

Linkage to atopy on chromosome 19 in north-eastern Italian families with allergic asthma

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Summary

Background Allergic asthma is a multifactorial disease for which there is a widely assessed, although poorly understood, genetic involvement. Genome-wide screens reported evidence for linkage of allergic asthma-related phenotypes to several chromosomal locations. Markers on chromosome 19 have been linked to allergic asthma phenotypes in different populations in independent studies.

Objective The aim of this study was to perform a genetic linkage analysis on chromosome 19 to search for DNA markers linked to phenotypes related to allergic asthma.

Methods Using non-parametric multipoint linkage analysis on a total of 22 random DNA markers in 2 stages, a sample of 111 families (542 subjects) from north-eastern Italy, recruited through an asthmatic allergic proband, was investigated. Phenotypes examined were: clinical asthma, total serum elevated IgE, skin prick test positivity, bronchial hyper-responsiveness, and atopy defined as skin prick test positivity and/or elevated IgE. Simulation studies were performed to confirm the significance of the results.

Results A novel linkage of atopy and skin prick test positivity to marker D19S601 (19q13.3) was found. Modest evidence for linkage of atopy, skin prick test positivity, and IgE was also found to marker D19S591 (19p13.3). Simulation analysis for atopy gave an NPL-Z > 3.326 in 2 replicates out of 1000 ($P = 0.002$) for D19S601, and an NPL-Z > 2.56 in 16 replicates out of 1000 ($P = 0.016$) for D19S591.

Conclusions On chromosome 19, suggestive linkage of atopy and skin prick test positivity with marker D19S601 (19q13.3) and modest evidence of linkage of marker D19S591 (19p13.3) to the atopic phenotypes investigated were found. These results suggest that these regions may contain susceptibility loci associated to atopic phenotypes.

Keywords: allergic asthma, BHR, IgE, SPT, atopy, linkage analysis, simulation, chromosome 19

Clinical and Experimental Allergy, Vol. 31, pp. 1220–1224. Submitted 5 September 2000; revised 2 November 2000; accepted 23 January 2001.

Introduction

Asthma is one of the most common multifactorial diseases in childhood, characterized by inflammation of airways,

wheeze, cough and bronchial hyper-reactivity to chemical and physical stimuli. Asthma is often associated with atopy [1], whose clinical features include total serum high levels of IgE antibodies, antigen-specific IgE responses, and positive skin prick test (SPT) to common airborne allergens [2,3]. The phenotypically variable expression of atopic asthma seems to be influenced not only by genetic factors

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such as population heterogeneity and varying penetrance but also by non-genetic factors such as uncontrolled environmental factors (i.e. airborne allergens), lifestyle, infections and last, but not least, selection criteria (i.e. age, sex and clinical evaluation) [4]. Since allergic asthma is phenotypically variable, the analysis of related but well defined phenotypes might be of importance in identifying genes involved in the different aspects of the disease.

Several genome-wide searches have been performed and candidate chromosomal locations have been reported as being possibly associated with allergic asthma phenotypes in different ethnic groups [5–9]. Three out of five genome searches have reported linkage on chromosome 19 ($P < 0.05$) for Hutterites [6,7, <http://genes.bsd.uchicago.edu/hutterite/>] and North American Caucasoids [5, <http://www.csga.org>]. Linkage was not found in German [8], Australian and British populations [9]. We previously reported preliminary results indicating the 19q13.3 region as possibly containing allergic asthma susceptibility loci [10]. In this study we present the results of a comprehensive linkage analysis of chromosome 19 markers with phenotypes related to atopic asthma in a sample of Italian families.

Materials and methods

Families and phenotypes

During a period of three years (1995–98), 111 families (542 genotyped subjects) from north-eastern Italy were enrolled in this study through the Paediatrics Clinic of the University of Verona and the Hospital of Bolzano. Families were recruited through an allergic asthmatic child, defined as having SPT positivity and asthma. Mean age of the probands was 9 years ($SD = 3$), and of the other participants was 25 years ($SD = 17$). This family panel was phenotyped for clinical asthma through a physician diagnosis according to the American Thoracic Society criteria [11], including positive response to a validated respiratory questionnaire [12]. Data regarding bronchial hyper-responsiveness (BHR) to methacholine, total serum IgE level, and skin prick test (SPT) reactivity against common aeroallergens, were collected. BHR was defined as a $\geq 20\%$ drop in baseline FEV_1 at 25 mg/mL ($PC_{20} < 25$ mg/mL) [13]. SPT was performed to standardized allergens, including house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), moulds (*Alternaria tenuis*, *Aspergillus mix*, *Penicillium mix*, *Candida albicans*, *Hormodendrum hordei*, *Phycomycetes mix*), gramineae (*Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Holcus lanatus*, *Cynodon dactylon*, *Zea mays*, *Poa pratensis*, *Sorghum alepense*, *Festuca elatior*) tree pollens (*Corylus avellana*, *Fagus sylvatica*, *Quercus*

