

ARTICLE

A detailed genetic map of the chromosome 7 bronchial hyper-responsiveness locus

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Non-specific bronchial hyper-responsiveness to various inhaled stimuli is a characteristic of asthma. We have previously shown linkage of bronchial responsiveness to methacholine (measured as dose-response slope (DRS)) and the peripheral blood eosinophil count (EOS) to chromosome 7. We have now further investigated these linkages by genotyping 49 microsatellite markers across the DRS locus on chromosome 7. The markers were spaced on average 2.6 cM apart and spanned a sex averaged cumulative genetic distance of 129 cM. Multipoint linkage to DRS was bimodal and dipped at the centromere. The two peaks of linkage were close to markers D7S484 ($P=0.0003$) and D7S669 ($P=0.006$) respectively. Separate testing for linkage to paternally and maternally derived alleles showed that the linkage near D7S484 was paternally derived ($P<0.00001$): maternally derived alleles did not exhibit significant linkage. The results indicate that two disparate loci may be influencing asthma from chromosome 7.

European Journal of Human Genetics (2002) 10, 177–182. DOI: 10.1038/sj/ejhg/5200787

Keywords: bronchial responsiveness; genetic map; chromosome 7; parent of origin effects

Introduction

Asthma is the most common chronic disease of childhood, and arises through interactions between genes and the environment.¹ These interactions are complicated by incomplete penetrance, phenotypic pleiotropy and environmental variation. Despite this complexity, segregation analysis² and twin studies³ have demonstrated a strong genetic component to asthma. In addition, many putative genetic loci influencing components of the asthma phenotype have been reported.¹

Asthma is usually recognised epidemiologically by standard symptom questionnaires or by physician diagnosis. Approximately 90% of children with asthma are atopic, and are allergic to common respirable proteins, called allergens. Atopy is detected by skin prick tests, or by measurement of

specific serum IgE titres against allergens with RAST or ELISA techniques, or by quantifying the total serum IgE. Atopic disease is accompanied by blood eosinophilia, and eosinophils are prominent effectors of inflammation in asthmatic airways.

Asthmatic airways are also typified by an increased non-specific constriction in response to a variety of inhaled substances. This non-specific bronchial responsiveness (NSBR) may be quantified, and bronchial hyper-responsiveness (BHR) was included in the 1962 American Thoracic Society definition of asthma. Early studies suggested that asthmatics could be differentiated from non-asthmatics by BHR. It is now apparent that bronchial responsiveness is a normally distributed variable within the general population, and there is a considerable overlap between individuals with asthma, atopy (IgE mediated allergy) and chronic obstructive pulmonary disease (COPD). Nevertheless, if COPD and smoking are excluded, BHR and asthma are very strongly correlated.⁴

A transmissible familial component of BHR has been long recognised,⁵ and segregation analysis has shown DRS to have a heritability of 30% and to have separate genetic determinants to atopy.⁶

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Received 15 May 2001; revised 11 January 2002; accepted 24 January 2002

Genetic linkage to bronchial responsiveness has been reported in humans⁷⁻⁹ and mice.^{10,11} In a genome-wide screen for linkage to quantitative traits underlying asthma we have reported linkage between DRS and chromosomes 4 and 7.⁸ The most significant single-point linkage to DRS was to the microsatellite marker D7S484 ($P < 0.0005$). The same marker was linked to the peripheral blood eosinophil count. A subsequent genome scan of French families with asthma showed some evidence of linkage of BHR to neighbouring markers,¹² as did a study of German children.¹³ The locus has been identified in two studies of founder populations: some evidence of linkage to asthma was found in Hutterites,¹⁴ and most recently linkage of asthma and the total serum IgE was identified to the same region in a Finnish population isolate.¹⁵ It is interesting to note that linkage of inflammatory bowel disease (IBD) has also been demonstrated in one study to D7S484 and surrounding markers.¹⁶ This might suggest that a gene or genes in the vicinity of D7S484 may be common to a number of inflammatory phenotypes.¹⁷

Here we report the results of a multipoint linkage study using dense map of microsatellite loci across human chromosome 7. Subsequently we speculate on candidate genes located within a 1 LOD score support interval around the peaks of linkage.

