A major susceptibility locus for atopic dermatitis maps to chromosome 3q21

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Atopic dermatitis (eczema) is a chronic inflammatory skin disease with onset mainly in early childhood[1]. It is commonly the initial clinical manifestation of allergic disease, often preceding the onset of respiratory allergies[2,3]. Along with asthma and allergic rhinitis, atopic dermatitis is an important manifestation of atopy that is characterized by the formation of allergy antibodies (IgE) to environmental allergens. In the developed countries, the prevalence of atopic dermatitis is approximately 15%, with a steady increase over the past decades[4,5]. Genetic and environmental factors interact to determine disease susceptibility and expression[6], and twin studies indicate that the genetic contribution is substantial[7]. To identify susceptibility loci for atopic dermatitis, we ascertained 199 families with at least two affected siblings based on established diagnostic criteria[8,9]. A genome-wide linkage study revealed highly significant evidence for linkage on chromosome 3q21 (Z_{all}=4.31, P=8.42×10^{-6}). Moreover, this locus provided significant evidence for linkage of allergic sensitization under the assumption of paternal imprinting (hlod=3.71, α=44%), further supporting the presence of an atopy gene in this region. Our findings indicate that distinct genetic factors contribute to susceptibility to atopic dermatitis and that the study of this disease opens new avenues to dissect the genetics of atopy.

Atopy is a complex disorder that results from interactions between heterogeneous genetic factors and the environment[6]. Previous genome-wide scans for genes predisposing to atopy have focused on asthma and high IgE levels as phenotypes[10–13]. Clinically, atopic dermatitis is characterized by an intensely pruritic rash with a typical morphology and distribution. The diagnosis of atopic dermatitis is straightforward and validated scoring systems are available to gauge disease severity[14]. In addition, epidemiologic studies show that parental atopic dermatitis confers a higher risk of atopic dermatitis to offspring than parental asthma or allergic rhinitis[15], indicating the presence of atopic-dermatitis–specific genes. So far, the genetic determinants of atopic dermatitis have received little attention and genetic investigations have been limited to association studies with few candidate genes[3,16–18].

We conducted a genome-wide scan for atopic dermatitis susceptibility loci in a set of 199 complete nuclear families of European origin composed of 839 individuals (Table 1). To enhance the contribution of genetic factors in our study group, we restricted ascertainment to affected siblings with an early age of onset (≤2 years) and severe to moderate disease expression. As the mode of inheritance is unknown, we conducted a nonparametric multipoint linkage analysis to search for chromosomal segments in which excess allele sharing was observed among affected relatives. We detected evidence for linkage on chromosome 3q21 near marker *D3S3606* (Z_{all}=4.31, P=8.42×10^{-6}). The probability of obtaining a Z_{all} score of greater than or equal to 4.31 by chance in a genome-wide scan was estimated by 10,000 simulations to be 0.0009. This result provides highly significant evidence for an atopic dermatitis susceptibility gene on chromosome 3q. No other region in the genome attained even the level of suggestive evidence for linkage (Fig. 1). As parametric linkage analysis may provide superior power to detect linkage when the inheritance is complex[19], we also carried out parametric linkage analyses using one dominant and one recessive inheritance model. Previous research indicates that genetic heterogeneity underlies atopic disorders[6]. We therefore carried out the parametric linkage analysis under the assumption of heterogeneity. We obtained the highest lod score on chromosome 3q21 under the dominant model (hlod=3.65) for a proportion (α) of 39% of linked families (Fig. 2*a*).

To test whether families unlinked to this major locus showed evidence for linkage elsewhere in the genome, we carried out an additional analysis in which weights 0 or 1 were assigned to each family according to a positive or negative linkage score contribution, respectively, on chromosome 3q21. This test revealed positive, but not suggestive, evidence for linkage on chromosome 1p near marker *D1S2876* (Z_{lr}=2.67, P=0.0038) and at the chromosome 19p telomere (Z_{lr}=2.70, P=0.0035) near marker *D19S209*. Moreover, parent-of-origin effects are suspected to have an important role in the development of allergic diseases[20]. As these effects may only be detectable if modelled adequately, we computed linkage scores using inheritance models for both paternal and maternal imprinting. Under the assumptions tested in this analysis, we found no evidence for paternal or maternal imprinting for atopic dermatitis at the new locus on 3q21 or elsewhere in the genome (data not shown).

Table 1 • European families with atopic dermatitis

	Sibpairs	Sibtrios	≥4 siblings	Total
Germany	101	15	2	118
Italy	35	1		36
Sweden	31	3		34
The Netherlands	10	1		11
Total	177	20	2	199

Summary of the European study cohort indicating the origin, number and size of sibships affected with atopic dermatitis.

