Original Paper

Eur J Hum Genet 1995;3:188–194

A Genetic Map of Chromosome 11q, Including the Atopy Locus

A.J. Sandford[a]
M.F. Moffatt[a]
S.E. Daniels[a]
Y. Nakamura[b]
G.M. Lathrop[c]
J.M. Hopkin[d]
W.O.C.M. Cookson[a]

[a] Nuffield Department of Medicine, John Radcliffe Hospital, Oxford, UK;
[b] Division of Biochemistry, Cancer Institute, Tokyo, Japan;
[c] CEPHB, Paris, France; and
[d] Osler Chest Unit, Churchill Hospital, Oxford, UK

Key Words
Atopy
Genetic map
Linkage analysis
Chromosome 11

Abstract

Atopy is a common and genetically heterogeneous syndrome predisposing to allergic asthma and rhinitis. A locus linked to the atopy phenotype has been shown to be present on chromosome 11q12-13. Linkage has only been seen in maternally derived alleles. We have constructed a genetic linkage map of the region, using 15 markers to span approximately 27 cM, and integrate previously published maps. Under a model of maternal inheritance, the atopy locus is placed within a 7-cM interval between D11S480 and D11S451. The interval contains the important candidate gene FCERIB.

Introduction

Atopy is a common familial syndrome which underlies allergic asthma and rhinitis. It is characterised by immunoglobulin E responses to common aero-allergens such as grass pollens or house dust mite. An atopy-associated phenotype may be defined by measuring prick skin test responses to these allergens, by measuring specific IgE responses and by estimating the total serum IgE. These variables are strongly correlated with each other and with the prevalence of symptoms [1].

A gene predisposing to atopy has previously been localised on chromosome 11q13 [2]. The linkage has been replicated in nuclear families [3], and has been independently confirmed in extended Japanese families [4], and Dutch asthmatic sib pairs [5]. All these positive linkage results were seen in families with severe symptomatic atopy. Linkage at this locus is made more difficult because it is seen predominately through maternal meioses [3–7]. Linkage is also confounded by a high population prevalence, and low penetrance in early childhood and late adult life [7].

In common with many studies of complex diseases, the ability to replicate the linkage has not been universal [8–10]. Despite some methodological difficulties with these nega-

Received: September 22, 1994
Revision received: February 10, 1995
Accepted: March 1, 1995

Dr. William Cookson
Nuffield Department of Medicine
John Radcliffe Hospital
Oxford OX3 9DU (UK)

1018–4813/95/
0033–0188$8 00/0

tive studies, particularly the size of the sample investigated, it is clear that significant genetic heterogeneity underlies the syndrome. The importance of the 11q13 locus with respect to other loci has not yet been determined.

The atopy locus has previously been mapped to the interval between the loci D11S97 and D11S451 [11]. More precise mapping required a comprehensive genetic linkage map of 11q13 and the surrounding area. Previous maps of this region [12, 13] have only one locus in common. To integrate markers from these two earlier maps, we have genotyped 15 loci from the region in 88 two-generation families segregating atopy. We have subcloned previously described cosmid probes [14, 15] to reduce the number of repetitive elements, and to facilitate genotyping.

Materials and Methods

Pedigrees

A mapping panel of 401 subjects from 88 nuclear families was studied as described previously [6]. The father was atopic in 31 families, the mother in 17, both parents in 21, and neither in 5; in the remainder, atopic status of one or both parents was uncertain or unknown. The families were recruited through probands with asthma or hay fever, or through media appeals for families with symptomatic atopy.

Phenotype

Atopy was defined as the presence of one or more of the following features: a positive skin prick test at least 2 mm greater than a negative control; a positive specific IgE titre, and a high total serum IgE concentration. Skin prick tests to a variety of common aero-allergens were performed as described previously [2]. Specific IgE was detected by enzyme-linked immunosorbent assay (ELISA; Phadezym RAST, Pharmacia) and the cut-off points for a positive titre were as used previously [2]. A high total IgE (Phadezym PRIST, Pharmacia) was taken to be greater than published normal values for children [16] or 100 kU/l in non-smoking adults [17]. High concentrations of IgE in smokers and ex-smokers, and IgE in the borderline range 85–100 kU/l was classified as unknown phenotype in the absence of other abnormal tests.

