The -17 T/C Chemokine Receptor 3 Genetic Polymorphism Is Associated Asthma but Not Atopy: Transmission and Association Studies

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We genotyped and identified the asthma and atopic status and related phenotypes of 154 nuclear families (453 individuals) each containing at least two affected children with physician-diagnosed asthma (PDA) in order to confirm or refute the possible relevance of known single nucleotide polymorphisms (SNPs) in the gene coding for the CCR3 receptor. Allelic quantification for each SNP by DNA pooling identified –17/TC as the only allele with a clinically relevant frequency in this population with a frequencies of 0.142 in cases of PDA and 0.035 in asymptomatic controls. The whole population frequency of the –17/TC polymorphism was 13.9% and the functional binding site analyses by MatInd and MatInspector programs found that it belonged to the same family as activating transcription factor 6 (ATF-6). The pedigree disequilibrium test (PDT) was applied in 34 informative families and the mutant allele was preferentially transmitted with PDA (*P*=0.0001) with methacholine bronchial hyperresponsiveness (BHR) (0.002) but not with markers of atopy as assessed by allergen skin prick tests (SPT) or elevated serum IgE. Case-control analyses in 303 unrelated parents (34-61y [median 43y]) revealed a significant association with both atopic and non atopic asthma (*P*=0.001), and in 150 unrelated child probands for non-atopic asthma (*P*= 0.001). The mutant allele was associated with BHR, with baseline Forced Expiratory Volume in the first second (FEV1) below the population median value but not with atopy defined SPT or elevated serum IgE (>100 IU/ml). The T17C chemokine receptor 3 polymorphism appears to be associated with asthma BHR and disease severity but not with atopy.

The chemokine pathway is one of several contributing to the airway inflammation associated with asthma. Along this pathway is the C-C type chemokine receptor 3 (CCR3) which belongs to family 1 of the G protein-coupled receptors (Daugherty *et al.,* 1996). An encoding gene of this receptor, which is CMKBR3 (GenBank AF247359), is clustered with other chemokine receptor genes as CCR2 and CCR5 (< 300kb span) on chromosome 3p21.3 (Daugherty *et al.,* 1997). CCR3 is highly expressed in eosinophils and basophils, and to a lesser extent in TH1 and TH2 cells, and airway epithelial cells (Combadiers *et al.,* 1995; Uguccioni *et al.,* 1997). This receptor binds and responds to a variety of chemokines, including eotaxin (CCL11), eotaxin-3 (CCL26), MCP-3 (CCL7), MCP-4 (CCL13), and RANTES (CCL5) Heath *et al.,* 1997. Knowing its function and its tissue expression, suggests that it may contribute to the accumulation and activation of eosinophils and other inflammatory cells, as it is the major ligand for eotaxin, which is in turn influences eosinophil trafficking during baseline and inflammatory processes (Ponath *et al.,* 1996). Eotaxin and CCR3 mRNA are expressed and co-localized in the bronchial mucosa of asthma and the intensity of their expression correlates with increased airway hyperresponsiveness (AHR) (Ying *et al.,* 1997). It is also known to be an entry co-receptor for HIV-1 virus (Ying *et al.,* 1997).

Genomic sequencing has identified four different single nucleotide polymorphisms (SNPs) of this receptor, namely –17 T/C, -21 G/A, -39 T/C, and -5′UTR G/A. Of these 4 SNPs only –17T/C has been found to occur with any potentially relevant frequency for a

common disorder such as asthma and/or atopy in a caucasian population (Zimmermann *et al.,* 1998). Although the functional affect of this SNP is as yet unclear it has been found to be associated with asthma independent of atopy in a British population but not in a Japanese population (Fukunaga *et al.,* 2001).

We and others have also found evidence that asthma, atopy, and related phenotypes may associate differently with these conditions in an age specific fashion in that some may be relevant to asthma and atopy in early life but not to the same clinical conditions in adulthood (Srivastava *et al.,* 2003; Khoo *et al.,* 2004).

We therefore intended to confirm the alleleic frequencies of the 4 known SNPs of the CCR3 receptor gene and based on these frequencies assess their possible relevance to asthma to atopy and related phenotypes in generation specific case control and in familial transmission analyses.

