Lack of association of CCR gene polymorphisms and left ventricular hypertrophy in essential hypertension

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ABSTRACT

Left ventricular hypertrophy (LVH) is a major determinant of heart damage. Scientific evidence suggests the influence of genetic factors, but these have yet to be completely clarified. This study investigates a possible relationship between LVH and two chemokine receptor (CCR) gene polymorphisms: CCR5Δ32 and CCR264I. Essential hypertensive out-patients (n=118, grade I–II, age 27–54) were recruited from the Catholic University Hypertension Centre. For each subject, clinical data on office blood pressure and M-mode/2D echocardiography were collected. Statistical analysis did not show a significant association between the CCR polymorphisms and LVH in the study population.

KEY WORDS: Genes, CCR. Hypertension. Hypertrophy, left ventricular. Polymorphisms (genetic).

Introduction

Left ventricular hypertrophy (LVH) is a major determinant of heart damage, is one of the most important complications of essential hypertension (EH), and is associated with an increased risk of cardiovascular morbidity and mortality.[1–3] Similar to EH, LVH appears to have a variety of determinants, including haemodynamic and non-haemodynamic factors, with an underlying genetic predisposition. Age, gender, body mass index (BMI), blood pressure (BP), growth factors, neurohormones, cytokines and environmental factors may all contribute to generate the cascade of molecular changes and the increase in protein synthesis that lead to LVH. [4,5]

However, these factors only partly explain LVH variability in the population. Current evidence suggests that the number of human cardiac myocytes is genetically determined and that myocardial architecture is constructed on the basis of genotype, but the degree of growth in cell size should be determined by other stimuli.[6]

Familial studies have documented the genetic predisposition of LVH in EH, and the introduction of molecular genetic technology has allowed the evaluation of the association of separate genes with EH and LVH, but this has produced conflicting results.[7]

New studies focusing on the role of chronic inflammation in the pathogenesis of cardiovascular damage show a relationship between LVH and some mediators of the inflammatory response.[8,9] Among the different gene polymorphisms associated with EH that may have a role in LVH are those at the chemokine receptors.[10–13]

Chemokine receptor (CCR) genes code for a subgroup of G-protein-coupled receptors involved in the modulation of the immune response. The presence of an inflammatory response appears to play a role in the development of hypertension through mechanisms involving vascular hypertrophy and macrophage infiltration, as shown by different epidemiological studies and *in vivo* observation in animal models.[14–17]

In order to evaluate the influence of CCR genes in the development of LVH, this study aims to analyse the effect of CCR5Δ32 and CCR264I in a group of essential hypertensive patients.

Materials and methods

Essential hypertensive out-patients (n=118: male 90, female 28; stage I–II, age range 27–54) were recruited using the following inclusion criteria: diagnosis of EH, based on careful clinical examination and routine laboratory studies, according to international guidelines;[18] age range 20 to 60 years; and not previously treated with antihypertensive drugs. Exclusion criteria were: evidence of coronary, valvular or primary myocardial disease; cerebrovascular accident; malignant hypertension or secondary form of hypertension; and diabetes.

All patients underwent office BP measurement and echocardiography. Blood pressure was measured using a mercury sphygmomanometer. Three readings were taken over a 10-minute period and systolic BP (SBP) and diastolic BP (DBP) were taken as the mean of the three

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Table 1. Results of bivariate analysis.

	With LVH (n = 53)	Without LVH (n = 65)	P
Gender			
Male	44 (83%)	46 (70.8%)	0.120
Female	9 (17%)	19 (29.2%)	
Age group			
< 40	9 (17%)	20 (30.8%)	
40-50	23 (43.4%)	22 (33.8%)	0.213
> 50	21 (39.6%)	23 (35.4%)	
Smoking			
Non-smoker	33 (62.3%)	41 (63.1%)	
Smoker	19 (35.8%)	23 (35.4%)	0.987
Ex-smoker	1 (1.9%)	1 (1.5%)	
CCR polymorphism			
wt/wt	33 (62.3%)	44 (67.7%)	
wt/Δ32	7 (13.2%)	7 (10.8%)	0.385
Δ32/Δ32	1 (1.9%)	2 (3%))	
wt64I	9 (17%)	12 (18.5%)	
wt64I/wtΔ32	3 (5.6%)	0 (0%)	

measurements. Blood pressure measurement was performed after five minutes' rest in a quiet environment, with the patient in a sitting position.

A M-mode/2D echocardiography (HP Sonos 1000) was performed with patients in a partial left decubitus position, using 2.5-mHz traducers on light-sensitive paper at 50 mm/sec. Left ventricular measurements were made according to the Penn Convention.[19,20] End diastolic measurements of interventricular septal thickness (IVS), left ventricular internal dimension (LVID) and posterior wall thickness (PWT) were taken, following the Penn Convention protocol, to measure left ventricular mass (LVM). This was calculated by a simple anatomically validated formula: LVM = 1.04 ([IVS +LVID + PWT]^3 – LVID)^3 – 13.6. To minimise the impact of variation in body size on LVM, it was indexed (LVMI) for body surface area (BSA). An LVMI cut-off value of 134 g/m^2 was selected for the detection of LVH in men and a value of 110 g/m^2 in women. Controls comprised patients with normal LVM and structure.