alba, *Pinus sp.*, *Ulmus campestris*, *Alnus glutinosa*, *Betula verrucosa*, *Fraxinus excelsior*, *Olea europea*, *Platanus orientalis*, *Quercus ilex*, *Populus nigra*, *Tilia cordata*, *Aesculus hippocastanum*, *Cupressus sempervirens*, *Acer negundo*, *Acacia floribunda*), herbs (*Artemisia absinthium*, *Plantago lanceolata*, *Parietaria judaica*, *Ambrosia elatior*, *Ambrosia trifida*, *Ambrosia bidentata*, *Artemisia vulgaris*, *Chenopodium album*, *Urtica dioica*, *Rumex sp.*, *Humulus lupulus*, *Amaranthus retroflexus*, *Iva ciliata*, *Kochia scoparia*, *Ricinus communis*, *Canabis Sativa*, *Heliantus annuus*), and animal dander (*Canis familiaris*, *Felis domesticus*, feathers mix, epithelia mix), all purchased from Bayer, AG (Leverkusen, Germany). Skin prick test was considered positive if the diameter of the weal was 3 mm larger than negative control (saline). A subject was considered to be SPT positive if reacting to at least one allergen. Elevated IgE were defined as a qualitative trait with serum concentration greater than 200 kU/L for adults and above the 90th percentile in an age-adjusted standard curve value for children under 10. Mean total serum log IgE level in the probands was 2.76 (SD 0.45, range: 1.69–3.91), and 1.96 (SD 0.66, range: 0.30–3.89) in the other participants. Atopy was defined as a qualitative trait with raised circulating total IgE level and/or SPT positivity.

Genotype determination

Genomic DNA was prepared from whole blood by phenol/chloroform extraction. DNA markers used for genotyping were the following: D19S591, D19S1034, D19S586, D19S593, D19S1037, D19S431, D19S719, D19S433, D19S587, D19S718, D19S541, D19S585, D19S601, D19S589 (first set, Research Genetics Inc., Huntsville, AL, USA; these markers correspond to those used in [10]), D19S571, D19S418, D19S210, D19S414, D19S220, D19S178, D19S420, D19S902 (second set, Perkin Elmer Europe B.V., Milan, Italy). DNA fragments obtained by PCR with fluorescent labelled primers were loaded on a ABI377™ DNA Sequencer (Perkin Elmer) and genotypes were determined. Genotyping was carried out in two stages. In Stage 1 the entire chromosome 19 was screened with 14 DNA markers (first set) spanning about 90 cM from D19S591 to D19S589. In Stage 2 the families were genotyped with 8 additional markers (second set) spanning 19q13.3.

Statistical analysis and computer programs

The multipoint linkage program Genehunter2 r2 was used [14]. Genehunter2 computes non-parametric linkage analysis (NPL) combining the score from each pedigree into a NPL-Z score. It performs a multipoint linkage analysis with a large number of highly polymorphic markers. It extracts

Table 1. Chromosome 19 linkage results for asthma and associated traits

Chromosome	Marker	cM	Asthma		BHR		IgE		SPT		Atopy		Simulation
			NPL-Z	P-value	NPL-Z	P-value	NPL-Z	P-value	NPL-Z	P-value	NPL-Z	P-value	
19q13.3	D19S601 (first set)	89.3	1.157	0.12	2.34	0.0096	2.039	0.021	2.81	0.0026	3.143	0.0009	
	D19S601 (all)		0.90	0.18	2.05	0.02	2.12	0.017	3.06	0.0012	3.326	0.00051	0.002
19p13.3	D19S591 (all)	18	1.19	0.117	0.36	0.357	2.23	0.013	2.02	0.022	2.56	0.0056	0.016

Genetic distance (cM) from p-ter was taken from the Genetic Location Database (LDB). (First set) refers to Stage 1 markers, while (all) refers to Stage 1 plus Stage 2 markers. BHR: bronchial hyper-responsiveness to metacholine defined as a $\geq 20\%$ drop in baseline FEV₁ at 25 mg/mL. IgE: total serum elevated IgE. SPT: skin prick test positivity to at least one allergen. Atopy: SPT positivity and/or raised circulating total IgE level.

