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Interleukin-1 receptor antagonist gene (IL1RN) polymorphism possibly associated to severity of rheumatic carditis in a Brazilian cohort

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ABSTRACT

Aims: To evaluate the IL1RN polymorphism as a possible marker for Rheumatic Fever (RF) susceptibility or disease severity. *Methods:* The genotypes of 84 RF patients (Jones criteria) and 84 normal race-matched controls were determined through the analysis of the number of 86-bp tandem repeats in the second intron of IL1RN. The DNA was extracted from peripheral-blood leukocytes and amplified with specific primers. Clinical manifestations of RF were obtained through a standardized questionnaire and an extensive chart review. Carditis was defined as new onset cardiac murmur that was perceived by a trained physician with corresponding valvae regurgitation or stenosis on echocardiogram. Carditis was classified as severe in the presence of congestive heart failure or upon the indication for cardiac surgery. The statistical association among the genotypes, RF and its clinical variations was determined. *Results:* The presence of allele 1 and the genotype A1/A1 were found less frequently among patients with severe carditis when compared to patients without this manifestation (OR = 0.11, $p = 0.031$; OR = 0.092, $p = 0.017$). Neither allele 1 nor allele 2 were associated with the presence of RF ($p = 0.188$ and $p = 0.106$), overall carditis ($p = 0.578$ and $p = 0.767$), polyarthritis ($p = 0.343$ and $p = 0.313$) and chorea ($p = 0.654$ and $p = 0.633$). *Conclusion:* In the Brazilian population, the polymorphism of the IL-1ra gene is a relevant factor for rheumatic heart disease severity.

1. Introduction

Rheumatic Fever (RF) is a late consequence of a pharyngeal infection by Group A *Streptococcus*. RF is still a great economical and social burden in developing countries, where the incidence remains close to 500,000 new cases each year [1]. The pathogenesis of RF is complex and mediated through both genetic and environmental factors [2]. It is estimated that only 0.3–3% of individuals who are known to be infected by rheumatogenic streptococcus will develop RF [2,3] and that one- to two-thirds of these individuals will develop rheumatic carditis [4–6]. The factors that lead to the susceptibility to RF are not well understood, but patterns of familial aggregation [7,8] and the stronger matching between identical over non-identical twins, both argue in favor of a genetic influence [9]. In the same way, the similarities of RF manifestation in siblings may represent their genetic proximity [9].

On the other hand, it is well known that RF is an immune-mediated disease in which pro-inflammatory cytokines play an important role. Exacerbated Interleukin-1 (IL-1) production seems to be an early event in the immunological abnormalities that are observed in RF, which is subsequently followed by the production of Interleukin-2 (IL-2) [9]. The production IL-1 and IL-2 by peripheral mononuclear cells is exacerbated in acute RF patients when compared with *Streptococcal* pharyngitis or chronic latent RF patients [9].

The Interleukin-1 receptor antagonist (IL-1ra) is an endogenous competitive inhibitor of IL-1 that acts by linking to the IL-1 receptor without initiating the intracellular cascade that leads to the inflammatory effects of the interleukins. The IL-1ra/IL-1 ratio is important in evaluating the intensity and duration of the inflammatory response [10].

Although many polymorphisms have been described for the IL-1ra gene (*IL1RN*) [11,12], the majority are in linkage disequilibrium with each other, such that a single polymorphism, the 86-bp tandem repeat in the second intron of *IL1RN*, is sufficient to evaluate the allelic variation of the gene [12]. It has been accepted that the number of repeats affects the cellular production of IL-1ra, and each allele may particularly influence the plasma concentra-

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tion of IL-1ra and its immune response. The majority of studies associate A2 with an exacerbated inflammatory activity and A1 with a relatively mild inflammatory state [13]. In fact, A2 has been related to a number of inflammatory and autoimmune diseases as well as to a greater resistance to infections [13].

Considering that RF is an inflammatory autoimmune disease that is triggered by a bacterial infection, we have evaluated the *IL1RN* polymorphism and its possible relevance to the susceptibility to RF and its clinical manifestations.

2. Materials and methods

2.1. Subjects

The study panel consisted of 84 consecutive chronic RF patients who fulfilled the Jones criteria and had their RF diagnosis confirmed by a follow-up after at least 3 years at the Pediatric Rheumatology Unit and Rheumatology Division, University of São Paulo, School of Medicine, Brazil. Exclusion criteria were a history of disease that may cause heart or valvular damage (such as hypertension, endocarditis, Chagas' disease and intravenous illicit drug use), neurological and psychiatric illnesses, juvenile idiopathic arthritis, rheumatoid arthritis and lupus and other inflammatory diseases.

