CCR5 and CCR2 gene polymorphisms in hypertensive patients

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ABSTRACT

Essential hypertension is a complex trait under polygenic control. Evidences suggests immune system involvement during pathogenesis. CC-chemokine receptor (CCR)5 and CCR2 are characterised by gene polymorphism. Variant alleles are derived from a deletion in the CCR5 gene (CCR5Δ32) and a substitution mutation at the CCR2 locus (CCR264I). CCR polymorphic forms have been studied extensively as invasion cofactors for HIV-1, but they have also been implicated in immuno-related disorders. Here, we evaluate the allelic distribution of CCR5 and CCR2 genes in essential hypertension in a case-control study. Genotype frequency in a group of essential hypertensive patients (stage I-II; n=120) and a group of unrelated, healthy Caucasian subjects (n=340) is compared. CCR gene polymorphism is analysed by polymerase chain reaction and restriction enzyme digestion. A statistically significant difference was observed for CCR5 and CCR2 mutant alleles in essential hypertensive patients, compared with the controls (P=0.004 and P=0.003, respectively). CCR5Δ32 and CCR264I alleles showed a 0.096 and 0.10 frequency among cases. To date, a role for the immune system in hypertension has not been clarified, nor has the predictive value of CCR polymorphisms.

KEY WORDS: Chemokine receptor. Genes. Hypertension. Immune system.

Introduction

Essential hypertension is a polygenic disease influenced by complex environment-gene interactions. Multiple candidate genes and chromosomal loci have been identified but their contribution to the development of hypertension remains incomplete.[1] There appears to be a relationship between immunological dysfunction and essential hypertension in humans and rats.[2,3] Primary or secondary activation of the immune system has been involved in determining pro-hypertensive effects,[4-6] and an altered profile of pro- and anti-inflammatory cytokines has been observed in patients with essential hypertension.[7,8]

In a recent study, Nguyên ñ *et al*.[9] found a statistically significant elevation in prevalence of hypertension among homozygotes for an allelic polymorphism at the chemokine receptor-5 (CCR5) gene. CCR genes code for a subgroup of G-protein-coupled receptors involved in modulating the immune response, and are characterised by different allelic forms.[10] A common 32 bp deletion in the CCR5 gene (CCR5Δ32) and a G-A nucleotide substitution in the CCR2 gene at position 190 (CCR264I) have been described.[11,12]

These variants were first associated with resistance to human immunodeficiency virus-1 (HIV-1) infection and a delay in progression to acquired immune deficiency syndrome (AIDS).[13,14] In addition, several multifactorial diseases have been associated with CCR gene polymorphisms, including asthma, insulin-dependent diabetes mellitus and pulmonary sarcoidosis.[15-17]

In the present study we evaluate the distribution of CCR5 and CCR2 polymorphisms in essential hypertensive patients.

Materials and methods

Chemokine receptor polymorphisms were determined in 120 patients with essential hypertension and 340 Caucasian control subjects. Case selection was made by strict clinical evaluation and laboratory investigation, according to international guidelines.[18]

All patients (mean age: 49.4 yrs) were characterised on the basis of ambulatory blood pressure monitoring.[19] The age-matched Caucasian control group consisted of unrelated healthy subjects, in whom the presence of hypertension or any other major clinical disease had been excluded.

After obtaining informed consent, whole blood (4 mL) was collected and genomic DNA was isolated from peripheral blood cells using standard methodology based on sodium dodecyl sulphate(SDS)/proteinase K lysis and phenol/chloroform extraction.[20]

CCR5 and CCR2 genotypes were determined by polymerase chain reaction (PCR), as previously described.[21] CCR5 wild-type and mutant alleles were detected using primers that flanked the 32 bp deletion (182 and 150 bp fragments, respectively). CCR2 gene amplification was performed using a primer with a diagnostic restriction site. After enzymatic restriction analysis with Bsa BI, the expected products of 183 and 165 bp were obtained.

