we conducted parametric multipoint analysis with three penetrances by means of multiple testing (MOD score) which allows the evaluation for each chromosomal region for the genetic model fitting best with regard to the highest LOD scores to be reached.

After this optimization we conducted multipoint analysis with four penetrances by means of multiple testing (MOD score) to find potential imprinting features.

The comparison of the results of the nonparametric as well as the parametric calculations based on the 603 microsatellite markers with our first genome-wide candidate region search (12) shows that three novel regions appear additionally to regions already found to be in linkage. The results of the nonparametric multipoint analysis as well as the results of the three-penetrance and four-penetrance parametric multipoint analysis (MOD scores) for these three regions are listed in Table 1.

One new region which appears to be significant was the region 2p12 next to the marker D2S1777 (103.1 cM). We obtained an NPL of 2.15 in the total population located at position 101.2 cM, and an NPL of 2.07 in the British population at position 105.8 cM. We obtained the highest MOD score with the four penetrance model with a MOD score of 3.04 in the total population, and a MOD score of 3.35 in the British population both located at position 105.8 cM. The second region, which appears to be significant, was the region 16q21 with the marker D16S2620 (79.0 cM). We obtained an NPL of 1.31 in the total population at position 79 cM and an NPL of 2.53 in the German population at the same position. We obtained the highest MOD score with the four penetrance model with a MOD score of 3.70 in the total population

Table 1. Results of the parametric (MOD score) and nonparametric multipoint linkage analyses for the phenotype sensitization to *Dermatophagoides pteronyssinus*

Population	Locus	Marker position (cM)	NPL/P-value	MOD	Position (cM)	
					NPL	MOD
Total population	D2S1777	103.10	2.15 0.01		101.20	
Model: 0.05/0.05/0.11/0.58 f (m): 0.005				3.04		105.80
Model: 0.04/0.04/0.50 f (m): 0.005				2.85		101.20
British population	D2S1777	103.10	2.07 0.02		105.80	
Model: 0.03/0.03/0.08/0.58 f (m): 0.001				3.35		105.80
Model: 0.04/0.04/0.50 f (m): 0.005				2.84		101.20
Total population	D3S3606	146.00	1.52 0.06		146.00	
Model: 0.03/0.03/0.18/0.66 f (m): 0.001				3.57		146.00
Model: 0.01/0.01/0.11 f (m): 0.02				2.91		146.00
British population	D3S3606	146.00	4.00 0.00061		146.00	
Model: 0.06/0.06/0.45/1.00 f (m): 0.05				4.51		146.00
Model: 0.00/0.06/0.49 f (m): 0.07				4.30		146.00
Total population	D16S2620	79.00	1.31 0.09		79.00	
Model: 0.15/0.40/1.00/1.00 f (m): 0.16				3.70		77.72
Model: 0.01/0.01/0.06 f (m): 0.03				0.60		9.60
German population	D16S2620	79.00	2.53 0.005		79.00	
Model: 0.03/0.32/1.00/1.00 f (m): 0.06				4.18		77.72
Model: 0.01/0.99/1.00 f (m): 0.27				3.26		79.00

The penetrances of the disease models obtained by MOD-score analysis are given in the order [$f_{+/+}$; $f_{m/+}$; $f_{+/m}$; $f_{m/m}$], with '+' denoting the wild type and 'm' denoting the mutant allele, and the paternally allele listed first. The most significant results are in bold.