the full inheritance information from the pedigree structure, analysing all the affected pairs. The Stage 1 reference map was built using 15 Centre d'Etude du Polymorphisme Humain (CEPH) families, while genotype data available in the CEPH database (<http://www.cephb.fr/cephdb>) were used to build the reference map with all markers after Stage 2 (104 cM, from D19S591 to D19S210). The map showing the best support has been chosen, even if other orders were acceptable since the difference of the supports among various combinations was not > 3 . CRIMAP2.4 was used for construction of the reference maps [15]. For the analysis, our panel of 111 families which included some multigeneration pedigrees ($n = 14$) was split into 140 nuclear two generation families. Therefore, some subjects were considered twice, once as a parent and once as a child. Thus, the families presenting at least one affected sib-pair (ASP) for the individual phenotypes were: 48 for asthma, 57 for IgE, 63 for BHR, 95 for SPT and 102 for atopy. For simulation analysis, genotypes of the family members for the map of linked markers (unlinked to the affection locus) were constructed with the SIMULATE computer program [16,17]. The simulation studies were performed on the atopy phenotype and gene frequencies were estimated from the family founder members. Only the genotypes of the typed individuals were used in simulation analysis wherein multigeneration pedigree of the original set were considered. After simulation, each replicate was split into nuclear two generation families and analysed. Although this method implies a certain loss of information it has permitted a rapid elaboration of the data. Due to the inherent composition of our familial sample, the simulation analyses are time consuming and have been performed only for atopy, i.e. the phenotype showing the highest significance.

Results

Five allergic asthma phenotypes (asthma, IgE, BHR, SPT and atopy) were investigated in a sample of 111 families (542 subjects) with sib-pairs affected by each individual phenotype considered. Table 1 summarizes the linkage results obtained for markers D19S601 and D19S591. Of 14 markers spanning the entire chromosome (Stage 1), the best evidence of linkage was at marker D19S601 (19q13.3) for atopy and SPT (Table 1, first set). Therefore, the region around D19S601 (from D19S433 to D19S210, 53 cM) was further analysed with 8 additional markers (Stage 2) and the results indicated linkage at marker D19S571 located near (2 cM) marker D19S601 (data not shown). The cumulative confirmatory results are given in Table 1 (all). Modest linkage was also observed at marker D19S591 (19p13.3) for atopy, elevated IgE and SPT (Table 1). A simulation analysis was performed on the whole chromosome with all 22 markers for the atopy phenotype: an NPL-Z > 3.326 (D19S601) was obtained in 2 replicates out 1000 ($P = 0.002$), while an NPL-Z > 2.56 (D19S591) was obtained in 16 replicates out of 1000 ($P = 0.016$). Figure 1 shows the multipoint linkage analysis plots for atopy and SPT along chromosome 19, obtained with all markers. Two peaks, corresponding to D19S601 and D19S591, are evident. The other phenotypes investigated displayed lower linkage values along the chromosome, and therefore they have not been shown. More detailed data are available at <http://medgen.univr.it/asthma>.

Discussion

In this report we present a linkage analysis on chromosome 19 for allergic asthma phenotypes in a representative

