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Rapid Communication

SNP/haplotype associations of *CCR2* and *CCR5* genes with severity of chagasic cardiomyopathy

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ABSTRACT

Background: Chronic inflammation plays a major role in the tissue injury seen in the chronic chagasic cardiomyopathy. The CCR2 and CCR5 chemokine receptors are involved with the type of cellular infiltrate present in cardiac tissue and *CCR5*-gene variants were previously associated with this pathology.
Methods and results: This is a replication study in an independent cohort with larger sample size. Nine SNPs of *CCR5* and *CCR2* were typified to confirm the association previously found with Chagas disease. Evidence of association with severity was found for the A allele of rs1799864 of *CCR2* (p_{ad} = 0.02; OR = 1.91, 95% CI = 1.10–3.30), the T allele of the rs1800024 of *CCR5* (p_{ad} = 0.01; OR = 1.95, 95% CI = 1.13–3.38), and the HHF*2 haplotype (p = 0.03, OR = 1.65, 95% CI = 1.03–2.65). These results were replicated in the study combined with previous data. In this analysis it was replicated the allele T of rs2734648 (p_{ad} = 0.009, OR = 0.52, 95% CI = 0.32–0.85) with protection. In addition, the allele G of rs1800023 (p_{ad} = 0.043, OR = 0.61, 95% CI = 0.38–0.98), and the HHC haplotype (p = 0.004, OR = 0.62, 95% CI = 0.44–0.86) were also associated with protection. In contrast, the allele A of rs1799864 of *CCR2* (p_{ad} = 0.009; OR = 1.90, 95% CI = 1.17–3.08); and the allele T of rs1800024 of *CCR5* (p_{ad} = 0.005, OR = 1.98, 95% CI = 1.22–3.23) were associated with greater severity. No evidence of association between symptomatic and asymptomatic patients was observed.
Conclusions: These results confirm that variants of *CCR5* and *CCR2* genes and their haplotypes are associated with the severity but not with susceptibility to develop chagasic cardiomyopathy.

1. Introduction

Chronic chagasic cardiomyopathy (CCC) is the most frequent and severe manifestation of the Chagas disease (CD). This pathology was recognised by World Health Organization (WHO) as one of the world's 13 most neglected tropical diseases [1] and has a substantial impact on public health in Latin America, thus becoming an emerging disease in non-endemic countries. The Pan American Health Organization estimates that 7.7 million people are infected with *Trypanosoma cruzi*, 109 million are at risk of infection and 12,500 deaths occur per year [2]. The number of new annual cases of vector-borne infection is 41,000 and congenital Chagas is 14,000 [3]. Recently, it was estimated that the economic burden of Chagas disease is similar or higher than other relevant global pathologies as rotavirus or cervical cancer [4], resulting in an estimated 750,000 productive life years lost and 1.2 billion dollars lost annually [5].

In the chronic form, only 10–30% infected individuals will go on to develop heart disease or mega-syndromes. The dilated cardiomyopathy is the most important and severe manifestation and is characterized by heart failure, ventricular arrhythmias, heart blocks, thromboembolic phenomena and sudden death [6]. In addition, CCC is associated with a worse prognosis and survival compared with idiopathic disease and other cardiomyopathies of non-inflammatory etiology [7].

Nowadays, the role of chronic inflammation and immune response has been fully accepted as mechanisms involved in CCC. The persistence of the inflammatory process is due to persistence of the stimulus (parasite presence) or absence of endogenous reg-

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ulatory mechanisms, may lead to chronic inflammation and ensuing tissue injury and fibrosis [8]. The CCR2 and CCR5, two CC chemokine receptors, are important players in the trafficking of T cells, monocytes/macrophages and in the functions of other cell types relevant in pathogenesis of cardiovascular diseases [9]. Recently, one study in heart tissue of autopsy samples found that Th1 cytokine pattern predominated in the cardiac inflammatory cell infiltrate of Chagas' disease patients associated with heart failure [10]. Therefore, the expression of chemokines and their receptors involved with the type of cellular infiltrate present in cardiac tissue may be related not only to control the infection, but also may be participating in tissue damage [11]. To unravel underlying common genetic risk factors for CCC, we previously conducted a case-control study in the endemic area in Santander, Colombia and identified *CCR2* and *CCR5* SNPs significantly associated with severity of CCC [12]. Other studies have reported association with individual genetic variants [11,13,14]. However, the population size has been small. In the present study, an independent cohort with a larger number of patients was used for a replication study to confirm the association of nine genetic variants of *CCR2* and *CCR5* genes and their haplotypes in the development of symptoms and severity of CD in the population of an endemic area of Colombia.