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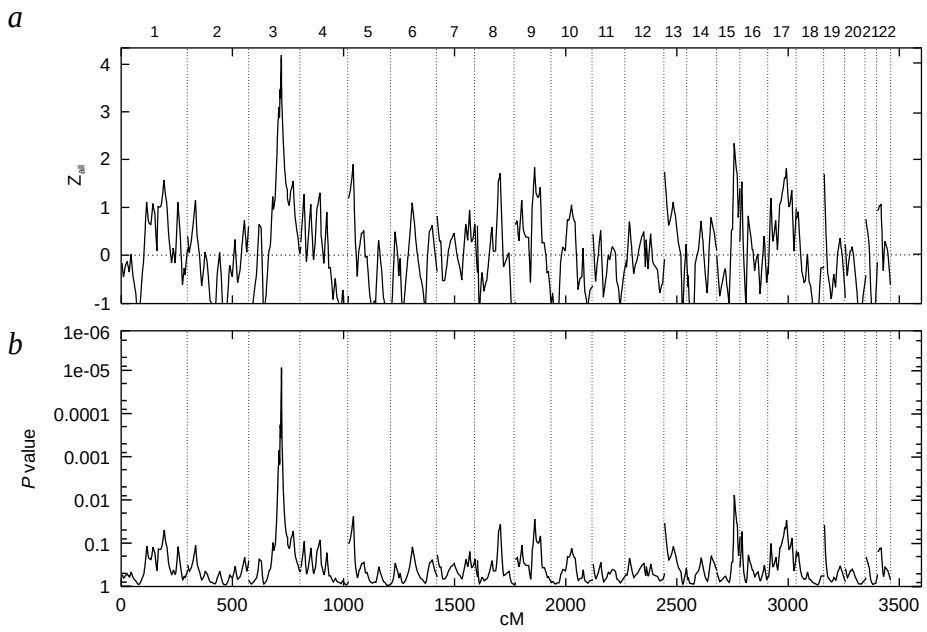

Fig. 1 Genome-wide scan for atopic dermatitis susceptibility loci. The *x* axis represents the length of all 22 autosomes from p to q telomere, and the *y* axis represents the nonparametric multipoint GENEHUNTER Z-score (Z_{all}) (***a***) and the calculated *P* value (***b***).

We have reported the localization of an atopic dermatitis susceptibility locus to chromosome 3q21. Moreover, we detected significant evidence for linkage of allergic sensitization to this locus under the assumption of paternal imprinting, further supporting the presence of an atopy gene in this region. The finding of a single major locus in the present, genetically diverse population is unexpected given the extensive genetic heterogeneity that seems to underlie other atopy-related phenotypes, even within founder populations[12]. Our study highlights the importance of careful family selection for familial cases with an early age at onset and a clearly defined, severe phenotype. Additional susceptibility loci are almost certainly involved in the aetiology of atopic dermatitis. Using different analytic approaches to account for genetic heterogeneity and putative imprinting effects, we found no evidence for linkage of atopic dermatitis to other chromosomal regions. It may be that the current cohort was not sufficiently large to identify additional loci of small or moderate effect. But the possibility of a single major locus underlying infantile atopic dermatitis, in contrast to asthma, should also be considered.

Moreover, we carried out a separate analysis for allergic sensitization as defined by elevated serum IgE levels. Nonparametric linkage analysis yielded suggestive evidence (Z_{all}=2.52, P=6.7×10^{-4}; Fig. 2*b*) and parametric linkage analysis under the dominant model revealed weak evidence for linkage (hlod of 1.26, α=29%) at the same locus on chromosome 3q21. It was only under the assumption of paternal imprinting that we detected significant evidence for linkage of allergic sensitization to this locus. Although we obtained an hlod of 0.08 (α=7%) for maternal imprinting, we found an hlod of 3.71 (α=44%; Fig. 2*b*) modelling paternal imprinting, indicating that this trait is predominantly transmitted by the mother. The genome-wide significance level of this result was estimated by 10,000 simulations to be less than 0.016.

Atopic dermatitis and allergic sensitization have been linked to the same locus on chromosome 3q21. Although linkage of one trait may not be independent of that of another closely associated phenotype, 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 that may be imprinted in a time- or tissue-specific manner. The candidate region on chromosome 3q21 is approximately 10 cM. Genes encoding two type-I membrane proteins of the immunoglobulin superfamily, CD80 and CD86 antigens, have been mapped to this region. Both CD80 and CD86 interact with CD28 to provide costimulatory signals for T-cell activation and have been implicated in the activation of the Th2 subset of CD4$^+$ T-helper lymphocytes that are thought to have a pivotal role in mediating allergic inflammation[21,22]. CD80 and CD86 are therefore positional and functional candidate genes for atopic dermatitis and allergic sensitization.