After obtaining informed consent, a blood sample (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.[21] CCR2 and CCR5 genotypes were determined by a polymerase chain reaction (PCR) technique described previously.[22]

Statistical analysis was performed using SPSS and STATA software. The χ^2 and Student's t test were applied for categorical and continuous variables, respectively. A multiple logistic regression was conducted, with LVH as the dependent variable (yes/no) and CCR5/CCR2 polymorphisms, age groups (<40 as reference group), gender and smoking habits as covariates. A stepwise regression method was used (backward elimination). Significance was set at $P<0.05$.

Results

In the group of 118 hypertensive patients studied, there were 53 with LVH (44 males, nine females) and 65 without LVH (46 males, 19 females) (Table 1). Among the case group, a frequency of 0.12 was observed for both the CCR5Δ32 and CCR264I mutant alleles. Among the controls, frequencies for Δ32 and 64I were 0.09 and 0.1, respectively (Table 2). Both cases and controls were in Hardy-Weinberg equilibrium for the CCR264I polymorphism at the CCR2 locus.

Controls did not exhibit a Hardy-Weinberg equilibrium for the CCR5Δ32 polymorphism at the CCR5 locus (P=0.01). The reason for this is unknown but did not appear to be due to technical inaccuracies and probably reflected a chance event.

Statistical analysis did not show a significant association between CCR5Δ32 and CCR264I polymorphisms and LVH in the study population (P=0.38; Table I). Multiple logistic regression did not show an association between *CCR5/CCR2* polymorphisms and LVH (OR=1.48, P=0.468 [CCR5 polymorphism]; OR=0.93, P=0.774 [CCR2 polymorphism]).

Discussion

Left ventricular hypertrophy is recognised as a major independent risk factor for cardiovascular morbidity and mortality.[1–3] Age, gender, BP, obesity, growth factors, neurohormones, cytokines and environmental factors, which are all important determinants of LVM, account only for part of the observed variance of LVH in the population.[4,5]

Scientific evidence shows that LVM is a familial trait, suggesting the influence of genetic factors.[23–27] Studies on monozygotic and dizygotic twins, by Adams *et al*., support the hypothesis that genetic background contributes to the regulation of cardiac hypertrophy.[28]

The introduction of molecular genetic techniques has allowed the evaluation of the association of separate genes with EH and LVH. To date, however, little is known of the genetics of LVM, other than that associated with the inherited cardiomyopathies, which are rare syndromes that show a different aetiology and geometry to the ventricular hypertrophy. These conditions are characterised by simple Mendelian patterns of inheritance, and the molecular basis of several examples has been elucidated.[29] Owing to absence, except in these rare syndromes, of classic Mendelian traits of inheritance, LVM is identified as a complex phenotype influenced by interacting genetic and environmental factors.

The genetic underpinnings of non-Mendelian forms of LVH have yet to be completely clarified, but there is an increasing interest in the investigation of potential candidate genes. The first studied are those encoding the proteins of the renin-angiotensin system; for example, I/D polymorphism of the angiotensin-converting enzyme (ACE) gene, A1166C polymorphism of the AT1 receptor gene, M235T polymorphism of angiotensinogen gene, and -6G/A polymorphism of its promoter region.[30–35] Also, the association of aldosterone synthase gene (CYP11B2) – 344 C/T, G-protein β-3 subunit 825T and β-1 adrenoceptor Gly389Arg gene polymorphisms and left ventricular structure has also been studied.[36–41].

However, studies are now focused on the role of inflammation and inflammation-associated vascular damage in the pathogenesis of LVH. A relationship between cardiac

Table 2. Frequencies of CCR5 and CCR2 genotypes and alleles in hypertensives with and without LVH.

		With LVH	Without LVH	*P*
		n=53	*n*=65	
CCR5 genotype frequency	CCR5/CCR5	42	56	
	CCR5/CCR5Δ32	10	7	
	CCR5Δ32/CCR5Δ32	1	2	0.436
	any CCR5Δ32	11	9	0.319
CCR5 allele frequency	CCR5	0.88	0.91	0.489
	CCR5Δ32	0.12	0.09	
CCR2 genotype frequency	CCR2/CCR2	41	53	
	CCR2/CCR264I	12	12	0.575
	CCR264I/CCR264I	0	0	
CCR2 allele frequency	CCR2	0.88	0.9	0.651
	CCR264I	0.12	0.1	

hypertrophy and endothelial dysfunction has been shown by Perticone *et al*. in untreated hypertensives in whom drug-induced vasodilation was inversely correlated with LVMI,[42]

Also, Pennica *et al*. demonstrated that a new cytokine, cardiotrophin 1, can induce hypertrophy of cardiomyocytes in mice,[8] and recent findings, by Losito *et al*. demonstrated an association between the interleukin (IL)-6 promoter polymorphism -174G/C and high BP and LVH in haemodialysis patients.[9]

All this supports the hypothesis that chronic inflammation is a mechanism of cardiovascular damage. However, previous investigations on the association of genetic loci and LVM have provided conflicting results and further studies on potential candidate genes are required.[32–34, 43]

Previously, this group described a relationship between CCR264I and CCR5Δ32 polymorphisms and essential hypertension,[16] and suggested a possible association between the same polymorphisms and LVH, which, similar to EH, is a complex trait, influenced by gene-gene interactions and environmental factors and associated with chronic inflammation.[4,5,8,9,42]

The present study did not demonstrate a significant association between LVH and the CCR264I and CCR5Δ32 polymorphisms, and this suggests different patterns of genetic predisposition for EH and LVH. However, large-scale association studies are required to predict the genetic risk for LVH and to further explore the hypothesis outline here. □

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