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Genetic contribution of chemokine receptor 2 (CCR2) polymorphisms towards increased serum total IgE levels in Indian asthmatics

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ABSTRACT

The chemokine (C–C motif) receptors (CCR) 2 and 5 are members of a large family of G protein-coupled receptors, playing important roles in asthma pathogenesis. Using standard sequencing techniques, a total of 15 single nucleotide and 8 insertion/deletion polymorphisms (DIPs) (5 novels) were identified in and around these two genes. None of the studied polymorphisms ($N=7$, selected on the basis on linkage disequilibrium) was associated with asthma in a case ($N=315$) – control ($N=337$) study and showed no evidence for non-random transmission to individuals with asthma/atopy in Indian pedigrees ($n=235$). However, multilocus haplotype analysis based on simulations yielded a $P=0.00005$ in the case–control study and a $P=0.03$ for the family-based association studies. Furthermore, rs3918356 and rs743660 polymorphisms in *CCR2* were found to be associated with total serum IgE levels in both the study designs. Thus, our study supports a significant role for chemokine receptor polymorphisms in genetic susceptibility to asthma.

Background

The chemokine (C–C motif) receptors; CCR2 (MIM#601267) and 5 (MIM#601373); are members of a large family of G protein-coupled seven-transmembrane domain receptors, playing important roles in asthma (NM#600807) pathogenesis [1,2]. CCR5 is expressed mainly on the T helper (Th) cells producing type 1 cytokines such as interferon-γ and tumor necrosis factor as well as on a major subset of $CD8^+$ cytotoxic T cells, [3] and binds the pro-inflammatory chemokines CCL5 (regulated on activation, T-cell expressed and secreted), CCL3, CCL4 and CCL2 [4], while CCR2 is expressed on monocytes, basophils and eosinophils. The primary agonist of CCR2 is MCP-1 (CCL2), although other lesser agonists of this receptor include CCL8, CCL7, CCL13, and CCL12 [1,5]. CCR5 plays a key role in the distribution of $CD45RO^+$ T cells and contributes to the generation of a T helper (Th) 1 immune response. Since Th2 immune responses are critical for the inflammatory reaction, it is hypothesized that the presence of CCR5 may delay the development of Th-2 mediated diseases like asthma. Evidently, CCR5 knockout mice demonstrated a shift towards type2-cytokine activation in a model of experimental colitis [6]. In contrary, an impressive accumulation of CCR5-positive T cells and macrophages is found in the lungs of the asthmatics after allergen challenge [2]. Also, the airways hyper responsiveness (AHR) was found to be significantly lower in *A. fumigatus*-sensitized $CCR5^{-/-}$ mice [7]. While, in a similar model, CCR2-deficient animals exhibited an increase in eosinophils and lymphocytes in the airways, serum IgE, Th2 cytokines and Th2-induced chemokines, AHR, and fibrosis in comparison to wild type mice [8]. However, neutralization of CCR2 using specific anti-CCR2 mAb leads to the reduction of antigen-induced bronchial HR and attenuates macrophage and eosinophil accumulation in the BAL of a primate model of asthma [9]. Thus, the role for both the chemokine receptors in asthma pathogenesis remains controversial.

Few genetic epidemiology studies have also documented a role for chemokine receptors and their ligands in the pathogenesis of asthma. The presence of the 64I allele of rs1799864: A>G (V64I) in *CCR2* conferred significantly lower risk for the development of asthma in a Korean population [10]. A 32-bp deletion in *CCR5* (Δ32), which causes loss of CCR5 cell surface receptors, has been associated with asthma and atopy in some studies [11,12], but not in others [13,14]. Recently, we have shown that the frequency of heterozygous mutant is much higher in patient group, in comparison to the control group ($P=0.0089$), thus shown a protective role for the deletion mutation [15]. However, because of its low frequency in our population, we were not able to establish the role of *CCR5* in atopic asthma. This observation, in part, motivated further identification of polymorphisms in the *CCR5* gene and its flanking regions including *CCR2*. Thus, we screened the complete gene-length of the *CCR5* and its flanking regions, and also sequenced the *CCR2* exonic region and performed the association

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studies on the selected polymorphisms with asthma and serum total IgE levels in case–control and family-based study designs.

Results

Resequencing of CCR2 and CCR5 gene and linkage disequilibrium

We sequenced all exons, introns and ≈1.8 kb of 5′ flanking region of the human *CCR5*, and the exonic regions of *CCR2* gene in 20 individuals irrespective of their disease status using the primers given in Table 1. Besides 32 bp deletion, we have observed seven insertion/deletion polymorphisms (DIPS) (five novel exonic variants) and 11 SNPs (1 novel) in *CCR5* and four exonic variants in *CCR2* in our sequencing panel. A "CAA" DIP ≈ 5 kb upstream of the translation start site of *CCR2* was also identified using *Repeatmasker* and confirmed by sequencing (Fig. 1A). None of the other reported mutations was found to be polymorphic in our sequencing panel, thus pairwise LD among the 17 polymorphisms (except for the rare polymorphisms) was calculated. A pattern of strong linkage disequilibrium was observed and three haplotype blocks were identified (Fig. 1B). Depending upon their functional significance and to capture maximum alleles based on LD, four polymorphisms from block 1, two from block 2 and one from block 3 were chosen for further genotyping.