DNA Markers

The characteristics of the markers used to construct the linkage map are summarised in table 1. The markers include two VNTR probes and two microsatellite repeats. Eight of the markers were originally cosmid clones. Although the cosmid from the D11S453 locus revealed alleles different to those previously reported in Japanese subjects [14], the probe was retained in the analysis because of its linkage to the other markers in the region.

Subcloning of Cosmid Probes and Detection of Polymorphism

Because the cosmid probes contained repetitive sequences, with subsequent difficulty in Southern hybridisation, subclones free of repetitive elements were developed for each probe.

As each cosmid was likely to contain a complete copy of one allele found at that locus, digestion of cosmids with the appropriate restriction enzyme allowed recognition of particular allele fragments by their size. Fragments of the correct size were excised from a gel and hybridised to suitable genomic blots. Fragments detecting the correct polymorphism were subcloned into pUC19 [18].

This procedure was successful for seven of the eight cosmids used in this study (table 2). Cosmid cCI11-44 did not however contain a *Pst*I fragment which was the same size as one of the alleles at this locus. This was probably due to an incomplete allelic fragment at one end of the genomic insert in cCI11-44. The ends of the insert were isolated using the two *Not*I recognition sequences flanking the vector cloning site [19]. Two fragments from a *Pst*I digest of cCI11-44 were cleaved by *Not*I. One of these was found to detect the polymorphism and subcloned as pCI11-44-AS (table 2).

RFLP and VNTR polymorphisms were detected using standard procedures described previously [11]. The α satellite centromere probe pLC11a was used under conditions designed to ensure that only chromosome-11-specific hybridisation occurred [20]. Two microsatellite markers (FcεRIβca and CI11-319ca) were isolated and typed as previously described [11, 21, 22].

Linkage Analysis

All autoradiograms were independently scored by two individuals without knowledge of the atopic status of the subjects. Linkage analysis was performed with the LINKAGE group of programs [23]. Estimates of allele frequencies at the marker loci were derived from the literature or from unrelated individuals in the mapping panel.

Table 1. Characteristics of RFLP/VNTR markers

Probe (locus)	Enzyme	Allele sizes kb	Allele frequency	PIC	Reference
pMS51	*Taq*I	VNTR 9 alleles		0.77	25
(D11S97)		1.3–4.3			
pLC11a	*Xba*I	abs	0.71	0.41	20
(D11Z1)		2.5+3.4	0.07		
		1.0	0.19		
		1.0+2.5+3.4	0.03		
pB1-21	*Msp*I	9.0	0.47	0.37	26
(CD20)		6.0	0.53		
p3C7	*Msp*I	10.0	0.30	0.32	27
(D11S288)		7.0	0.70		
PGA101	*Eco*RI	13.5+17.2+17.8	0.54	0.48	28
(PGA)		17.2+17.8	0.37		
		22.0	0.06		
	*Bgl*II	6.3			
		1.6			
pCJ52.92-AS	*Msp*I	4.7	0.47		
		3.9	0.53		
pCI11-4-AS	*Taq*I	VNTR 6 alleles		0.70	14
(D11S427)		2.5–3.5			
pCI11-8-AS	*Pst*I	4.8	0.42	0.36	14
(D11S429)		3.1+1.7	0.58		
pCI11-44-AS	*Pst*I	3.5	0.58	0.36	14
(D11S443)		2.5	0.42		
pCI11-222-AS	*Taq*I	4.5	0.33	0.34	14
(D11S451)		2.4	0.67		
pCI11-231-AS	*Taq*I	7.8	0.58	0.36	14
(D11S453)		4.0	0.42		
pCI11-318-AS	*Rsa*I	2.5	0.42	0.36	14
(D11S479)		1.8	0.58		
pCI11-473-AS	*Taq*I	6.2	0.50	0.37	15
(D11S585)		3.1	0.50		
CI11-319ca		0.201	0.07	0.74	22
(D11S480)		0.199	0.15		
		0.197	0.31		
		0.195	0.10		
		0.193	0.04		
		0.191	0.02		
		0.189	0.30		
FcεRIβca		0.128	0.03	0.63	11
(FCERIB)		0.124	0.01		
		0.122	0.23		
		0.120	0.28		
		0.118	0.01		
		0.116	0.42		
		0.112	0.02		

abs = Absent.