2. Materials and methods

2.1. Patients

This is a replication study with an independent cohort and a higher number of patients, to confirm the association of polymorphisms previously identified in *CCR2* and *CCR5* genes [12]. The study was conducted from 2007 to 2013 and included 476 patients of whom 206 were classified as asymptomatic and 270 as symptomatic. All the patients were seropositive to *T. cruzi* antigens. The serological diagnosis was based on results of two independent tests, enzyme-linked immune sorbent assay (ELISA) (BioELISA Chagas, Biokit, Spain) and indirect hemagglutination test (IHA) (Wiener Lab, Argentina). Seropositive individuals were classified for cardiologist of Colombia Cardiovascular Foundation according to classification proposed previously [15]. The control group was conformed by seropositive and asymptomatic patients, without cardiac symptoms and with normal electrocardiogram (ECG) and echocardiogram (ECHO) ($n = 206$); whereas patients whose clinical evaluation, ECG, and ECHO showed conduction alterations and/or structural cardiopathy, they were included in the cardiomyopathic or symptomatic group as: II ($n = 96$, minor symptoms, minor ECG alterations, without ECHO alterations), and III ($n = 174$, considerable ECG and ECHO alterations and heart failure). All participants are older than 18 and all the individuals are from the same geographic region and having lived there for more than 10 years. The population from this region does not have concentration of ethnical groups.

2.2. Combined study

To increase the robustness of this study, we combined the populations of this and previous study (607 patients in total). Of these patients, 239 were in asymptomatic group and 368 in CCC group. There were no overlapping participants between these two studies. The CCC group was stratified according to disease severity (CCII = 111 and CCIII = 257). All patients and controls of two studies live in the same geographic region.

2.3. Ethics statement

Patients and controls were included in the study after written informed consent, according to the declaration of Helsinki. All participants are adults and over 18. Ethical Committees from Fundación Cardiovascular de Colombia and Universidad Industrial de Santander approved the study.

2.4. SNPs and genotyping

DNA from patients and controls was obtained by using standard methods [16]. The chemokine receptor cluster is located on chromosome 3p21 and contains at least 12 genes including *CCR5* and *CCR2*. Nine single-nucleotide polymorphisms (SNPs) of *CCR5* and *CCR2* genes were selected. In previous study, we found association of *CCR5*−2733 and −2554 polymorphisms with CCC [12]. The SNPs located in both coding and promoter regions have been described forming major haplotypes and associated with infectious diseases especially AIDS progression, tuberculosis and CCC [12,17,18]. Some of these haplotype blocks have been associated with higher *CCR5* expression levels [18,19]. In this study the SNPs: rs2856758 (−2733), rs2734648 (−2554), rs1799987 (−2459), rs1799988 (−2135), rs41469351 (−2132), rs1800023 (−2086), rs1800024 (−1835) for *CCR5* gene; and rs1799864 (+190) and rs3138042 for *CCR2* gene were analysed. All SNPs were genotyped using a TaqMan SNP genotyping assay by following the conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA). Each batch of up to 96 samples included four non-DNA template controls and eight duplicate samples, enabling us to calculate the percentage of contamination (i.e., 0%) and concordance rates (i.e., 99%) per batch.

2.5. Statistical analysis

All control and patient groups were tested for all markers on Hardy–Weinberg equilibrium (HWE), by means of the Fisher Exact test or χ^2 when necessary [20]. The case-control association study was performed by using a 2×2 contingency table with χ^2 to obtain P values, odds ratios (ORs), and 95% confidence intervals (CIs). P values <0.05 were considered statistically significant. All SNPs were assumed to be independent, and Bonferroni correction was used to adjust for multiple testing and statistical significance was assessed using a False Discovery Rate cut-off of 0.05, in order to adjust for multiple comparisons. Using a logistic regression model with cases or control as the dependent variables assessed the genetic effect of each polymorphism with chagasic cardiomyopathy, and where age and gender were additional covariates included in this model. Statistical analyses were carried out with PLINK v1.07 [21]. Pairwise linkage disequilibrium (LD) (D' and r^2) and haplotypes were estimated with an expectation–maximization algorithm implemented in Haploview 4.2 software [22].

3. Results

3.1. Population description

The proportion of men was similar to women (1:1). The mean symptomatic age was 59.5, whereas the mean age of the asymptomatic patients was 51.13. In the group of symptomatic patients 174 were classified in CCIII clinical stage and 96 as CCII. The population shared the same environmental and socioeconomic living conditions.

3.2. CCR2 gene variants in CD

A total of 270 patients with CCC and 206 asymptomatic individuals were genotyped for rs1799864 (+190 V64I) *CCR2* polymorphism. This SNP causes a valine-to-isoleucine substitution at position 64 (V64I or G190A). The genotypic and allelic frequencies