Methods

Subjects and phenotypes

Two hundred and thirty-two randomly ascertained West Australian nuclear families (1020 individuals, 484 sib-pairs) were studied. Families were identified via the electoral role, targeting adults aged 55 and under. Children under the age of 5 years were excluded since they could not complete respiratory testing. Clinical testing of the entire data set took place in the Australian winter months of May, June and July in 1992. Eighty families (363 individuals) were sub-selected for linkage analyses from the recruited population to include families with sib-ships of three or greater with both atopic and non-atopic members represented.⁸

'Asthma' was defined as a positive answer to the questions 'Have you ever had an attack of asthma?' and 'If yes, has this happened on more than one occasion?'. 'Atopy' was defined by the quantitative phenotypes of skin test index (STI) and radioallergosorbant test (RAST) index (specific IgE to Timothy grass and house dust mite) and total serum IgE as described previously.⁸ Eosinophils in peripheral blood were Coulter-counted and the values \log_e transformed before analysis. Bronchial responsiveness to methacholine (up to 12 micromoles) was measured in by the rapid method of Yan.¹⁸ The provocative dose of methacholine to produce a 20% fall in FEV1 (PD20) was measured by interpolation of the dose-response curve. This measure is strongly censored, as most individuals will not

show a 20% decline in FEV1 despite a maximal dose of methacholine. We therefore estimated the slope of the dose-response curve (DRS) as the (pre-dose forced expiratory volume in 1 s (FEV1)–last FEV1)/cumulative dose of methacholine. A constant of 0.01 was added to each calculation of bronchial responsiveness to allow for natural logarithmic normalisation of the data.¹⁹ Moderate bronchial hyper-responsiveness was defined as a dose of methacholine producing a fall in 20% of FEV1 (PD₂₀) ≤ 4 micromoles.²⁰ Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform extraction procedures.

Saturation mapping of chromosome 7

Forty-seven microsatellite markers were typed over chromosome 7. Of these, six: D7S484, D7S486, D7S629, D7S524, D7S519 and CFTR, were included in our previous genome screen.⁸ Four markers, GATA4B03, GATA86D01, GATA7A04 and GATA84F06, were Co-operative Human Linkage Center (CHLC) tetranucleotide markers (<http://lpg.nci.nih.gov/CHLC>). The remaining 37 were dinucleotide repeats from Dib *et al.*²¹ Details of these can be found at the GDB (<http://gdbwww.gdb.org/>) and CEPH databases (<http://www.cephb.fr/cephdb>).

Protocols for genotyping microsatellites were as previously described.²² Briefly, one primer for each microsatellite primer pair was labelled with a fluorescent tag (either 6-FAM, HEX, TET, Perkin Elmer Biosystems, Warrington, U.K.). Genomic samples (50 ng) were PCR amplified in a total reaction volume of 10 μ l also containing: 40 μ M dNTPs (Sigma Aldrich Co. Ltd., Poole, U.K.), 0.3 μ M of each primer, 1.5 mM MgCl₂, 1 \times PCR reaction buffer and 0.1 U Amplitaq[®] (all supplied by Perkin Elmer Biosystems Ltd.). All reactions were overlaid with mineral oil to prevent evaporation during thermal cycling. Cycling conditions were: 95°C for 1 min, 55°C for 1 min and 72°C for 45 s, repeated 25 times, and followed by an incubation for 10 min at 72°C. Reactions were performed on Hybaid Omnigene[®] thermal cyclers (Hybaid Ltd., Ashford, U.K.). PCR products from the same individuals were pooled then separated on an ABI 373A sequencer (Perkin Elmer Biosystems Ltd.). Allele sizes were determined using GENESCAN[®] (ver 3.1) and GENOTYPER[®] (ver 2.5) software (both Perkin Elmer Biosystems Ltd.).

Statistical analysis

All data was converted into numbered alleles and then checked for Mendelian inheritance using GAS version 2.1 (A Young 1996, <http://linkage.rockefeller.edu/soft/list2.html#g>). Genotypes from families with apparent non-Mendelian patterns of inheritance were checked and retested if necessary. Two-point linkage of the markers with DRS was tested using the SIBPAL routine of SAGE (v3.1).²³ Multipoint model-free linkage to DRS was performed using GAS. The use of GAS allowed the separate estimation of linkage to maternally and paternally derived alleles.

Results

Phenotype of subjects

The 80 families contained a total of 203 offspring forming 172 sib-pairs. The mean age of the children was 12.6 years (\pm SE 1.3) and their geometric mean IgE was 55.7 IU/L \pm 1.1. 12.4% of the children were asthmatic and 25% admitted to wheeze. Fifty-eight percent were atopic, and 10.9% had moderate bronchial hyper-responsiveness. These results are consistent with other studies of young Australians.²⁰ More than 90% of subjects were of Western European stock, based on family surnames.