Table 2. Subclones of cosmid probes.

Subclone	Insert fragment
pCI11-4-AS	2.7 kb *Pvu*II
pCI11-8-AS	1.7 kb *Pst*I
pCI11-44-AS	5.3 kb *Pst*I
pCI11-231-AS	7.8 kb *Taq*I
pCI11-318-AS	2.5 kb *Rsa*I
pCI11-222-AS	4.5 kb *Taq*I
pCI11-473-AS	3.1 kb *Taq*I
pCJ52.92-AS	3.9 kb *Msp*I

Pairwise recombination estimates and lod scores were calculated between all possible pairs of loci. Map construction was started with sets of three informative markers, which were tested for all possible orders. Orders with odds of 1,000:1 better than any other order were chosen as a framework onto which all other markers were mapped. Each additional marker was tested in every possible position and added if placement was favoured by odds of a least 1,000:1, thus extending the map in a sequential manner. The map was constructed assuming sex-equal recombination fractions for each interval. Once an initial locus order was determined, the data were analysed to identify any individual chromosomes with multiple recombination events. Markers were retyped when these recombinants involved closely linked adjacent loci.

To minimise the possibility of an incorrect order being accepted, the map was constructed several times using different starting sets of loci. Once an order was established; the odds against inversion of adjacent loci were calculated. For markers whose placement was not favoured by odds of 1,000:1, a 1-lod-unit support interval was estimated (approximating to a 95% confidence interval).

Linkage of atopy to the markers was assessed independently for maternally and paternally derived alleles by affected sib-pair methods. All sibling pairs from multiple sibships were regarded as independent [24]. Significance was calculated on the assumption that the expected proportion of sibling pairs sharing an allele from the specified parent was 0.5.

To derive a 1-lod-unit support interval for the localisation of atopy from the multipoint map, a maternal model of inheritance of atopy was imposed on the data. The analysis assumes that paternal inheritance of atopy does not take place at this locus, and that non-atopic mothers are 'carriers' who are nevertheless capable of transmitting an allele conferring disease to their offspring (as is the case of an imprinted allele derived from the maternal grandfather).

The LINKAGE program was forced to consider only maternal alleles for the transmission of atopy by classifying all fathers as having a normal phenotype with a penetrance of 1.00. To allow for the presence of carriers, the mothers were assumed to have a penetrance < 1, arbitrarily 0.60 in the present analysis. The children were classified as having a penetrance of 0.99 for heterozygotes. The gene frequency of atopy was set at 0.25 and only affected offspring were considered. The LINKMAP program was then used to define a 1-lod support interval for atopy.

Results

Two-Point Analysis

Pairwise recombination estimates and lod scores were calculated between all 15 loci. Differences in sex-specific recombination rates were examined for each adjacent locus pair in the map. A significant difference in sex-specific recombination was only found between D11S451 and FCERIB ($\chi^2 = 5.06$, $p < 0.059$), which showed an excess of female recombination.

Multipoint Analysis

The results allowed the construction of a genetic linkage map spanning a distance of 27 cM (sex-equal length) on chromosome 11. Eleven of the 15 markers could be ordered with odds of at least 1,000:1 (fig. 1). The overall length of the map between CJ52.92 and D11S288 was 37 cM in female meioses and 21 cM in males (female/male ratio 1.8).

The framework loci used for the construction of the map were D11S97, D11S480 and D11S429. The mapping procedure was repeated with three different sets of framework loci, giving the same results. Odds against the inversion of adjacent loci are shown in figure 1. This figure demonstrates that the weakest evidence is for the relative placements of D11S97 versus D11S443, D11S427 versus D11S480 and D11S585 versus FCERIB. The low odds for these orders arise because the recombination rates between these loci are very low. Four markers could not be placed on the map with 1,000:1 odds, either because they were not very informative, or because of their close proximity to other markers. Figure 1 shows their most likely positions.