Materials and Methods

Subjects

Population characteristics have been described elsewhere (Al-Abdulhadi *et al.,* 2005). Briefly we identified 154 unrelated nuclear families (598 individuals including children and parents) from the Local Grampian population in 2001, containing at least 2 children and young adults between 8-24 yrs, the majority of whom had a physician diagnosis of asthma (PDA) and current symptoms (wheeze in the past 12 months). All 154 families were white Caucasian and were drawn from a population in which the UK 2001 census showed that 84% were born in Scotland, 12 % in other parts of the UK and only 4% out of the UK.

Atopy was defined as the presence of at least one positive Skin prick test (SPT) with a wheal size of ≥ 3 mm among 5 inhalant allergens (cat, dog, house dust mite, grass and alternaria) referenced to negative control and serum IgE (Pharmacia and Upjohn Diagnostics, Milton Keynes, UK) [10], categorised as above or below 100 IU/ml. Bronchial hyperresponsiveness (BHR) was assessed by methacholine challenge (Cookcroft *et al.,* 1977), only omitting this test in subjects with a baseline Forced Expiratory Volume in the first second (FEV1) below 70% predicted. A positive response and hence significant responsiveness was defined as a fall in baseline FEV1 of 20% or more at a dose of less than 8mg/ml of inhaled methacholine. Predicted FEV1 values for adults ≥ 18 years were from Crapo *et al.,* (1981) and for children < 18 years from Wang *et al.,* (1993). Baseline FEV1 was categorized as above or below the median whole population % predicted value. DNA was isolated from EDTA anticoagulated whole blood using the phenol-chloroform method.

Ethical approval for all studies was awarded by the Grampian Research Ethics Committee.

DNA Pooling and Allelic Quantification (AQ)

Diluted genomic DNA samples were pooled (pre-PCR pools) separately for cases and controls. Six pre-PCR pools were produced by randomly sampling 200 individuals from cases and controls with pools of 100 random even and odd numbered individuals. Each pre-PCR pool was amplified by PCR for all 4 candidate SNPs in triplicate using the following PCR conditions: 50μl volumes containing 100ng of genomic DNA; 10 × PCR Buffer; 25 mM $MgCl_2$; 10 mM dNTPs; 10 pmol/μl of each primer (Table 1). For each run 5 U of *Taq* DNA polymerase was used with PCR conditions of 40 cycles of 95°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1.5 minutes. The PCR products (post-PCR pools) were inspected for clearly scored product using 1.5% agarose gel electrophoresis stained with ethidium bromide. Thirty microliters of each post-PCR pool was then used to determine the allelic frequencies using Pyrosequencing™ technology (PSQ) (Quadt *et al.,* 1995). The PCR products were prepared for PSQ using a PSQ96 Sample Prep tool and PSQ reactions were performed using the PSQt96 SNP reagent kit (Biotage AB, Sweden) according to the manufacturer's instructions with two different sequencing primers for each SNP (Table 1) and each run was performed in triplicate. Each PSQ96 plate contained one negative control (no template) and one positive control per genotype. The program PSQt96 evaluation allelic quantification (AQ) software was used to obtain the ratio of each separate allele peak height to the sum of height of both allele peaks. To allow the conversion of this peak height ratio to allele frequency for the DNA pools, a standard curve based on individual samples was established and the ratios from pooled samples plotted against individual sample frequency. The equation of the linear regression best-fit line was then determined for each SNP and used to convert the allele peak height ratios to allele frequencies in the DNA pools.

Genotyping and Sequencing

Primers and probes informed by the allelic frequency results were designed by Assay-by-design (Applied

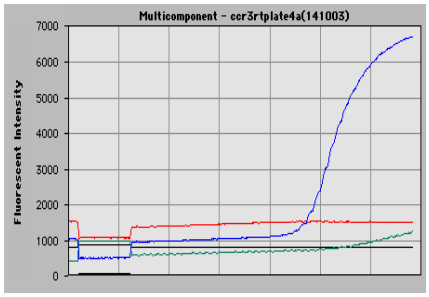
Biosystems, California, USA) (Table 1). Probe lengths were adjusted such that both probes had approximately the same melting temperature (67.0ºC). The probe melting temperature was 7.0-8.0 ºC above the primer melting temperature of 60 ºC. Two-fluorogenic allele-specific probes were used one matching the wild-type sequence, and one matching the mutant sequence. Each probe was labelled at the 5´end with a fluorescent reporter dye and at the 3´end with the quencher dye NFQ. The reporter dyes in this study were VIC for the wild-type sequence and FAM for the mutant sequence. TaqMan universal master mix (Applied Biosystems, USA) was used at a final concentration of 1× and volume of 25 µl along with 50 ng of genomic DNA with forward and reverse primers for wild-type and mutant probes. Optical plates (Applied Biosystems, USA) were thermal cycled in the Prism 7700 Sequence Detection System (SDS) for real-time detection and (Fig 1,a,b,c) end-point analysis (Fig 2). Direct sequencing was performed in random samples of each genotype and in all cases were identical to the genotypes inferred from PCR RFLP.