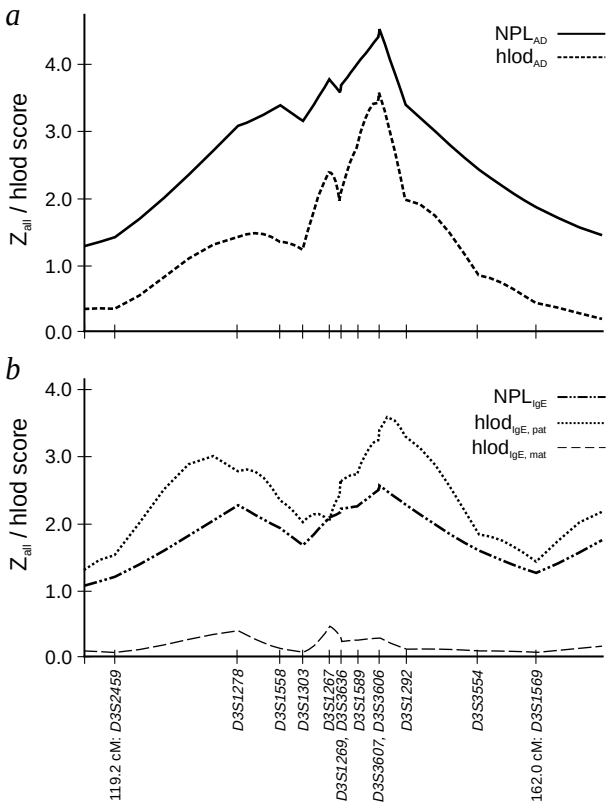

Fig. 2 Linkage analysis on chromosome 3q. The *x* axis represents the genetic distance along the chromosome. Markers are arranged in map order according to the final Généthon human linkage map[23]. The *y* axis depicts the Genehunter nonparametric Z_{all} (NPL_{AD}) and hlod score ($hlod_{AD}$) assuming a dominant inheritance model (0.001, 0.9, 0.9) for atopic dermatitis (***a***), as well as the Z_{all} score (NPL_{IgE}) and the hlod assuming paternal ($hlod_{IgE, pat}$) and maternal ($hlod_{IgE, mat}$) imprinting for elevated IgE levels (***b***).

Notably, the locus on chromosome 3q21 is distinct from any previous linkage reports for atopy phenotypes. Atopic dermatitis, asthma and allergic rhinitis are closely associated and show strong familial and intra-individual clustering, indicating common disease aetiology. Clinically, however, they differ substantially, affecting different organ systems and showing distinct epidemiological characteristics. The factors influencing target organ selectivity are poorly understood. Our findings indicate that distinct genetic factors predispose to atopic dermatitis. The magnitude of the effect at this locus is expected to facilitate the identification of the disease gene and the molecular mechanisms by which it contributes to the development of atopic dermatitis. The characterization of the genetic factors involved in this common, chronic disorder may provide important clues to its relationship to asthma and allergic rhinitis and is ultimately hoped to lead to more effective interventional strategies.

Methods

Families. The institutional review boards of the participating European centres approved the study protocol. After obtaining informed consent, we ascertained 199 complete nuclear families composed of 839 individuals with at least 2 affected children with atopic dermatitis in 7 clinical centres in Germany, Italy, Sweden and the Netherlands. We also included 14 unaffected siblings in the study. Of the affected children, 52% were male and 48% were female aged 7.5±5.6 years (mean±s.d.); 48% of the children were ≤5 years old. Among the affected children, 24% had asthma, 29% had allergic rhinitis and 14% had both. We questioned the parents about a history of atopy. We excluded two of four twin pairs because they were monozygous twins.

Clinical evaluation. The diagnosis of atopic dermatitis was made according to standard criteria[8,9] in the presence of a chronic or chronically relapsing (>3 months) pruritic dermatitis with the typical morphology and distribution and a family history of atopic disease. We assessed disease severity using the SCORAD system[14]. We limited inclusion to severe to moderate disease as defined by an objective SCORAD of >15 or involvement of >20% of the body surface[9] and an age at onset of ≤2 years. We determined total IgE and levels of specific IgE against grass and birch pollen, ribwort, cat and dog dander, mold (*Cladosporidium herbarum*, *Alternaria tenuis*), hen's egg, cow's milk, fish, peanut and house dust mite using CAP-RAST-FEIA (Pharmacia). A proband was defined as sensitized if specific IgE to at least one allergen was detected (detection limit 0.35 kU/l) or if the total IgE was elevated above the age-specific norm. Serum IgE levels were available in 95% of the probands. The proportion of sensitized individuals was 61% of all probands and 74% of the affected children. We did not perform skin prick testing.