Localisation of Atopy

No significant excess of shared paternal alleles was present for any locus (table 3). However, for the maternal meioses, all the

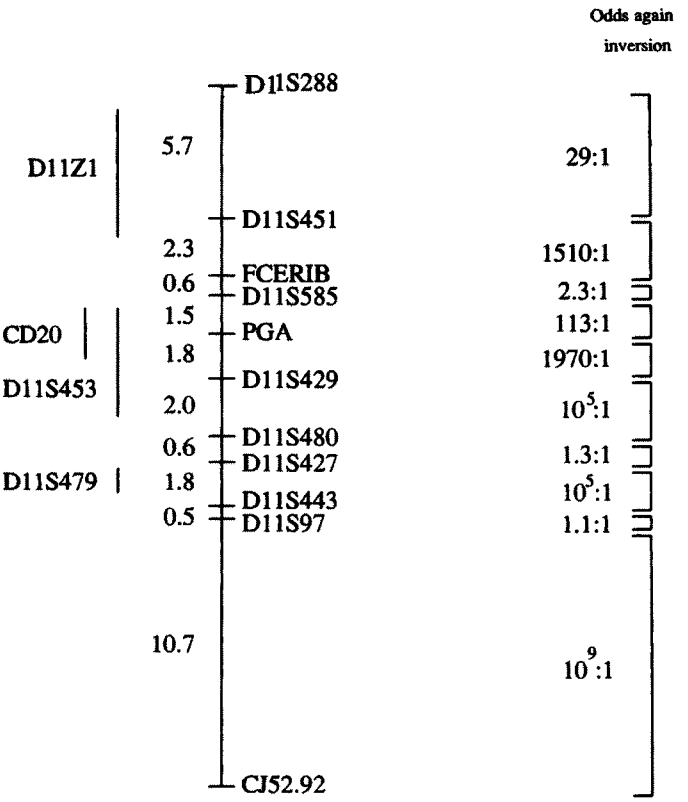

Fig. 1. Genetic linkage map of chromosome 11q, constructed assuming sex-equal recombination fractions for each interval. Distances are indicated in cM (Kosambi mapping function). Markers with only regional localisation are displayed on the far left. The bars delimit the 1-lod-unit support interval for localisation. Odds against inversion of adjacent loci are shown on the right of the map.

markers showed an excess sharing of alleles. The results extend our previous observation that atopy at the 11q locus is inherited through maternal meioses [6]. The degree of excess sharing was highest in markers centromeric to D11S97, but simple inspection of the data did not give precise localisation of the locus. To use multipoint information from the extended chromosome 11 haplotype, and to generate a confidence interval for the localisation of atopy, the LINKMAP program was used as described above, exclusively utilising information from maternal alleles in affected children. This analysis defined a 7-cM confidence interval for atopy excluding the flanking markers D11S480 and D11S451 (fig. 2).

Discussion

We have constructed a genetic linkage map of 15 markers on chromosome 11. Two other linkage maps of this region have been published [12, 13]. The map presented here shares 3 loci in common with that of Julier et al. [12] and 5 with that of Fujimori et al. [13]. The order of the markers for all the maps is entirely consistent. The map presented here has integrated marker loci from the two previous maps and has assigned positions to loci not previously ordered.

The families in this study were characterised by severe atopy, with a high level of respiratory symptoms. Other methods of ascertain-

Table 3. Alleles shared by affected atopic sibling-pairs.

Locus	Paternal alleles			Maternal alleles		
	1	0	χ^2	1	0	$\tilde{\chi}^2$
D11S288	8	13	1.19	12	6	2.00
D11Z1	20	18	0.10	29	7	13.44**
D11S451	16	17	0.03	6	5	0.09
FCERIB	30	33	0.14	53	9	31.23**
D11S585	17	17	0	36	4	25.60**
CD20	8	13	1.19	12	3	5.40*
PGA	20	14	1.06	8	3	2.27
D11S453	19	15	0.47	17	5	6.54*
D11S429	21	19	0.10	9	5	1.14
D11S480	44	42	0.05	58	11	32.01**
D11S427	23	23	0	50	12	23.29**
D11S479	11	13	0.17	31	2	25.48**
D11S443	21	24	0.20	27	2	21.55**
D11S97	42	41	0.02	70	25	21.32**
CJ52.92	15	13	0.14	14	9	1.09

* $p < 0.05$; ** $p < 0.001$.