Table 1. Allelic Quantification (AQ), TaqMan Assay and Direct Sequencing Oligonucleotide Sequences.

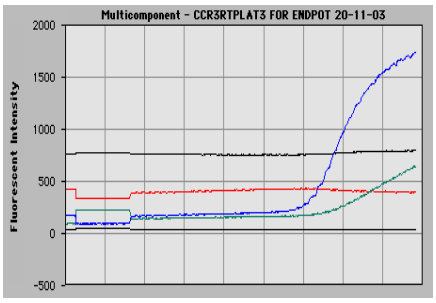
SNPs	Forward Primer Sequence (5´→3´)	Reverse Primer Sequence (5´→3´)	Tm* (ºC)
Allelic Quantification (AQ):			
CCR3-primers	Biotin-CACATGTGGCATCTTTGTTG	GGCCATCAGTGCTCTGGTAT	55
s-SNP1: G21D	CTTTTTCACAGAGCAGG		
s-SNP2: 5´UTR	TGAGGTTGTCATTTCACTT		
SNP3-primer:	CACATGTGGCATCTTTGTTG	Biotin-GGCCATCAGTGCTCTGGTAT	57
s-SNP3: Y17Y	CCTTTGGTACCACATCCT		
SNP4-primer:	CCTGCTCTGTGAAAAAGCTGA	Biotin-GATCATCACCACCACCACAT	57
s-SNP4: T39C	CCAGTTTGTGCCCC		
TaqMan Assay:			
CCR3Y17Y	AGTTGAGACCTTTGGTACCACATC	ATCAGTGCTCTGGTATCAGCTTTT	92
Props	VIC-CCACGTCATCGTAGTAG-NFQ	FAM-CCACGTCATCATAGTAG-NFQ	
Direct sequencing:			
CCR3Y17Y	CCCTAGGCTGCTATCACAT	CATCACCACCACCACATT	57

* Tm = Optimum annealing temperatures; s = Sequencing primers.

a) CCR3 1,1 Genotype:



b) CCR3 1,2 Genotype:



c) CCR3 2,2 Genotype:

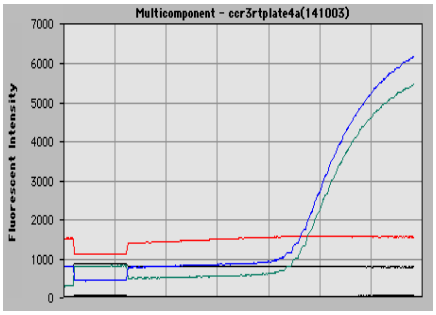


Figure 1. CCR3-17T/C SDS genotyping by real-time sequencer shows: the genotype discrimination as (a) single fluorescence peak (VIC) as 1,1 genotype. (b) Two fluorescence peaks (VIC) and (FAM) as 1,2 genotype. (C) Single fluorescence peak (FAM) as 2,2 genotype. Fluorescence intensity for both dyes.