Description of microsatellite loci and genetic map

Characteristics of the microsatellite loci are described in Table 1. Dinucleotide markers had a mean heterozygosity and number of alleles of 78% and 10, respectively; tetranucleotide markers were less informative and had respective mean heterozygosity and allele numbers of 70% and 6. The mean sex-averaged genetic distance between markers was 2.6 cM. Two intervals, at the centromere where markers are scarce and distal to the significant linkage, have genetic distances > 10 cM. Between 90% (D7S690) and 97% (D7S667) of all alleles in each marker (average 93%) were unambiguously assigned.

Results of linkage analysis

Two point linkage data are also shown in Table 1. Two clusters of significance were observed. The first cluster, around D7S2250, demonstrated maximal linkage to EOS at marker D7S656 ($P=0.0006$) and to DRS at D7S484 ($P=0.0003$). The second cluster of linkage centred about D7S1870 and showed a peak of linkage to both EOS and DRS at D7S663 ($P=0.007$ and 0.005 , respectively). Multipoint linkage results of the saturation data for DRS are shown in Figure 1a. The maximum $-\log_{10}$ of P value (LOP) was 3.85 and occurred at marker D7S484. The 1 LOD support unit for localisation extended between gata86d01 and D7S494 for the short-arm peak were, a distance of 34 cM. For linkage peak on the long arm the markers defining a 1 LOD support unit were D7S1870 and D7S527 encompassing 13.6 cM.

We also separately tested multipoint linkage in paternally (Figure 1b) and maternally (Figure 1c) derived alleles. A very wide peak of linkage was seen in paternally derived alleles between markers D7S506 and D7S494 with a LOP of 5.2. Maternally derived alleles only showed significant evidence of linkage on the long arm of the chromosome.

Discussion

Bronchial hyper-responsiveness is an important component of allergic asthma. We have previously shown that bronchial responsiveness to methacholine has a high heritability⁶ and that a locus showing strong linkage to DRS is located on chromosome 7.⁸ In the present study we genotyped a further 41 multi-allelic markers over a large genomic region.

We observed a bimodal distribution of significances for DRS about the centromere of chromosome 7. One LOD score support intervals around the peaks of linkage to DRS subtended wide genetic distances. It is recognised that the significance of linkage to a locus should be assigned by the width as well as the height of the linkage curve,²⁴ suggesting that these chromosome 7 loci have a strong effect on DRS. The broad peak however poses problems for identification of the genes responsible for the linkages.

The bulk of the linkage on the short arm originated from male meiotic events, and the linkage curve appeared as a single broad peak extending over the centromere when paternally derived alleles alone were examined. Broman *et al*²⁵ have previously observed a region of absence of paternally derived allelic recombination over approximately 20 Mb at 7 cen in the CEPH families. This anomalous behaviour was not seen with maternally derived alleles in their study and led to an overall under-representation of recombinations at 7 cen by twofold. Similar suppression of male recombination may explain why there is no dip in linkage observed to DRS at the centromere when considering paternal meioses alone.

Many studies have shown that parent of origin effects are important in genetic traits.²⁶ Maternal atopic status has been consistently shown to be significantly more predictive than paternal status in determining the atopic status of a child,²⁷ and preferential transmission or sharing of maternal or paternal alleles has been recognised at several loci involved in asthma and other diseases.¹ The mechanisms commonly proposed are parental allele-specific transcription (imprinting) and some unknown *in utero* mechanism.²⁷

We found that linkage to the peripheral eosinophil count closely mirrored the linkage to DRS, suggesting that the locus influences both phenotypes. Evidence for linkage in this region has been seen to inflammatory bowel disease (IBD).¹⁶ The dip in our interval map at the centromere was not seen in the corresponding IBD data, where however no markers were typed in the centromeric region.¹⁶

Three particularly interesting candidate genes have been mapped within the linked region on chromosome 7. Interleukin 6 (*IL6*) and the T cell antigen receptor gamma (*TCR γ*) are located close to D7S497 and D7S484, and elastin (*ELN*) lies close to D7S663. *ELN*, which maps to 7q11.23,²⁸ is expressed in the lung, skin and large blood vessels and plays a vital role in tissue pliability and remodelling.²⁹ Asthmatic lungs have been shown to contain degraded elastin³⁰ with decreased lung recoil that in turn could result in wheezing.³¹ *IL6* is a pro-inflammatory cytokine and is therefore a plausible candidate gene for the linkages to both DRS and IBD. A microsatellite marker near to *IL6* has been linked with osteopaenia and osteoporosis³² and a promoter SNP polymorphism has been associated with juvenile onset rheumatoid arthritis.³³ Circulating levels of *IL6* have been found to be elevated in asthmatics,³⁴ and *IL6* has been shown to be strongly expressed in murine airway epithelial cells.³⁵ *TCR γ*