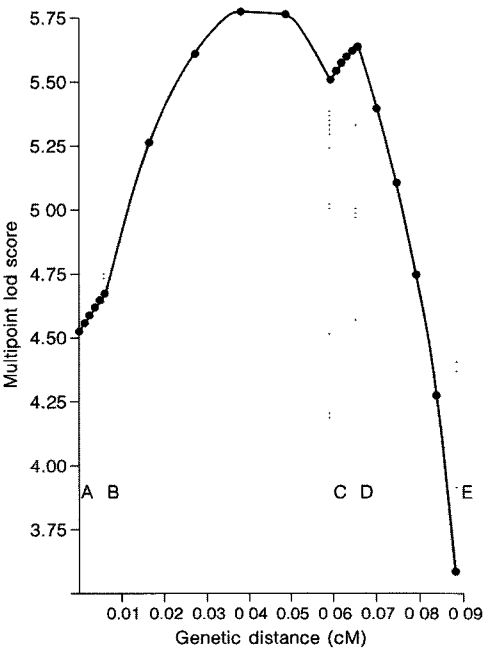

Fig. 2. Localisation of the atopy locus on chromosome 11q. Vertical lines show positions of markers. A = D11S427; B = D11S480; C = D11S585; D = FCERIB; E = D11S451.

ment, as from a population sample, may produce different degrees of linkage. The evidence for linkage has been assessed by sib-pair methods. These show, as previously [6, 11], that linkage to chromosome 11q13 in our subjects is exclusively through the maternal line. This sex-specific effect was limited to the atopy phenotype, as the recombination rates between markers displayed a slight excess of female recombination. A possible explanation for these results is that the atopy locus on 11q is subject to genomic imprinting, although maternal modification of the atopy phenotype through the placenta or breast milk is also possible [6].

This study has allowed better localisation of the chromosome 11 atopy gene, based on maternally derived alleles in affected children. The most likely position of this locus has been defined as a 7-cM interval between D11S480 and D11S451. Significantly, this interval contains FCERIB, an important candidate gene for atopy. The map presented here will assist in the design of future studies of the atopy locus on 11q, and will allow accurate estimation of the degree of heterogeneity. The map will also help in the study of other disease loci in this region.

References

1 Cookson WOCM, Hopkin JM: Dominant inheritance of atopic immunoglobulin-E responsiveness. Lancet 1988;ii:86–88.

2 Cookson WOCM, Sharp PA, Faux JA, Hopkin JM: Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet 1989;i: 1292–1295.

3 Young RP, Sharp PA, Lynch JR, Faux JA, Lathrop GM, Cookson WOCM, Hopkin JM: Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. J Med Genet 1992;29:236–238.

4 Shirakawa T, Hashimoto T, Furuyama J, Morimoto K: Linkage between severe atopy and chromosome 11q13 in Japanese families. Clin Genet 1994;46:228–232.

5 Collee JM, de Vries HG, Kliphuis JW, Bouman K, Scheffer H, Gerritsen J: Allele sharing on chromosome 11q13 in sibs with asthma and atopy. Lancet 1993;342:936.

6 Cookson WOCM, Young RP, Sandford AJ, Moffatt MF, Shirakawa T, Sharp PA, Faux JA, Julier C, Le Souef PN, Nakamura Y, Lathrop GM, Hopkin JM: Maternal inheritance of atopic IgE responses on chromosome 11q. Lancet 1992;340: 381–384.

7 Moffatt MF, Sharp PA, Faux JA, Young RP, Cookson WOCM, Hopkin JM: Factors confounding genetic linkage between atopy and chromosome 11q. Clin Exp Allergy 1992;22: 1046–1051.

8 Lympany P, Welsh K, MacCochrane G, Kemeny DM, Lee TH: Genetic analysis using DNA polymorphism of the linkage between chromosome 11q13 and atopy and bronchial hyperresponsiveness to methacholine. J Allergy Clin Immunol 1992;89:619–628.