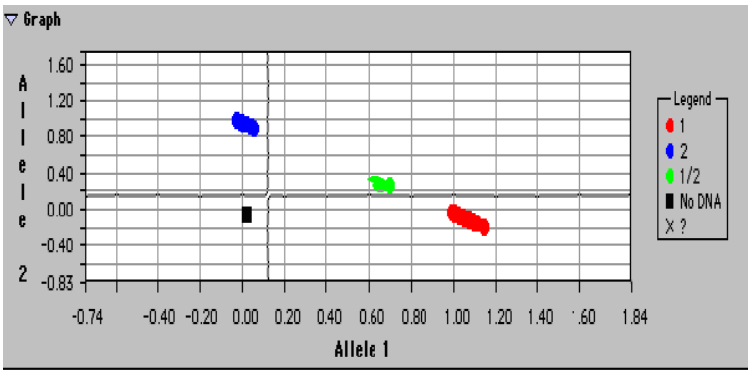


Figure 2. CCR3-17T/C endpoint assay: Allelic discrimination plots shows clusters CCR2 genotypes as 1,1 (red), 1,2 (green), and 2,2 (blue). Not template DNA (blank) clustered as shown black color. Fluorescence wavelength for each dyes for allele 1 (Y access) and allele 2 (X access). The merge fluorescence length between the two accesses indicated the genotype cluster

Functional Binding Site within CCR3 Gene

MatInspector and Matlnd, new fast and versatile bioinformatics tools for detection of consensus matches in nucleotide sequence data were used. The online MatInspector, identifies binding sites with variable spaces provided that sufficient sequences for the generation of multiple matrices (Spielman *et al.,* 1993), is available at (http://www. genomatix.de/cgi-bin/matinspector/matinspector.pl), and was used to identify transcription factor consensus binding sites in putative promoter sequences. Matlnd was used to carry out alignment and thus allows identification of subsets of binding sites according to their space length. MatInspector identifies binding sites with variable spaces provided that sufficient sequences for the generation of multiple matrices are available (Spielman *et al.,* 1993).

Statistical Analysis

- Family-Based Association Tests (FBATs)

In order to assess familial transmission of the alleles, the pedigree disequilibrium test (PDT) was used as this test has advantages over the classical transmission/disequilibrium test (TDT) (Martin *et al.,* 2000; Martin *et al.,* 2001). TDT is not a valid test of association if related nuclear families and/or siblings from larger pedigrees are used (Martin *et al.,* 2000; Martin *et al.,* 2001; Spielman *et al.,* 1993). PDT overcomes these problems by treating triads (parent-child trios) as independent entities in contrast to TDT which treats the contribution from heterozygous parents as independent (Martin *et al.,* 2000; Martin *et al.,* 2001), and which makes this test less susceptible than TDT to confounding by phenocopies or heterogeneity.

PDT was applied separately for PDA, BHR, % predicted FEV-1 below the population median, atopy defined as a least one-skin prick test (SPT) at least 3mm greater than negative control, and for serum IgE greater than 100 Iu/ml.

- Case-Control Study

Of the 598 available subjects from the 154 families, 453 contributed to the case control study, as participants had to be unrelated to each other. None of the parents were related thus providing 212 cases of PDA and 96 controls. For the case control study in the younger generation the first identified child within each family was selected yielding, 83 cases and 67 controls.

Allele frequencies for the wild type (wt) and the mutant allele were compared in 4 diagnostic categories, namely, atopic asthma, non atopic asthma, non-asthmatic atopic, and non asthmatic non atopic, for both parent and child generations.

Genotype and allele frequencies among different groups were tested by Chi-square, Odds Ratios (OR's) and 95% CI's were determined after adjustment for age and gender. Univariate analyses were also performed for non-atopic asthma, atopic asthma, and atopy without asthma.

Hardy–Weinberg equilibrium was tested in a contingency table of observed genotype frequencies (http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/

kitest.htm). Association analyses were carried out using SPSS version 11 (SPSS Inc., Chicago, IL, USA) with Chi-square tests applied to 2 × 2 genotype/phenotype tables and PDT with version 3.12 (http://www.rfcgr.mrc.ac.uk/Registered/Option/pdt.html).

Results

Of the 154 unrelated probands (age 8-24y [median 14y]) 150 had complete data and of 308 parents (age 34-61y [median 43]) 303 had complete data. Of the whole population (parents and children) 211 were defined as atopic asthmatic, 61 as non-atopic asthmatic, 171 as atopic without asthma, and 155 as neither atopic nor asthmatic. The overall mutant allele frequency was 13.9% (Table 3). From the pooling technique the allelic frequencies in cases of PDA and asymptomatic controls were 0.002 and 0.016 for the –21A SNP, 0.001 and 0.005 for the -39C SNP, and 0.002 and 0.003 for the 5′UTR SNP. These frequencies were so low that further analyses of these SNPs were not pursued in our population. This was in contrast to the –17C SNP, which had respective allelic frequencies of 0.142 in cases of PDA and 0.035 in asymptomatic controls.