Table 1 Two-point linkage analysis of 47 polymorphic markers to bronchial responsiveness to methacholine (DRS) and peripheral blood eosinophil count (EOS)

Markers	Cumulative map position (cM)*	Intermarker distance (cM)*	Heterozygosity (%)	Number of observed alleles	Linkage (P-value)	
					EOS	DRS
d7s531	12.2	3.8	78	7	–	–
d7s513	16.0	2.0	84	8	–	–
d7s493	18.0	0.9	88	13	–	–
d7s629	18.9	1.8	73	7	–	–
d7s2564	20.7	0.2	77	11	–	–
d7s2515	20.9	0.4	67	10	–	–
d7s2496	21.3	4.5	75	11	–	–
d7s526	25.8	0.8	75	10	–	–
d7s690	26.6	0.0	62	6	–	–
d7s2252	26.6	0.1	79	11	–	–
GATA4B03	26.7	1.9	67	4	0.03	0.04
d7s656	28.6	0.0	67	6	–	–
GATA86D01	28.6	0.0	64	4	0.0006	0.02
d7s683	28.6	0.4	55	6	0.03	0.0009
AFM067xa9	29.0	0.1	63	5	–	–
d7s497	29.1	0.0	53	6	–	–
d7s484	29.1	0.0	70	9	0.04	0.0003
d7s2250	29.1	0.1	79	11	0.02	0.009
d7s2251	29.2	0.3	83	16	–	0.02
AFM312za5	29.5	1.1	41	6	–	–
GATA7A04	30.6	1.1	70	6	–	–
GATA84F06	31.7	0.0	74	7	0.003	0.001
d7s528	31.7	1.1	65	7	–	0.01
d7s2497	32.8	1.6	81	15	–	–
d7s2507	34.4	0.0	72	10	–	–
d7s668	34.4	0.5	68	13	–	–
d7s510	34.9	0.0	79	7	–	–
d7s485	34.9	5.7	74	7	–	0.03
d7s519	40.6	8.8	80	6	–	0.05
d7s506	49.4	13.2	87	10	–	–
d7s494	62.6	8.2	78	11	–	0.01
d7s520	70.8	3.4	68	8	–	–
d7s502	74.2	0.0	84	13	–	0.01
d7s482	74.2	0.0	67	10	0.009	0.03
d7s663	74.2	4.4	91	12	0.007	0.005
d7s639	78.6	8.8	73	9	–	0.008
d7s653	87.4	0.8	79	12	0.04	0.007
d7s1870	88.2	4.1	78	15	–	–
d7s669	92.3	6.2	79	15	–	0.006
d7s524	98.5	3.8	73	7	0.02	0.05
d7s527	102.3	7.4	74	8	–	–
d7s486	109.7	2.4	80	12	–	0.01
CFTR	112.1	9.5	73	8	–	0.01
d7s530	121.6	10.8	78	7	–	–
d7s684	132.4	7.6	81	12	–	–
d7s483	140.0	1.5	83	11	–	–
d7s550	141.5	–	83	12	–	–

Map positions were derived from Marshfield data.²⁵ Linkage to quantitative traits was calculated using the Haseman/Elston method as implemented in SAGE (ver 3.1). Non significant linkages ($P > 0.05$) are not shown. *Genetic distances are sex-averaged.

forms a heterodimer with TCR δ in γ/δ +T cells. γ/δ +T cells reside in epithelial tissues, and, unlike α/β T cells, recognise antigen without the need for MHC presentation. Polymorphism at this locus could modulate an antigen-specific cause of DRS in these subjects, and it may be of interest that linkage of the region to allergen-specific IgE levels has been observed in other studies.^{13,14,36} A further candidate gene, CFTR, is just beyond our support interval for localisation on the long arm,

and some evidence already exists for a role of this gene in asthma.³⁷

Our results suggest the presence of loci of strong effect but the dense genetic map has identified broad peaks of linkage that would not be resolved further by typing additional markers. Gene identification at the locus might depend on pooling of data from other studies or a comprehensive SNP map testing for association rather than linkage. Screening of

