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ARTICLE

Positive association to IgE levels and a physical map of the 13q14 atopy locus

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Linkage of atopy and associated traits to a locus on chromosome 13q14 has been identified by several studies in diverse populations. We have previously shown the putative atopy gene to be contained within an interval of approximately 5 Mb flanked by *D13S328* and *D13S1269* and centred on *D13S273*. We have now extended this work using a top-down approach to physical mapping. A YAC contig was constructed covering the *D13S328* and *D13S1269* interval. Thirty-one ESTs were mapped to the contig. We constructed a BAC and PAC contig flanking *D13S273* by ~750 kb in either direction. The interval contained 27 of the 31 ESTS from the YAC contig. Seven previously unknown microsatellites were recovered and then typed in two subject panels. A positive association between the total serum Immunoglobulin E concentration and the novel *USAT24G1* microsatellite was discovered ($P_{corrected} < 0.005$) and replicated in a second panel of families. The discovery of a region of positive association within the BAC/PAC contig will permit identification of the atopy gene from this locus.

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Introduction

Atopic asthma is the most common chronic disease of childhood in the UK.[1] Atopy and asthma result from an interaction between environmental and genetic factors and show a complex mode of inheritance. Segregation analyses and twin studies indicate that asthma and atopy have a significant genetic component.[2,3] These diseases are likely to be influenced by genes of moderate effect in addition to polygenes. Several regions of genetic linkage have been recognised by different groups, and a number of definite associations have been made to candidate genes.[2]

We have previously identified a linkage region on chromosome 13q14 in a genome screen for asthma and atopy and their associated traits in two populations.[4] The maximum linkage to atopy was identified at the microsatellite marker D13S153. No obvious candidate gene is known to exist within this region. A report of genetic linkage to the total serum Immunoglobulin E concentration (IgE) at the nearby Esterase D locus pre-existed this work,[5] and linkage has also been reported in other families with asthma and allergy.[6,7] Linkage to 13q14 has also been observed to house dust mite allergy in children with asthma[8] and to children with atopic dermatitis.[9] Kimura *et al*[7] showed linkage in Japanese families to D13S153, and identified a possible positive transmission association to atopic asthma at this marker, suggesting the proximity of a gene influencing these phenotypes.

We have subsequently created a high-density saturation genetic map of 7.5-cM of the 13q14 locus, and have shown localisation of the gene to be bordered by D13S328 and D13S1269.[10]

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Cytogenetic abnormalities at 13q14 have been noted in around 25% of cases of B-cell chronic lymphocytic leukaemia (B-CLL).[11] The most commonly deleted region extends 1 Mb between the markers D13S272 and D13S25,[12,13] although the locus has been placed more in a more centromeric position between D13S273 and D13S272 by other authors.[14] The B-CLL locus therefore extends into our region of interest, and we have integrated physical maps of this locus into our results.

Materials and methods

Families and phenotype

Two panels of subjects, AUS1 and UK1, were used in the genetic analysis of the locus. Their recruitment and phenotypic characteristics have previously been described.[4] Our genome screen was based on the AUS1 Panel (the primary data set) comprising 80 nuclear families containing 364 subjects with 172 sib-pairs.[4] The UK1 Panel (the replication set) contained 77 nuclear and seven extended pedigrees comprising 380 subjects with 268 sib-pairs.[15]

The total serum immunoglobulin E concentration was $\log_e$ transformed and analysed as a quantitative trait ($\log_e$IgE).