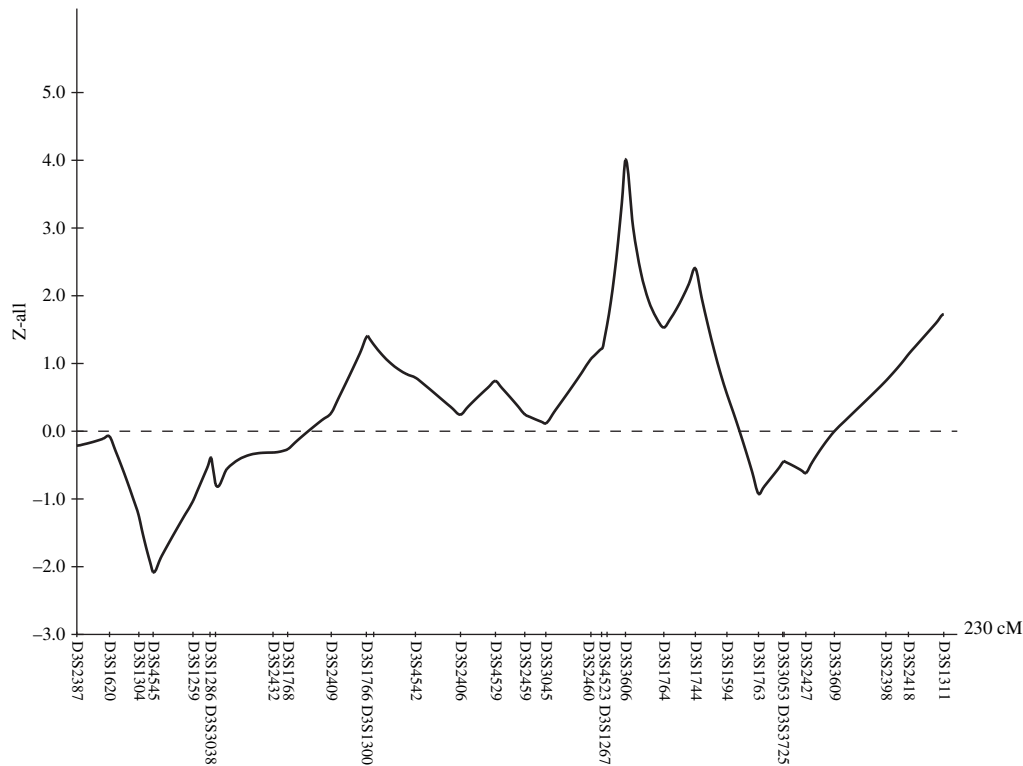


Figure 1. Linkage analysis on chromosome 3 in the British population. The x-axis represents the genetic distance according to Marshfield comprehensive human genetic linkage map. The y-axis depicts the nonparametric Z_{all} score obtained by GENEHUNTER.

and a MOD score of 4.18 in the German population both located at position 77.72 cM. The most significant result was seen for the region 3q21.3. We obtained an NPL of 1.52 in the total population and an NPL of 4.00 in the British population. We again obtained the highest MOD score with the four-penetrance model with a MOD score of 3.57 in the total population and a MOD score of 4.51 in the British population.

The graphical result of the nonparametric multipoint analysis of chromosome 3 in the British population is shown in Fig. 1, the graphical result of the four penetrance MOD-score analysis of chromosome 3 in the British population is shown in Fig. 2. In Fig. 3, the graphical results of the four penetrance MOD-score analysis in the German population is shown.

Discussion

A clear-cut phenotype definition might be the most essential basis for any genetic study. Dissecting complex diseases in the underlying distinct traits, and studying these for their genetic basis, might enhance the power as well as the specificity to detect disease genes (17–19). These phenotypes are known as intermediate phenotypes. Given atopic asthma, this is genetically defined by at least two subsets of genes, those genes defining the status

atopy, and a distinct set of genes, which defines the mere inflammatory response typical to asthmatic reactions, but also to other inflammatory diseases. Sensitization to mite allergens is such an intermediate phenotype with a high predictive value for the development of asthmatic symptoms (6).

This genome-wide scan in our reduced population showed in comparison with our first genome-wide candidate region search (12) that three novel regions additionally appeared to be significant. The region on 2p12 with the marker D2S1777 (103.1 cM) was so far not shown to linked with any asthma or atopy phenotypes. The nearest region for which linkage to asthma or any atopy phenotypes has previously been identified is 2p13 with the marker D2S2113 (92.3 cM) (2, 20). Interestingly this region was also linked to the phenotype specific IgE. So it remains to be seen whether it concerns the same genetic locus or a different one.

It is a well-known fact that the power to detect linkage is reduced if the trait model specified for a parametric (LOD score) analysis is not sufficiently close to the true underlying model (14). This effect is even more pronounced in the context of multi-marker analysis (21). Therefore, especially when looking at a complex trait such as atopy, a MOD-score analysis, where LOD scores are maximized with respect to the disease model parameters, presents an absolutely promising route.