χ^2 analysis and Fisher's exact test were used to compare

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Table 1. Distribution of CCR5 and CCR2 alleles

		Patients	Controls
CCR5 genotypes	CCR5/CCR5	100 (83.3%)	315 (92.6%)
	CCR5/CCR5D32	17 (14.2%)	24 (7.1%)
	CCR5Δ32/CCR5D32	3 (2.5%)	1 (0.3%)
CCR5 allele frequency	CCR5	0.904	0.96
	CCR5Δ32	0.096	0.04
CCR2 genotypes	CCR2/CCR2	96 (80%)	308 (90.6%)
	CCR2/CCR264I	24 (20%)	30 (8.8%)
	CCR264I/CCR264I	0	2 (0.6%)
CCR2 allele frequency	CCR2	0.90	0.95
	CCR264I	0.10	0.05

data between cases and controls and test for conformity to the Hardy-Weinberg equilibrium.

Results

A different distribution of CCR5 and CCR2 mutant alleles was seen between cases and controls. CCR5Δ32 and CCR264I allelic frequencies were significantly higher in hypertensive patients than in the control subjects ($P = 0.004$ and $P = 0.003$, respectively). CCR5 and CCR2 genotypes and allelic frequencies are summarised in Table I.

In the patient group, 17 (14.2%) out of 120 were heterozygous CCR5/CCR5Δ32 and three (2.5%) were homozygous CCR5D32/CCR5D32, with a 0.096 frequency for the CCR5D32 mutant allele. CCR5 genotype distribution showed a slight deviation from Hardy-Weinberg equilibrium (χ^2=3.998; P=0.0046).

In the control group, 24 (7.1%) out of 340 were classified as heterozygous CCR5/CCR5Δ32 and one (0.3%) was homozygous CCR5Δ32/CCR5Δ32, with a 0.04 frequency for the CCR5 mutant allele. CCR5 genotype frequencies did not deviate from Hardy-Weinberg equilibrium (χ^2=0.550; P>0.05).

The following CCR2 genotype distribution was observed in the patient group: 96 (80%) out of 120 were homozygous for the CCR2 wild-type allele and 24 (20%) were heterozygous CCR2/CCR264I, with a 0.10 frequency for the CCR264I allele. Thirty (8.8%) out of 340 controls were heterozygous CCR2/CCR264I and two (0.6%) were homozygous CCR264I/CCR264I, with a 0.05 frequency for the CCR2 mutant allele. CCR2 genotype distribution was in equilibrium in both the patient and control groups, according to the Hardy-Weinberg equation (χ^2=1.481 and 1.724, respectively; P>0.05).

Discussion

This study showed an association between chemokine receptor gene polymorphisms and essential hypertension. Both CCR5Δ32 and CCR264I mutant allele frequencies were significantly higher in the patient group studied. Our results agree with preliminary evidence from a phenotypic expression study of subjects with a CCR5Δ32/CCR5Δ32 homozygous genotype, in whom hypertension was one of the most frequent diagnoses.[9]

Deviation from the Hardy-Weinberg equilibrium may be due to chance, but the limited sample size makes it impossible to exclude or confirm the presence of selective factors influencing the CCR5 distribution in the patients studied.

Several reports suggest the possible involvement of the immune system in the pathogenesis of essential hypertension.[2-8] To date, however, no definite mechanism has been found to explain the observed association between CCR5 and CCR2 mutant alleles and the hypertensive phenotype.

Bush *et al*. reported a critical role for macrophage recruitment in the pathogenesis of hypertension, supported by experiments in mice deficient for the CCR2 gene.[22] This and other results implicate the CCR genes in several pathogenetic pathways involving the immune system through vascular hypertrophy and macrophage infiltration.

The functional relevance and predictive value of chemokine receptor polymorphisms requires further assessment, using longer survey periods and less strict inclusion criteria. New studies are currently underway and preliminary results suggest implications for prognosis and pharmacogenetics. □

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