Functional Binding Site within CCR3 Gene

The Matlnd and MatInspector programs found the matrix number V$CREB/ATF6.02 was the only one that matched the inserted sequence of CCR3-17Y with matrix similarity greater than 0.85. This binding site sequence corresponded to the IUPAC string ctacgatGACGtgggcctgct of the SNP –17Y polymorphism with a core similarity of 1.00 and matrix similarity of 0.987.

Family Based Association Test (FBATs)

Informative child parent trios were assembled from the 150 pedigrees with complete data and with at least one heterozygous parent for the mutant polymorphism. In these informative families, the mutant (T-C allele) was preferentially transmitted at high levels of significance to children with PDA (affected = 52 vs. non-affected = 26, *P*=0.0001) and to children with positive BHR (affected = 49 vs. non-affected = 25, *P*=0.002). Borderline levels of transmission were noted for SPT (affected = 47 vs. non-affected = 23, P=0.087) and FEV1 below the population median (affected = 32 vs. non-affected = 28, *P*=0.056), but with no suggestion of significance for elevated s-IgE (affected = 35 vs. non-affected = 31, *P*=0.267) (Table 2). Transmission of the mutant allele to affected children was therefore higher than expected following Mendelian laws of transmission with the strongest associations for PDA and BHR rather than for baseline lung function and markers of allergy.

Case-Control Analysis

Table 3a,b shows the CCR3 genotype and allele frequencies in the clinical categories by family generation, namely probands and their parents. In 303 unrelated parents the presence of the C allele was significantly associated with both non atopic asthma (P=0.001, OR=12.8, 95% CI=3.51-51.20), and atopic asthma (*P*=0.001, OR=9.963, 95% CI=2.93-46.71) but not with atopy alone (*P*=0.899) (Table 3a).

In 150 unrelated child probands the mutant C allele was significantly associated with non atopic asthma (*P*=0.001, OR=14.50, 95% CI=3.9-52.64), and marginally with atopic asthma (*P*=0.056) but not with atopy alone (*P*=0.430) (Table 3b).

Case control analyses of associations among genotype, allele frequencies and asthma-related phenotypes revealed no significant associations between the CT genotype and positive skin test in both unrelated children (*P*=0.849), unrelated parents (*P*=0.735) neither with proband nor parental elevated serum IgE, (*P*=0.892) and (*P*=0.589), respectively (Table 4).

For BHR highly significant associations were found between the CT genotype in both probands (*P*=0.02, OR=4.09, 95% CI= 1.159-14.47) and their parents (*P*=0.006, OR=3.07, 95% CI=1.34-7.03) (Table 4).

In the whole population with PDA the median predicted FEV1 was 83.5% and when severity of asthma was assessed as values below this median value significant associations were found between the CT genotype and reduced baseline FEV1 in unrelated child probands (*P*= 0.02) and in their parents (*P*=0.001) (Table 4).

Table 2. PDT Analysis of Nuclear Families with at Least Two Asthmatic Children.

Phenotype	Informative Pedigrees	Parent Genotype		Children affected	Allele Transmission		Z-score	P_{pdt}
		CC CT	TT		C (%)	T (%)		
PDA	34	23	11	52	78.0	22.0	3.713	0.0001
SPT	30	20	10	47	56.6	43.4	1.067	0.087
s-IgE	28	19	9	35	47.9	52.1	0.957	0.267
FEV1	25	17	8	32	67.9	32.1	2.578	0.056
BHR	32	22	10	49	73.8	26.2	3.687	0.002

The table evaluates only alleles that were transmitted from heterozygous parents (C/T) to affected children. PDA = Physician diagnosed asthma, SPT = Skin prick test, s-IgE = Serum IgE >100 Iu/ml, FEV1 = < 83.5% predicted, BHR = PC 20 <8mg/ml methacholine.

Table 3. CCR3T17C Genotypes and Allele Frequencies in (a) Unrelated Parents; (b) Unrelated Children.