DNA analysis. We prepared genomic DNA from whole blood by standard methods. We conducted the genome scan in 2 stages, beginning with 89 families in the first, and 110 families in the second set. The countries of origin were equally represented in each set. We carried out fluorescence-based semi-automated genotyping using 380 autosomal microsatellite markers selected from the final Généthon linkage map[23] with an average heterozygosity of 0.8. We typed 12 additional markers in regions where positive evidence for linkage (P<0.001) was observed in the first data set. The data presented were computed using the combined data sets of 199 families. After individual PCR amplification, we pooled and size fractionated the PCR products by electrophoresis on Prism 377 DNA sequencers (Applied Biosystems). We determined allele sizes using the Genescan 2.1.1. and Genotyper V2 software (Applied Biosystems).

Statistical analysis. All marker genotypes were checked for mendelian inheritance using the PedCheck software[24]. We carried out separate linkage analyses using atopic dermatitis and allergic sensitization, defined as elevated IgE levels, as traits. As the mode of inheritance is unknown, we performed nonparametric multipoint linkage analysis with Genehunter V2.0 (ref. 25) using marker distances according to the Généthon linkage map[23] and uniform allele frequencies. We assumed a disease allele frequency of 0.01 throughout the analysis. Genehunter uses the nonparametric Z_{all} statistic to estimate the statistical significance of sharing alleles identical by descent between all affected relatives. Pointwise P values were derived by computing the exact probability distribution of the overall Z score under the null hypothesis of no linkage.

As parametric linkage analysis is more powerful when the specified model is close to the true underlying mode of inheritance[19], we carried out parametric linkage analyses using one dominant (0.001, 0.9, 0.9) and one recessive (0.001, 0.1, 0.9) inheritance model. For a polygenic trait, evidence for linkage at a given locus may not be detectable assuming genetic homogeneity, that is, in the complete data set of linked and unlinked families. We have therefore computed heterogeneity lod scores (hlod) for varying proportions of linked families (α) using Genehunter V2.0. The proportion of linked families represents an estimate that is dependent on the genetic models used in this analysis. Due to the small size of the individual families investigated (nuclear families), each family contributes relatively little linkage information, making it difficult to divide them into distinct categories of linked and unlinked families. The 1-lod-unit support interval for the size of the region on chromosome 3 was based on the parametric linkage analysis and the most likely model for atopic dermatitis and allergic sensitization where all map locations with a Z score >Z–1 are considered, Z being the peak hlod[26].

Moreover, to model locus heterogeneity, we carried out an additional analysis using the Genehunter-Plus V1.3 modification[27]. This software performs a likelihood ratio test for allele sharing in affected relative pairs and computes the nonparametric Z_{lr} score. Genehunter-Plus allows a linkage analysis that can be weighted according to the evidence of linkage at a particular location. To detect evidence for locus heterogeneity, weight 1 was assigned to families with Z_{all} score <1 at marker *D3S3606*, weight 0 was assigned to families with Z_{all} score ≥1 at marker *D3S3606*.

As parent-of-origin effects are suspected to be important in the development of atopy[20], the Genehunter-Imprinting software[28] was used to model imprinting. Genehunter-Imprinting allows the specification of two heterozygote penetrance parameters reflecting the penetrance of the paternal and maternal allele, respectively. We computed multipoint lod scores assuming paternal (0.001, 0.1, 0.9, 0.9) and maternal (0.001, 0.9, 0.1, 0.9) imprinting.

We assessed the genome-wide significance level of the linkage results empirically using Allegro V1.0 (ref. 29). Using all pedigrees and all genetic markers used in the actual analysis, we generated 10,000 unlinked replicates, and conducted nonparametric as well as parametric analyses under the dominant and recessive models used in the actual analysis. We calculated an empirical genome-wide significance level as the proportion of replicates for which the maximum Z_{all} score or hlod score was greater than that obtained in the real data set. Similarly, we assessed the significance of the imprinting results by generating 10,000 unlinked replicates for chromosome 3 only. We used Genehunter-Imprinting[28] to compute hlods under the paternal and maternal imprinting models used in the actual analysis. We determined the empirical genome-wide significance level as above and adjusted for the number of markers tested throughout the genome. Suggestive, significant and highly significant evidence for linkage was defined according to published criteria[30] as statistical evidence expected to occur once, 0.05 and 0.001 times at random per genome screen, respectively.

Acknowledgements

We thank the families for participation; R. Nickel for help in initiating this project; M. Schwarz for technical assistance; T. Jakob and R. Mayrl for help with family recruitment; and G. Nürnberg for preparing the figures. This work was supported by Bundesministerium für Forschung und Technologie and the German Human Genome Project.

Received 8 August; accepted 30 September 2000.

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