Association of CCR2 and 5 polymorphism with asthma and IgE

Allelic and genotypic frequencies for the five SNPs viz. rs1799864: A>G, rs1799865: C>T (VAL64ILE), rs743660: G>A of *CCR2* and rs1799988: T>C and rs1800023: A>G of *CCR5*, and two DIPs viz. a -/CAA (rs3918356) 5082 bp upstream of *CCR2* and -/CAA (rs41515644) in the 370 bp upstream of *CCR5* translation start site were determined in all the recruited individuals (Table 2). Genotype frequencies of the polymorphisms in cases and controls are given in Table 3. All the polymorphisms were found to be in Hardy–Weinberg equilibrium in both groups, when analyzed separately from distinct geographical areas and also from the combined meta-analysis (data not shown). None of the seven polymorphisms showed significant association with atopic asthma ($P > 0.007$) in any cohort. In the families, also, none of the alleles demonstrated significant transmission distortion. Among the West-Indian families, rs1800023 and rs41515644 polymorphisms demonstrated a weak evidence of transmission disequilibrium (unadjusted $P = 0.01$, using recessive model). This distortion was not statistically significant (particularly after correction for multiple testing) and was not observed North and North-east families (data not shown).

Total serum IgE levels were found to follow a log normal distribution. When the cases and controls were compared with respect to the log total serum IgE levels, a highly significant difference was obtained ($t = 7.4$; $df = 5.71$; $P < 0.0001$). Because increased serum total IgE level is one of the major characteristics of atopic asthma, the genetic effects of chemokine receptor gene polymorphisms were tested on serum total IgE levels. None of the CCR5 variant was associated with TsIgE levels (Table 4). On the other hand a significant effect was observed at the genotypic level for the rs3918356 and rs743660 in the patients ($P = 0.0028$ and 0.0002, respectively). The mean $\log_{10}$ TsIgE levels in individuals with BB (with insertion) at rs3918356 and with AA at rs743660 genotype were highest, followed by individuals with rs3918356_AB/rs743660_GA and rs3918356_AA/rs743660_AA genotypes (Table 4). Similar results were obtained when QTDT was used for family-based association test of genotypes with $\log_{10}$ TsIgE (Table 4). The data was highly significant ($P = 4 \times 10^{-9}$ for rs3918356 and $P = 2 \times 10^{-10}$ for rs743660: G>A SNP) when analyzed including dominance effect in the orthogonal model of Abecasis et al. (Table 4) [16].

Haplotype association with asthma

The permutation test for significant differences in haplotypes frequency in case and control group using PHASE resulted in a *P* value of 0.001 (data not shown) from the North cohort and 0.01 in

Table 1
Primers and PCR conditions used for sequencing and genotyping the *CCR2* and *CCR5* polymorphisms.

Primer name	Segment /polymorphism	Primer sequence	Annealing temperature (°C)	Size of PCR product (bp)
CCR5_1FP	1	5′-TCCTATGGGGTGTCCGAATGT-3′	58	1278
CCR5_1RP		5′-GCAAACTAGAAGCTGAAAAGGTAA-3′		
CCR5_2FP	2	5′-GTCTTGATCGCTGGGCTATTTCTA-3′	58	1278
CCR5_2RP		5′-TTTGTTCTCTGCTCATCCCACTAC-3′		
CCR5_3FP	3	5′-TCATTTGCTTCTTGGATAGTAATTT-3′	58	1266
CCR5_3RP		5′-CCAAGTCCCAGAGAAGGTCATA-3′		
CCR5_4FP	4	5′-TATTTCTTGGGTATGTATGACAACT-3′	58	1269
CCR5_4RP		5′-GAGGCGGGCTGCGATTT-3′		
CCR5_5FP	5	5′-TTCATGGAGGGCAACTAAAT-3′	58	1208
CCR5_5RP		5′-CCAGCCCAGGCTGTGTAT-3′		
CCR5_6FP	6	5′-ACGCTTCTGCAAATGCTGTTCTAT-3′	58	1070
CCR5_6RP		5′-CTCCCTCCTTCCCATCCTTACGA-3′		
CCR5_7FP	7	5′-GGCATTGCTCCGTCTAAGTCAT-3′	58	959
CCR5_7RP		5′-CCCCCACCCCCATTCAGTC-3′		
CCR5_8FP	8	5′-AACCACAGGCAGCATTTAGCA-3′	58	630
CCR5_8RP		5′-CTCACCGTTCATATTCAGAGGC-3′		
CCR2_1FP	9	5′-CTTTTCCCTGCCTTGCCACT-3′	62	963
CCR2_1RP		5′-TTTATAAACCAGCCGAGACTTCCT-3′		
CCR2_2FP	10	5′-CTTTTCCCTGCCTTGCCACT-3′	68	1168
CCR2_2RP		5′-AGTTCTGCTCTGTCCCCACTTCTT-3′		
CCR5_SS1	rs1799988: T>C	5′-TGAGAAAAGCCCGTAAATAAAC-3′	55	23
CCR5_SS2	rs1800023: A>G	5′-GAAGAACTGTTCTCTGATT-3′	50	20
CCR2_SS1	rs1799864: A>G	5′-TTTTGCAGTTTATTAAGATGAGGA-3′		
CCR2_SS2	rs1799865: C>T	5′-AAGGTGTTCAGGAGAATGACAAT-3′	55	24
CCR2_SS4	rs743660: G>A	5′-CCAGTGGGAACTCCTAAATCAA-3′	55	23
CCR5_M1_FP	rs3918356: (−)>(CAA)	5′-AGCCCGGGCAACATGACAAAACC-3′	68	313
CCR5_M1_RP		5′-CATTTGATTGTTGTTGGCTCTAGCAGTGG-3′		
CCR2_M2_FP	rs41515644: (−)>(CAA)	5′-GTGTGGTGGCGCCTGTAGTCC-3′	62	272
CCR2_M2_RP		5′-GAAAGCACCATCTCACCAAATAAAATCT-3′		

Ref Seq: *CCR5* (accession no. NM_000579); *CCR2* (accession no. NM_000647).