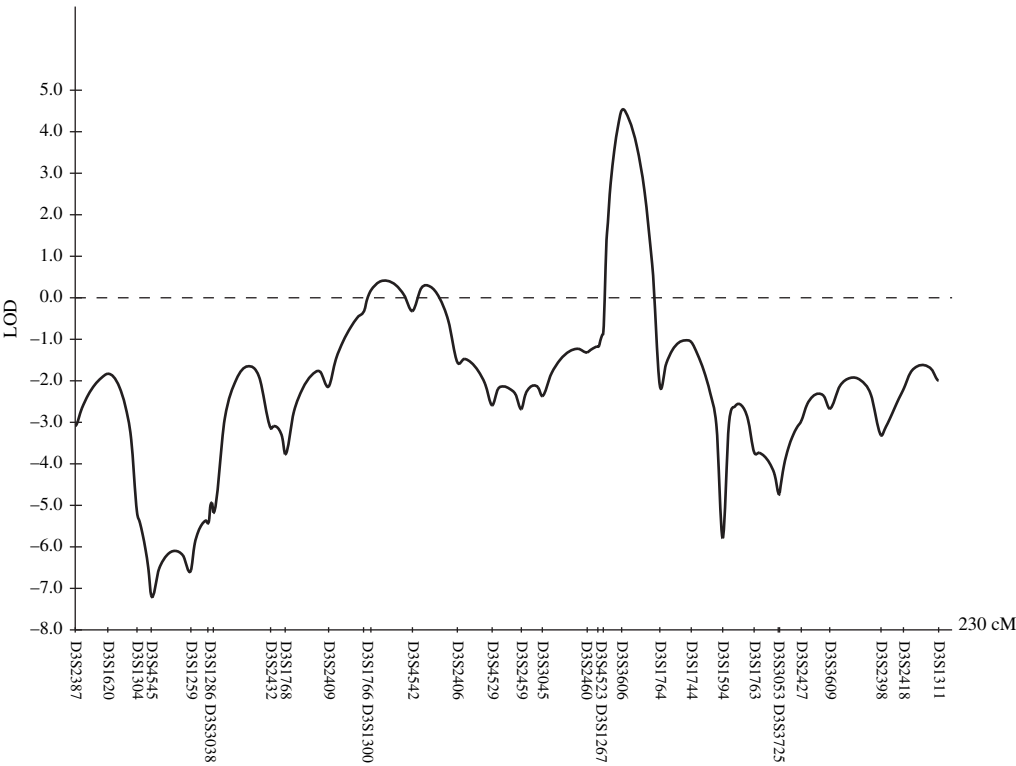


Figure 2. LOD-score plot for the optimized model obtained by MOD-score analysis (penetrances [0.06; 0.06; 0.45; 1.00], disease allele frequency 0.05) in the British population on chromosome 3.

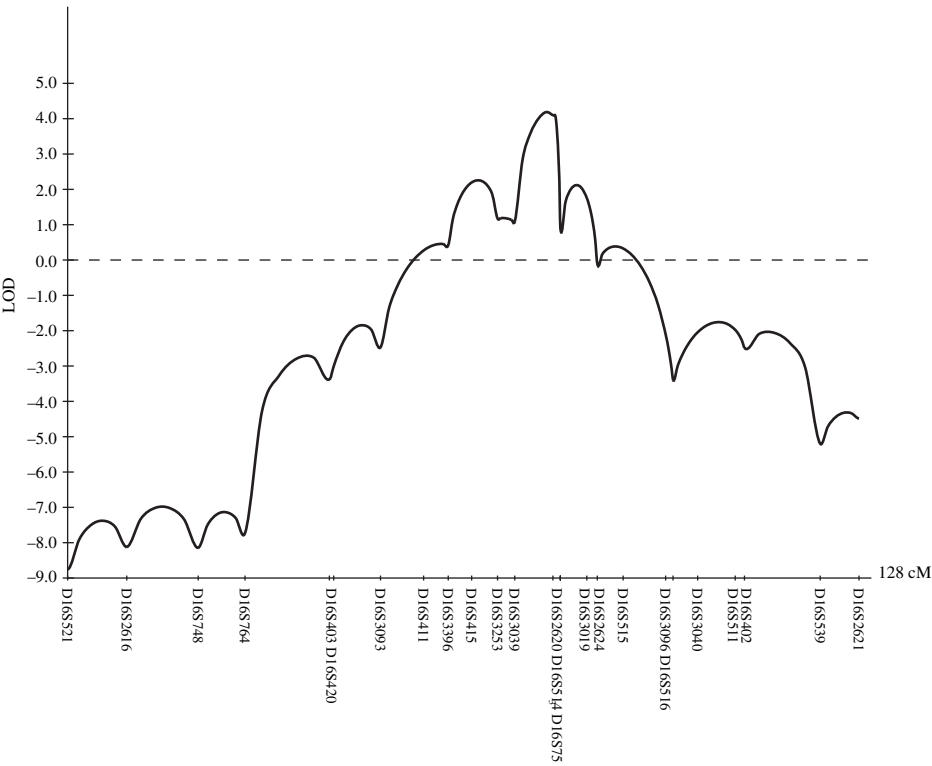


Figure 3. LOD-score plot for the optimized model obtained by MOD-score analysis (penetrances [0.03; 0.32; 1.00; 1.00], disease allele frequency 0.06) in the German population on chromosome 16.