Libraries and library screening

The yeast artificial chromosomes (YACs) used were from the CEPH mega YAC library (www.cephb.fr/services). YACs were identified by electronic screening for STSs (http://carbon.wi.mit.edu.8000/cgi-bin/contig/phys_map) and by PCR screening as described.[16,17] The human Bacterial Artificial Chromosome (BAC) and P1-derived Artificial Chromosome (PAC) libraries were the CITB BAC Pools Release IV library (www.resgen.com/products/CITBBAC_pf.php3) and the Pieter DeJong Human PAC library (www.resgen.com/products/RPCIHum.php3). PCR screening and subculturing of the libraries was performed as described by the distributor (Research Genetics Inc., Huntsville, AL, USA). All YACs, BACs and PACs used in this project were subcultured to purity, checked for the presence of correct insert by PCR and then stored in LB broth with 30% glycerol at -80°C.

Construction of contigs

YACs were sized by pulsed field gel electrophoresis (PFGE) and Southern blotting, and were assayed for the presence of *Not*I and *Sal*I restriction sites. A preliminary single-linked contig was generated between markers *D13S328* and *D13S1269* by selecting YAC clones for known microsatellites (*D13S161*, *D13S153*, *D13S1307*, *D13S165*, *D13S273*, *D13S272*, *D13S1269*, *D13S284*, *D13S1325* and *D13S1305*). A higher density of PCR markers including further microsatellites, STSs and ESTs were then added using the YACs as DNA template. ESTs and STSs putatively located within the region were derived from a genome-wide radiation hybrid map (www.ncbi.nlm.nih.gov/genemap).

The BAC and PAC contig was centred on the marker D13S273, which was close to the peak of linkage.[10] The YAC framework map indicated that 20 PCR markers (microsatellites, ESTs and STSs) were present within this region. These makers were used to screen the BAC and PAC libraries. Gaps were closed by IRE bubble PCR[18,19] to recover BAC/PAC clone ends then designing new STSs and re-screening the libraries. *Not*I and *Sal*I restriction sites were used to align the BAC and PAC clones with one another and also with the YAC map. We aimed to achieve a greater than 2-fold depth to the entire contig. PACs, ESTs and STSs from the B-CLL locus contig[20,21] were integrated into the right hand side (qtel) of our contig.

Microsatellite identification and genotyping

IRE bubble linkers were employed to rescue novel microsatellites.[19] Repeats were obtained by PCR using the bubble primer and the following dinucleotide primers: $(CA)_{11}A$, $(CA)_{11}T$, $(AC)_{11}T$, $(CA)_{11}G$, $(AC)_{11}G$, $(AG)_{11}A$, $(AG)_{11}T$, $(AG)_{11}G$, $(AG)_{11}C$; trinuceotide primers: $(GGC)_8$, $(AAC)_8$, $(AGC)_{10}$ and tetranucleotide primers: $(AAAB)_{10}$, $(AATG)_5$, $(AGAT)_5$, $(AAAG)_5$, $(ATCC)_5$, $(AAGG)_5$ and $(ACAT)_5$. Discrete PCR products were sequenced and the length of microsatellite repeat was assessed.

Informative microsatellites were typed in both study panels A and B. Genotyping was carried out using a high throughput fluorescent PCR based method.[22] Primers are available on http://www.well.ox.ac.uk/asthma/.

Statistical Analyses

Mendelian inheritance was checked using Genetic Analysis Software version 2.1 (GAS)[23] (http://www.linkage.rockefeller.edu/soft/list2.html#g) and discrepancies were re-examined and resolved using GENOTYPER™. Statistical analyses of association to the total serum IgE were carried out by the QTDT program, which allowed variance-components testing of family-based samples for association and transmission disequilibrium as described by Abecasis *et al*[24] Association was tested in all family members (Total Association Model[24]) to maximise use of the available data. To minimise multiple comparisons, multi-allelic tests of association were performed.[24] *P* values in the primary dataset were corrected by a Bonferroni multiplication with the number of markers tested (n=22).