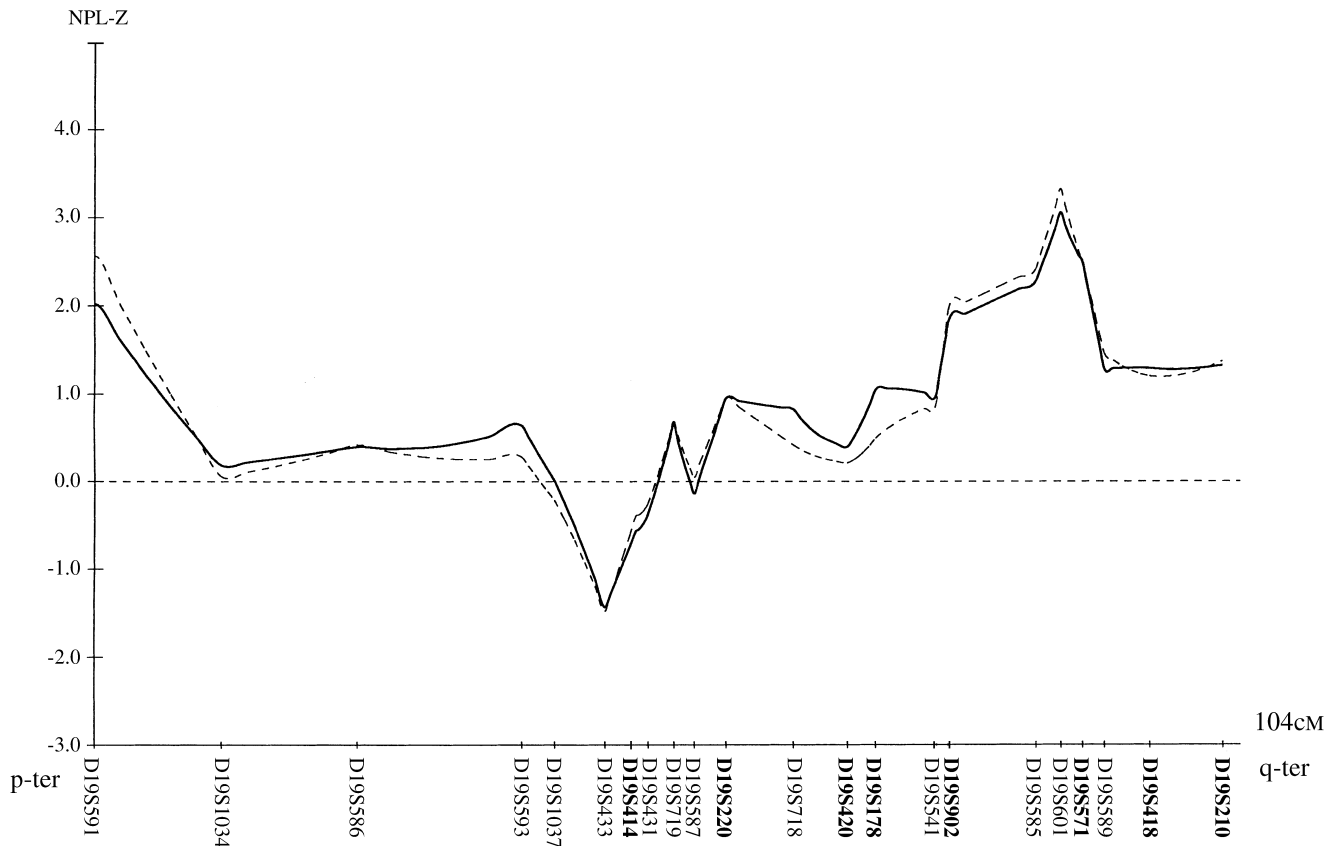


Fig. 1. Multipoint linkage analysis plots for atopy (broken line) and SPT (continuous line) against a genetic map of markers on chromosome 19. On the X-axis markers are displayed left to right from p-ter to q-ter. Stage 1 markers are presented in normal type, while Stage 2 markers are displayed in bold type.

sample of the Italian population. Due to little evidence for candidate genes on chromosome 19, we have undertaken a chromosome-wide gene-scan rather than a candidate gene approach. We found some evidence for linkage to atopy and SPT phenotypes with marker D19S601 in 19q13.3 ($P = 0.00051$ and $P = 0.0012$, respectively). By reference to the published criteria on threshold levels for linkage in complex traits [18,19], the observed linkage for atopy can be considered suggestive. However, the criteria for the determination of a threshold based on a whole genome search are still a subject of debate, particularly when a limited number of markers are considered in individual chromosomal searches [18,20–22]. We also found modest evidence of linkage with marker D19S591 in 19p13.3 for atopy ($P = 0.0056$), SPT ($P = 0.022$) and IgE ($P = 0.013$). Statistical significance was supported through a simulation analysis which showed a decrease in, but not an invalidation of, the statistical significance of the linkage results. Therefore, these remain suggestive for D19S601 while requiring further investigation for D19S591.

Three genome-wide screens have indicated the potential

involvement of chromosome 19 in the aetiology of the disease in US Caucasoids [5] and in the Hutterites [6,7]. The Collaborative Study on the Genetics of Asthma (CSGA), in US Caucasoids, reported the best evidence of linkage for asthma to 19q13.3 at marker D19S178, while no linkage was evident for D19S601 [5]. Another genome-wide search described linkage for asthma and BHR at D19S178 in the Hutterites [6]. D19S178 was included in the second set of markers, but no evidence of linkage was found. The D19S601 region identified in this study as potentially linked to atopy and SPT lies approximately 17 cm [5, this study] from the region identified by the two above mentioned genome screens for asthma and BHR, suggesting that asthma and atopy may be associated with different susceptibility loci. Alternatively, these studies may have sufficient power to detect linkage, but their resolution is limited; therefore the localization remains imprecise and the two markers may indicate the same susceptibility locus. Interestingly, Ober and colleagues [7] reported $P < 0.05$ for specific allergens in the region around D19S589 (approximately 3 cm from D19S601). In

the region comprising D19S601 the interleukin (IL)-11 and the interferon regulatory factor (IRF)-3 genes map. In the region around D19S591, the low affinity receptor for immunoglobulin E (FC ϵ R2 or CD23) gene has been recently shown to be associated to a high level of IgE [23]. Further analysis is needed to identify the possible susceptibility loci located in the linked region on chromosome 19.

Acknowledgements

This work was supported by Telethon Italy, the Italian Ministry of University and Research, and the Italian C.N.R. Target Project Biotechnology. S. V. acknowledges the support and advice of Dr P. A. Battaglia, Istituto Superiore di Sanità, Roma.

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