A MOD-score analysis represents one of the most thorough ways to analyze linkage data, and minimizes the type II error. On the contrary, MOD-score analysis is clearly explorative, and so it is difficult to control the type I error. Weeks et al. (22) and Hodge et al. (23) have found, by simulation, that for MOD scores, a critical LOD score of 3 should be adjusted by some value in the range of 0.3–1.0 to maintain a similar type I error, with the upper boundary being rather conservative.

Referring to the MOD score results for the region 2p12 (3.04 in the total population and 3.35 in the British population), this means that we have found borderline significance for this region.

The second region on 16q21 with the marker D16S2620 (79.0 cM) was so far not found to be linked to any asthma or atopy phenotypes either. The nearest region, which has shown linkage to asthma or any atopy phenotypes so far, was the region on 16q23.1 with the marker D16S289 (2, 24). This region was linked to the phenotype total IgE as well as atopy and asthma. The genetic model fitting best with regard to the highest MOD scores to be reached implied paternal imprinting, in the total population as well as in the German population. The corresponding MOD scores were 3.70 and 4.18, respectively. Penetrance was estimated to be complete for individuals who inherited the disease allele from the mother. Without the assumption of imprinting, the optimized MOD score was only 0.60 in the total population and 3.26 in the German population. Interestingly Daniels et al. (24) found linkage to maternal meioses between the mentioned marker D16S289 in region 16q23.1 and atopy.

The third region 3q21.3 with the marker D3S3606 was the only region which so far showed linkage to an atopic phenotype. A genome-wide screen for atopic dermatitis (AD) (25) found significant evidence for linkage between the mentioned phenotype and the marker D3S3606. Furthermore, they carried out a separate analysis for allergic sensitization defined by elevated serum IgE levels. Lee et al. (25) obtained significant evidence for linkage of allergic sensitization to this locus under the assumption of paternal imprinting. We obtained the highest MOD score in the total population with the four-penetrance model [0.03; 0.03; 0.18; 0.66] pointing to reduced penetrance and showing a tendency of paternal imprinting. In the British population, significant linkage (MOD score: 4.51) was found with the same marker, with penetrances [0.06; 0.06; 0.45; 1.00]. Furthermore, the nonparametric multipoint analysis showed significant linkage with this marker with an NPL of 4.0. The mentioned four-penetrance model in the British population interestingly points to paternal imprinting, i.e. reduced penetrance for maternally inherited alleles. The fact that we found the highest MOD score also with a paternal imprinting model indicates that we focussed on the same locus with our study.

Lee et al. (25) assume that the finding of two distinct genetic models for these traits indicates either the presence of two genes, each influencing one trait, or the pleiotropic effect of a single gene may be imprinted in a time- or tissue-specific manner. Another explanation could be the maternal–fetal immune communication that, for example, maternal antibodies regulate the clonal development of immune cells (26), which sensitivity to particular antigens can be transferred to the fetus (27), and that maternal immunization with ovalbumin in mice protects against IgE responses in offspring (28).

Linkage of chromosome 3q21 has also been shown in other genome screens. Bradley et al. (29) could also show linkage to AD, Enlund et al. (30) could show linkage to psoriasis, and Haahrup et al. (31) could show linkage to asthma. A further genome screen for AD (32) could not confirm the finding of the region 3q21.

Along with asthma and allergic rhinitis, AD is an important manifestation of atopy that is characterized by the formation of antibodies (IgE) to environmental allergens. Eighty percentage of cases of infantile eczema have elevations of the total serum IgE concentration (33), and asthma is present in 60% of children with severe disease (34). Furthermore, population studies suggest that AD is a response to different determinants influencing most of the common forms of asthma (35). Therefore, it is not surprising that we found linkage to a major susceptibility locus for AD, which could mean that the region 3q21 harbors a major locus for atopy.

In conclusion, it was the aim of this study to focus on chromosomal regions harboring genes responsible for the development of an atopic phenotype and at the same time regions linked to one of the most important sources of the asthma pathogenesis. The regions found are partially distinct from those in asthma searches that have been found to linked to asthma as well as to other inflammatory diseases. Furthermore, our findings showed that atopy, allergic asthma, allergic rhinitis and AD on the one hand are distinct traits on both the clinical and genetic basis, but on the other hand, our results also underline that these traits are genetically closely related diseases concerning the atopic basis of the traits.

Approval

The study was approved by the Ethical Commission of the University of Freiburg. A statement of informed consent was obtained from all participants or their parents.

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