Results

YAC contig construction

A YAC clone contig was constructed across the region spanning *D13S328* and *D13S1269*. Thirteen YAC clones (903a12, 847g8, 830c8, 851f1, 775c8, 868f11, 959a12, 745e3, 747b8, 857c5, 895b11, 879f5 and 935g2) were identified by electronic and PCR screening. The contig extended for approximately 6 Mb between *D13S328* and *SHGC-13595*. The approximate size of the region between the extreme microsatellites (*D13S328* and *D13S1269*) was 4 Mb. FISH showed two clones (775c8 and 745e3) to be chimeric: the remaining clones were used to draw a non-chimeric,

multiply linked, minimum tiling path between *D13S328* and *SHGC-13595*. The contig contained 21 polymorphic markers, five anonymous STSs and 31 ESTs arranged into 12 bins: WI-6333, WI-9598 and D13S272 could not be binned. The microsatellite order was estimated using a combination of contig STS content and MULTIMAP to be: *D13S328* – *D13S168* – *D13S287* – *D13S161* – *D13S153* – *D13S164* – *D13S1307* – *D13S165* – *D13S273* – *D13S272* – *D13S1269* – *D13S262* – *D13S284* – *D13S788* – *D13S268* – *D13S1325* – *ATA17CO6* – *D13S1305*.

BAC and PAC contig construction

Chromosome walking produced a complete contig for the region *D13S1307* to *D13S272* (Figure 1). The contig consisted of 28 BACs and 8 PACs. There were three regions with only a single clone spanning a gap which could not be deepened despite repeated library screening. FISH determined that 5 of 41 clones were not present on 13q14. These clones were discarded. *Not*I and *Sal*I restriction digest data was completed for 34 clones (Figure 1). The average insert size of the BACs and PACs was 117.9 kb and 117.4 kb respectively. We identified five *Not*I sites and seven *Sal*I sites. They clustered in two locations, one near the junction of clones HPAC35C13, HBAC339L13 and HBAC401J1, and the other just centromeric to HBAC352L21. Summing the restriction fragments together with non-overlapping clones gave an estimate of the contig size to be ~1.5 Mb. In addition, the physical distances separating the four pre-existing microsatellites could be estimated to be: *D13S1307* – 600 kb; *D13S165* – 600 kb; *D13S273* – 300 kb; *D13S272*. Twenty-seven of the 31 ESTs placed on the YAC map were proven to be on the PAC and BAC contig (Figure 1).

Microsatellite recovery

Twenty-five novel microsatellites were recovered, seven of which were polymorphic when typed in 32 unrelated healthy volunteers. These seven new markers had mean heterozygosities between 0.601 and 0.748.

Family panel characteristics

The mean age of the children in the primary (AUS1) panel was 12.6 years (±SE 1.3), their geometric mean IgE was 55.7±1.1 IU/l: 12% were asthmatic. The mean age of the AUS1 parents was 39.9±0.36 years, their geometric mean IgE was 24.8±1.1 IU/l: 14% were asthmatic. The mean age of the children in the replication panel (UK1) was 16.8±10.9 years and their geometric mean IgE was 115.2±1.1 IU/l: 56% were asthmatic. The mean age of the UK1 parents was 39.6±0.70 years, their geometric mean IgE was 56.6±1.1 IU/l: 47% were asthmatic.

Genetic analyses

The seven novel microsatellites were typed together with 15 microsatellites already in the public domain (all that were