9 Rich SS, Roitman-Johnson B, Greenberg B, Roberts S, Blumenthal MN: Genetic analysis of atopy in three large kindreds: No evidence of linkage to D11S97. Clin Exp Allergy 1992;22:1070–1076.

10 Amelung PJ, Panhuysen CIM, Postma DS, Levitt RC, Koeter GH, Francomano CA, Bleecker ER, Meyers DA: Atopy and bronchial hyperresponsiveness: Exclusion of linkage to markers on chromosomes 11q and 6p. Clin Exp Allergy 1992; 22:1077–1084.

11 Sandford AJ, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA, Young RP, Nakamura Y, Lathrop GM, Cookson WOCM, Hopkin JM: Localisation of atopy and β subunit of high-affinity IgE receptor (FcεRI) on chromosome 11q. Lancet 1993; 341:332–334.

12 Julier C, Nakamura Y, Lathrop M, O'Connell P, Leppert M, Litt M, Mohandas T, Lalouel J, White R: A detailed genetic map of the long arm of chromosome 11. Genomics 1990; 7:335–345.

13 Fujimori M, Wells SA, Nakamura Y: Fine-scale mapping of the gene responsible for multiple endocrine neoplasia type 1 (MEN1). Am J Hum Genet 1992;50:399–403.

14 Tokino T, Takahashi E, Mori M, Tanigami A, Glaser T, Park JW, Jones C, Hori T, Nakamura Y: Isolation and mapping of 62 new RFLP markers on human chromosome 11. Am J Hum Genet 1991;48:258–268.

15 Tanigami A, Tokino T, Takiguchi S, Mori S, Glaser T, Park JW, Jones C, Nakamura Y: Mapping of 262 DNA markers into 24 intervals on human chromosome 11. Am J Hum Genet 1992;50:56–64.

16 Kjellman NIM, Johannson SJO, Roth A: Serum IgE levels in healthy children quantified by a sandwich technique (PRIST). Clin Allergy 1976;6.51–59.

17 Holford-Strevens V, Warren P, Wong C, Manfreda J: Serum total immunoglobulin E levels in Canadian adults. J Allergy Clin Immunol 1984;73:516–522.

18 Sambrook J, Fritsch E, Maniatis T: Molecular Cloning: A Laboratory Manual, ed 2. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1989.

19 Wahl GM, Lewis KA, Ruiz JC, Rothenberg B, Zhao J, Evans GA: Cosmid vectors for rapid genomic walking, restriction mapping and gene transfer. Proc Natl Acad Sci USA 1987;84:2160–2164.

20 Waye JS, Grieg GM, Willard HF: Detection of novel centromeric polymorphisms associated with alpha satellite DNA from human chromosome 11. Hum Genet 1987; 77:151–156.

21 Daniels SE, Shirakawa T: A dinucleotide repeat polymorphism in the FCERIB gene. Hum Mol Genet 1994;3:213.

22 Moffatt MF: Dinucleotide repeat polymorphism at the D11S480 locus. Hum Mol Genet 1993;2:492.

23 Lathrop GM, Lalouel JM, Julier C, Ott J: Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 1984;81:3443–3446.

24 Blackwelder WC, Elston RC: A comparison of sib-pair linkage tests for disease susceptibility loci. Genet Epidemiol 1985;2:85–97.

25 Jeffreys AJ, Wilson V, Neumann R, Keyte J: Amplification of human minisatellites by the polymerase chain reaction: Towards DNA fingerprinting of single cells. Nucleic Acids Res 1988;16:10953–10971.

26 Charmley P, Nguyen J, Tedder TF, Gatti RA: A frequent human CD20 (B1) differentiation antigen DNA polymorphism detected with *Msp*I is located near 11q12-13. Nucleic Acids Res 1990;18:207.

27 Leach R, White R: Isolation of polymorphic DNA sequences from chromosome 6. Cytogenet Cell Genet 1994;37:521.

28 Boudi FH, Lothe RA, Taggart RT: Human pepsinogen A (PGA): An informative gene complex located at 11q13. Hum Genet 1990;84:293–295.