Clinical manifestations of RF were obtained through a standardized questionnaire and an extensive chart review. Carditis was defined as new onset cardiac murmur that was perceived by a trained physician with corresponding valvae regurgitation or stenosis on echocardiogram. Arthritis was defined as tenderness, swelling, heat, redness or limitation of motion in the large joints, as determined by a rheumatologist. Chorea was defined as purposeless, involuntary, rapid movements of the trunk and/or extremities that may or may not be associated with muscle weakness and emotional lability [14].

All patients underwent transthoracic echocardiography on at least two occasions during periods of inactive disease, confirming or not the rheumatic heart disease. Heart rheumatic disease was graded as severe or mild/moderate. Carditis was defined as severe if accompanied by symptoms of congestive heart failure (CHF), a severe valvular lesion on echocardiography or the indication for valvular surgery, with the latter based upon the American College of Cardiology/American Heart Association 1998 Guidelines for the Management of Patients With Valvular Heart Disease [15]. Carditis was considered mild/moderate in patients without significant valvular hemodynamic impairment or heart symptoms.

Race was defined based on the self-reported race of ascendants until the second generation by each participant, as previously validated for the Brazilian population [16]. The presence of both Caucasian and African ascendants in a patient's heritage determined them as 'mixed race'. In the absence of information about the grandparents, race was determined by the race of the parents. Descendants of other races were excluded.

Eighty-four race-matched, healthy blood donors older than 35 years (to minimize the possibility of including individuals who were yet to show susceptibility to RF) from Hospital Bandeirantes (HB), São Paulo, Brazil, were invited to participate as controls. The exclusion criterion was a history of chronic or recurrent disease.

The study was approved by the Local Ethical Committee, and patients and controls (or their respective legal responsible) signed a consent form.

2.2. IL1RN genotyping

Genomic DNA was extracted from peripheral-blood leucocytes using the *GFX Genomic Blood DNA Purification Kit* (Amersham Bioscience, Little Chalfont, UK), following specifications of the manufacturer. Genotyping was performed through the analysis of the number of 86-bp tandem repeats in *IL1RN* intron 2, according to Tarlow et al. [17], using standard primers (5′-ctc agc aac act cct at-3′ and 5′-tcc tgg tct gca ggt aa-3′). Ethidium bromide-stained polymerase chain reaction (PCR) products were visualized on a 1% agarose gel.

2.3. Statistical analysis

Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, USA). Allele frequencies (number of occurrences of the investigated allele in the population divided by the total number of alleles) and carriage rate (proportion of individuals who have at least one copy of the investigated allele) were calculated. Hardy–Weinberg equilibrium was tested for the IL1RN polymorphism using the χ^2-test. The χ^2 statistic was also used to verify the existence of an association between the analyzed parameters and the presence of the alleles [18]. For 2×2 tables of data that included less than five individuals per cell, Fisher's exact test was used. A 5% significance level was considered to indicate statistical significance.

3. Results

3.1. Patient characteristics

The age of RF patients varied from 7 to 41 years old (median = 18.6), and the median disease duration was 9.4 years (3–22). Race distribution in 84 RF patients revealed a clear predominance of Caucasians (44/52.4%) and mixed race (38/45.2%), with a small representation of African descendants (2/2.4%).

Carditis was observed in 67 (79.8%) patients. The majority (79.1%) of carditis was graded as mild/moderate, and 14 (20.9%) cases were graded as severe (Table 1). The severe carditis cases all had CHF symptoms, with 10 of those also indicated for surgery. Multivalvar damage was observed in 26 (38.8%) patients, with 1 patient having 4 damaged valves, 2 patients 3 damaged valves and 23 patients having 2 damaged valves. The three patients with three- or four-valve involvement had severe cardiac disease, as determined by the adopted criteria. The mitral valve was compromised in 66 patients (98.5%), the aortic valve in 20 (29.8%), the tricuspid valve in 9 (13.4%) and the pulmonary valve in one (1.5%).

The other clinical manifestations that were observed in RF were 54 (64.3%) patients with polyarthritis, 38 (45.2%) with chorea, 9 (10.7%) with erythema marginatum, 8 (1.2%) with subcutaneous nodules, 63 (75.0%) with arthralgia and 58 (69.0%) with fever. Seventeen (20.2%) had solely non-cardiac manifestations of RF (Table 1).

3.2. Genotyping results

For the allelic analysis, a total of 84 RF patients and 84 controls were evaluated. For genotype purposes, a total of 82 patients and 78 controls were evaluated, since two patients and five controls

Table 1
Clinical characteristics of Rheumatic fever patients.

Heart involvement (%)		Non-cardiac manifestations (%)	
Carditis	67 (79.8)	Polyarthritis	54 (64.3)
Severe carditis	14 (20.9)	Chorea	38 (45.2)
Valvar surgery indication	10 (11.9)	Erythema marginatum	9 (10.7)
Cardiac failure	14 (20.9)	Subcutaneous nodules	8 (9.5)
Multivalvar lesions	26 (38.8)	Arthralgia	63 (75)
		Fever	58 (69)