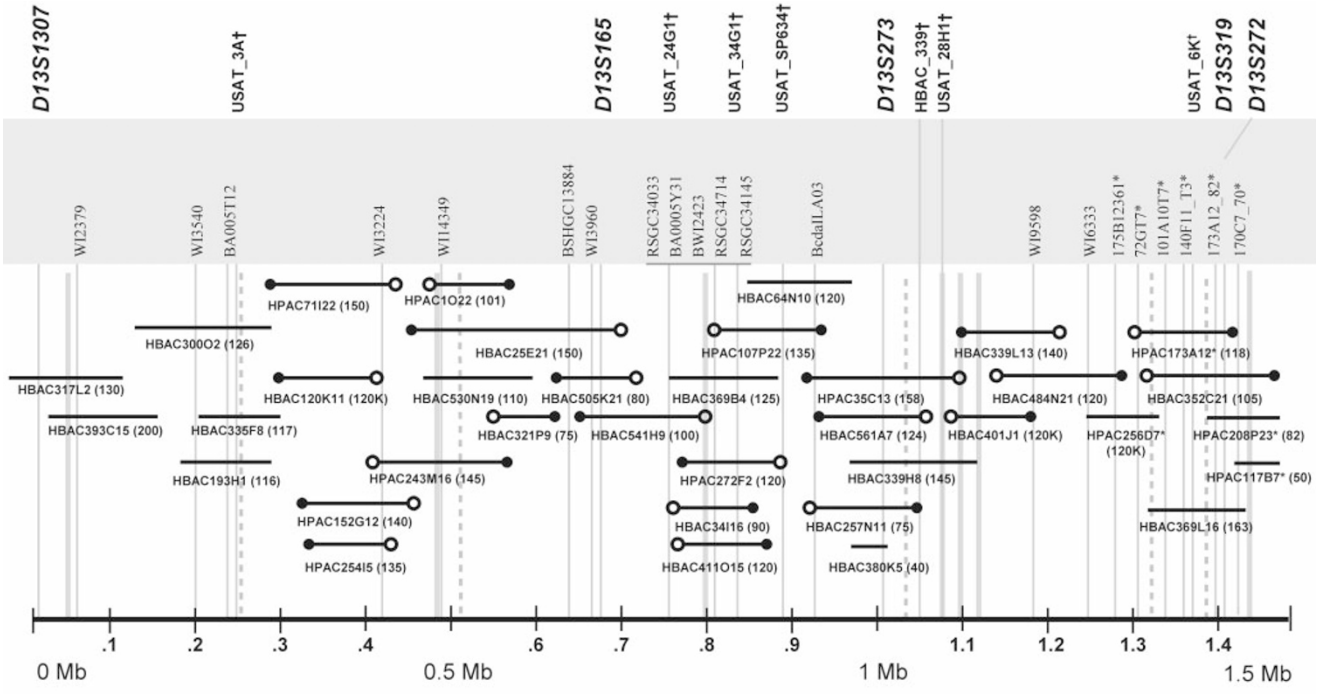

Figure 1 13q14 atopy locus BAC/PAC contig. The *Not*I restriction sites are delineated by thick dashed lines and *Sal*I by thick full lines. The size (kb) of the BAC/PAC is given in parenthesis. SP6 BAC/PAC ends are represented by the open circles, T7 by filled circles. ESTs, STSs and PACs derived from the CLL locus are denoted by * (Kalachikov *et al*[21]). Novel microsatellites are denoted by †.

available at the time of typing) (Table 1). We tested these markers for evidence of association to the quantitative trait of total serum IgE concentration (Table 1). A highly significant association was detected to the $\log_e$IgE at *USAT24G1*, within 200 kb of *D13S273*. This figure remained significant when corrected for multiple testing of 22 markers ($P_{corrected}$ =0.0044). Parent of origin effects were not detected. Adjoining microsatellites in the *USAT24G1 – D13S273* interval showed weaker association. The associations were replicated in the second panel of families.

Discussion

The results of our study build upon the previously demonstrated linkage of atopy and related phenotypes to chromosome 13q14.[4,6,7,25] The finding of a positive association between the total serum IgE concentration and microsatellites in a limited region of 200 kb represents a further step in fine localisation of the disease from the 5 cM, 1 LOD score support unit identified by Bhattacharyya *et al*.[10]

The construction of the PAC/BAC map was assisted by the work of various groups investigating the B-CLL locus.[14,20,21,26] Kalachikov *et al*[21] constructed a 13 clone PAC contig and a cosmid contig across the minimally deleted region in B-CLL at 13q14.3. This PAC contig spanned 560 kb between markers *D13S1150* and *D13S25* and is proximally overlapping with our atopy locus by approximately 200 kb at the distal (telomeric) end of our contig (around marker *D13S272*). Clones from the Kalachikov contig were validated and then incorporated into the distal 200 kb of our contig.