Patient group	Genotypes			Alleles				
	TT	CT	CC	T	C	P[a]	OR[a]	95% CI[a]
(a)								
Non asthmatic non-atopic	93 (96.9%)	2 (2.1%)	1 (1.0%)	188 (97.9%)	4 (2.1%)			
Non-asthmatic atopic	124 (96.9%)	3 (2.3%)	1 (0.8%)	251 (98.1%)	5 (1.9%)	0.899	1.125	0.814-6.869
Asthmatic Non-atopic	3 (27.3%)	6 (54.5%)	2 (18.2%)	12 (54.5%)	10 (45.5%)	0.001	12.810	3.510-51.206
Asthmatic atopic	50 (73.5%)	17 (25.0%)	1 (1.5%)	117 (86.0%)	19 (14.0%)	0.001	9.963	2.937-46.718
(b)								
Non asthmatic non-atopic	35 (92.1%)	2 (5.3%)	1 (2.6%)	72 (94.7%)	4 (5.3%)			
Non-asthmatic atopic	25 (86.2%)	3 (10.3%)	1 (3.4%)	53 (91.4%)	5 (8.6%)	0.430	2.10	0.326-13.508
Asthmatic Non-atopic	5 (38.5%)	7 (53.8%)	1 (7.7%)	17 (65.4%)	9 (34.6%)	0.001	14.5	3.932-52.642
Asthmatic atopic	55 (78.6%)	13 (18.6%)	2 (2.8%)	123 (87.8%)	17 (12.2%)	0.056	4.136	0.880-19.446

Crosstabulation analysis. Reference category (non-asthmatic, non-atopic), [a] C vs T allele.

Table 4. CCR3T17C Genotype and Allele Frequencies According to Asthma Related Phenotypes: (a) Unrelated Parents; (a) Unrelated Children

	Genotypes						Alleles				
	TT	CT	CC	P[a]	OR[a]	95% CI[a]	T	C	P[b]	OR[b]	95% CI[b]
(a)											
Skin test											
Negative	79 (84.9%)	12 (12.9%)	2 (2.2%)				170 (91.4%)	16 (8.6%)			
Positive	191 (90.9%)	16 (7.6%)	3 (1.4%)	0.735	0.871	0.391-1.938	398 (94.8%)	22 (5.2%)	0.145	0.587	0.301-1.146
IgE level											
< 100 IU/L	187 (90.3%)	18 (8.7%)	2 (1.0%)				392 (94.7%)	22 (11.5%)			
> 100 IU/L	83 (86.5%)	10 (10.4%)	3 (3.1%)	0.589	1.252	0.554-2.828	176 (91.7%)	16 (8.3%)	0.144	1.707	0.874-3.333
BHR											
> 8mg/ml	160 (94.1%)	9 (5.3%)	1 (0.6%)				329 (96.8%)	11 (3.2%)			
≤ 8mg/ml	110 (82.7%)	19 (14.3%)	4 (3.0%)	0.006	3.071	1.340-7.038	239 (89.8%)	27 (10.1%)	0.001	3.379	1.644-6.946
FEV1 %											
> 83% predicted	135 (95.0%)	5 (3.5%)	2 (1.4%)				275 (96.8%)	9 (3.2%)			
≤ 83% predicted	135 (83.8%)	23 (14.3%)	3 (1.8%)	0.001	4.076	1.592-10.43	293 (90.9%)	29 (9.0%)	0.004	3.024	1.407-6.504
(b)											
Skin test											
Negative	31 (81.6%)	6 (15.8%)	1 (2.6%)				68 (89.5%)	9 (11.8%)			
Positive	89 (79.5%)	19 (16.9%)	4 (3.6%)	0.849	1.103	0.404-3.013	197 (87.9%)	27 (12.1%)	0.987	1.036	0.464-2.312
IgE level											
< 100 IU/L	32 (78.0%)	7 (17.1%)	2 (4.9%)				71 (86.6%)	11 (13.4%)			
> 100 IU/L	88 (80.7%)	18 (16.5%)	3 (2.8%)	0.892	0.935	0.357-2.448	194 (88.9%)	24 (11.0%)	0.550	0.799	0.372-1.714
BHR											
> 8mg/ml	43 (91.5%)	3 (6.4%)	1 (2.1%)				89 (94.7%)	5 (5.3%)			
≤ 8mg/ml	77 (74.7%)	22 (21.4%)	4 (3.9%)	0.02	4.095	1.159-14.47	176 (85.4%)	30 (14.6%)	0.020	3.034	1.138-8.088
FEV1 %											
> 83% predicted	78 (86.7%)	10 (11.1%)	2 (2.2%)				166 (92.2%)	14 (7.8%)			
≤ 83% predicted	42 (70.0%)	15 (25.0%)	3 (5.0%)	0.02	2.316	1.119-4.793	99 (82.5)	21 (17.5%)	0.016	2.515	1.224-5.170

Cross-tabulation analysis. [a]CT + CC genotype compared with TT genotype.