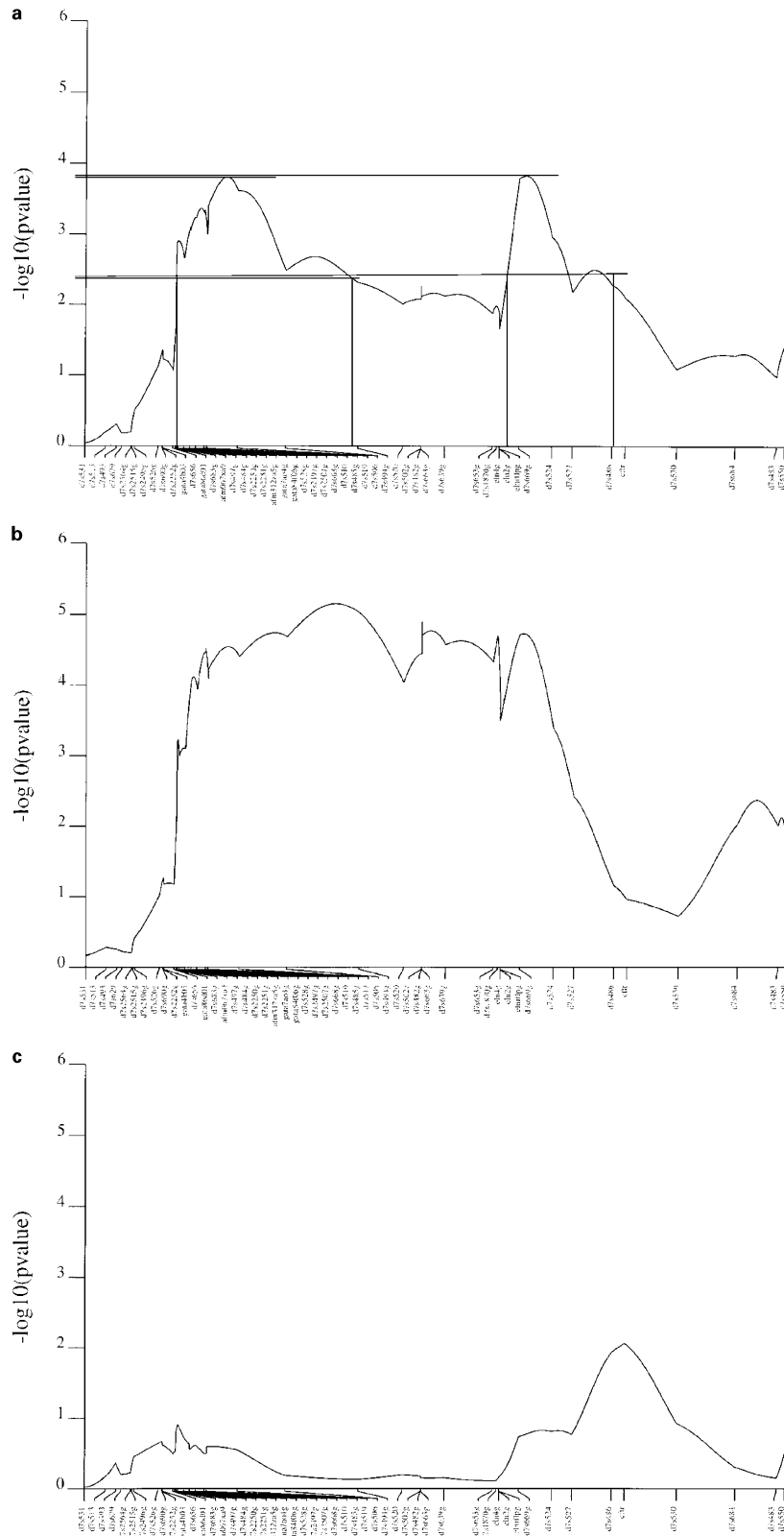


Figure 1 Multipoint linkage to bronchial responsiveness to methacholine (DRS): **a** using all alleles showing 1 LOD score support interval; **b** paternally derived alleles; **c** maternally derived alleles.

the three candidate genes *ELN*, *IL6* and *TCR γ* for association with BHR in these subjects is a priority.

Acknowledgments

The Wellcome Trust funded this work. We thank the Busselton families and all those who have helped study them. Some of the results of this paper were obtained by using the program package S.A.G.E., which is supported by a U.S. Public Health Services Resource Grant (1 P41 RR03655) from the National Centre for Research Resources.

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