A *Not*I and *Sal*I restriction map was created to help orientate and estimate the overlap and total size of the clones in the contig and to identify potential CpG islands at the 5′ end of genes.[27,28] Five *Not*I and seven *Sal*I sites were identified and appeared to cluster in two regions, one immediately centromeric to *D13S272* (in the critical deletion of the B-CLL locus) and the second 50–150 kb telomeric to *D13S273*. A number of putative transcripts are close by the first cluster of CpG islands but the second cluster is only close to the EST WI9598. This may indicate that there are further transcripts to be identified from the region.

The distribution of ESTs mapped to the locus was irregular, perhaps reflecting variations in gene density or the detection of multiple fragments of genes. A cluster of ESTs was observed around BAC 346I16 and a second cluster around marker *D13S272*. The latter cluster is likely to be at least in part due to the large transcript mapping effort made by the groups investigating the B-CLL locus. It is likely that not all 27 ESTs represent unique genes, and may represent multiple fragments or splice variants of the same transcripts, contaminating genomic sequence or pseudogenes.

Seven novel microsatellites were recovered from the final BAC/PAC map and genotyped together with public domain microsatellites. The most significant association to the serum IgE in the primary dataset was to the novel microsatellite *USAT24G1*. The association to this marker remained highly significant even after correcting for the number of markers tested, and was also seen in the replication panel of families.

The extent and distribution of LD is not constant over the genome, but 'useful' LD (that is able to detect association with disease) extends for an average of approximately 50 kb in this region.[29] The limit of detection of linkage disequilibrium (LD) between a disease and a marker given our sample size is likely to be less than 100 kb,[29,30] suggesting that an atopy gene is within 100 kb in either direction of *USAT24l*.

The statistical significance of the weak associations of IgE levels to other microsatellites in the 200 kb *USAT24G1 – D13S273* interval is uncertain, but may be taken as an indication that this entire interval should be investigated with a dense SNP map, and for the comprehensive characterisation of all transcripts. The chromosome 13q14 region encompassing our locus is being currently sequenced at the Sanger Centre, Cambridge (http://www.sanger.ac.uk/HGP/Chr13/). The results are in draft form at present.

Table 1 Summary of association testing between the total serum IgE concentration and microsatellite markers across the YAC contig covering the chromosome 13q14 atopy locus

Marker	*Heterozygosity (%)*	*Location (Mbp)*	*Association to IgE (multi-allelic test)*	
			AUS1	UK1
D13S168	81.2	0	–	
D13S161	64.3	0.20	–	
D13S287	66.2	0.30	–	
D13S153	89.4	0.80	–	
D13S1307	69.6	1.35	–	
USATEA	43.0	1.40	–	
D13S165	81.6	1.45	–	
USAT24G1	61.7	1.50	0.0002	0.028
USAT34G1	49.4	1.60	0.082	–
S34	36.1	1.65	–	0.095
D13S273	75.5	1.70	0.094	0.023
USAT28H1	74.7	1.75	–	
USATHBAC339	65.0	1.80	–	
MGG	70.6	1.90	–	
USAT6K	91.2	1.95	–	
D13S272	70.5	2.05	–	
D13S1269	70.3	2.75	–	
D13S262	80.5	2.85	–	
D13S284	88.0	3.35	–	
D13S1325	81.0	3.95	–	
D13ATA17	60.0	4.55	–	
D13S1305	74.0	5.15	–	

Novel microsatellites recovered from the contig are shown in bold. Non-significant results ($P>0.1$) are shown as –. Markers were tested in the primary (AUS1) panel of families, and those showing positive results were tested in the secondary (UK1) panel

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