[b]A compared with G alleles. Gender and age accounted for in the model.

Discussion

The present study confirms the previously reported association of the CCR3 Y17Y mutation in a caucasian population with asthma rather than with atopy (Fukunaga *et al.,* 2001). However the relevance of association is strengthened by the observed preferential familial transmission of the mutation with asthma and asthma related phenotypes rather than the atopy phenotype. The observation that the association was similar across 2 generations within the same families also points to its potential relevance to disease expression across the whole life span.

The inheritance of asthma and atopy is likely to be complex and be confounded by relatively small contributions from a number of different genes, by partial penetrance, asymptomatic carriers who have not yet been exposed to the relevant environmental hazard, and by disease phenocopies. All of these factors are likely to be operating and thereby decrease the power of linkage/association analysis because of the inherent assumption that the inheritance of asthma and atopy follow simple Mendelian inheritance.

However, despite these potential problems the present study found that the common CCR3T17C polymorphism was not only transmitted preferentially to affected children but that in their parents the allele was associated with asthma, with BHR and with disease severity, as assessed by baseline FEV1, but not with atopy. Similar observations were seen in child probands suggesting that this polymorphism might play a role in the expression of asthma but not atopy. Although this association was not found in a Japanese population (Fukunaga *et al.,* 2001) we did find a similar but not identical trend of association with a previously reported British population (Fukunaga *et al.,* 2001). Such race specific differences could be explained by population heterogenesis and differing allelic frequency.

The chemokine receptor 3 is known to be one of the principal receptors for the chemokine eotaxin, which is a potent eosinophil chemoattractant (Uguccioni *et al.,* 1997). High expression of CCR3 in eosinophils, as well as high expression in airway epithelium from asthmatic patients samples have been detected using immunohistochemical analysis of inflammatory and noninflammatory tissue (Stellato *et al.,* 2001). CCR3 expression in airway epithelium has also been shown to corral with expression of the CCR3 ligands, eotaxin and RANTES (Stellato *et al.,* 2001). Northern blot analysis has revealed upregulation of CCR3 expression on bronchial epithelial cell lines in response to tumour necrosis factor-alpha (TNFα) stimulation and expression could be further upregulated by gamma-interferon (IFNγ) (Stellato *et al.,* 2001). This finding suggested that the presence of CCR3 and its ligands in epithelial cells may contribute to the accumulation and activation of eosinophils and other inflammatory cells in the allergic airway (Stellato *et al.,* 2001).

The functional binding site analysis suggested that the CCR3T17C polymorphism belongs to the same family as activating transcription factor 6 (ATF-6) which responds to cAMP element binding protein 1 (CREB1). ATF-6 is the second messenger pathway involved in many processes such as cellular growth, differentiation, and cell signalling. Intracellular cAMP is stimulated by cell surface receptors and intracellular phosphodiesterases (PDEs) that play a key role of smooth muscle relaxation (Torphy *et al.,* 1991; Giembycz *et al.,* 1991) and its expression is significantly associated with chronic inflammatory process in the asthmatic airway (Bachelet *et al.,* 1991).

However, the matching technique we used may find false positive associations as large

genomic sequences are scanned and the sensitivity of the method remains to be established by comparison with experimental data (Quadt *et al.,* 1995). Despite these limitations, the close matching we observed suggests that the –17Y CCR3 polymorphism is functional and that it is likely to have a role in upregulating airway tone and associated inflammatory processes.

FBAT provided a robust method of confirming or refuting the contribution of genetic candidates to complex diseases such as asthma as they effectively eliminate many of the confounding factors associated with case control studies. PDT has also shown its advantage in allowing more data to be included from informative families in addition to its ability to account for mixed genetic models. Polymorphisms in CCR3 may well prove to be a target for intervention in the expression of asthma and related phenotypes although further work is required with regard to the functional mechanisms of the common CCR3 Y17Y polymorphism.

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