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Association study involving polymorphisms in IL-6, IL-1RA, and CTLA4 genes and rheumatic heart disease in New Zealand population of Māori and Pacific ancestry

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ABSTRACT

Introduction: Rheumatic fever (RF) incidence among New Zealand (NZ) individuals of Polynesian (Māori and Pacific) ancestry remains among the highest in the world. Polymorphisms in the IL-6, IL1RN, and CTLA4 genes have been associated with RF, and their products are modulated by new medications. Confirmation of these previous associations could help guide clinical approaches. We aimed to test IL-6, IL-1RA (IL1RN), and CTLA4 functional SNPs in 204 rheumatic heart disease (RHD) patients and 116 controls of Māori and Pacific ancestry.

Material and method: Self-reported ancestry of the eight great-grandparents defined ancestry of participants. Severity of carditis was classified according to the 2012 World Heart Federation guideline for the echocardiographic diagnosis of RHD. The IL-6 promoter rs1800797, IL1RN rs447713 and CTLA4 rs3087243 SNPs were genotyped by Taqman. Correlations were assessed by logistic regression analysis adjusting for gender and ancestry.

Results: The IL-6 rs1800797 variant was significantly associated with RHD with carriers of the GG genotype 6.09 (CI 1.23; 30.23) times more likely to develop RHD than the carriers of the AA genotype ($P = 0.027$). No significant associations with RHD were found for the IL1RN rs447713 and CTLA4 rs3087243 SNPs. Patients carrying the G allele (GG plus AG genotype) for the IL1RN rs447713 SNP had 2.36 times (CI 1.00; 5.56) more severe carditis than those without this allele (the AA genotype) ($P = 0.049$).

Conclusion: The IL-6 promoter rs1800797 (−597G/A) SNP may influence susceptibility to RHD of people of Māori and Pacific ancestry living in NZ. The IL1RN rs447713 SNP may influence the severity of carditis in this population.

1. Introduction

Rheumatic fever (RF) remains a major medical and social problem in the developing world and in the so-called hotspots, where the incidence is from 20 to 51 per 100,000 habitants or higher, causing around 500,000 deaths each year [1]. New Zealand (NZ) has a diverse population in which 22% of people identified themselves with at least one ethnicity of Polynesian ancestry. This population is composed of 68.2% indigenous Māori, and 31.8% Pacific people (2013 census, http://www.stats.govt.nz), the latter mostly derived from Samoa and Tonga immigration in the 1970s. The notification rate of ARF in NZ the remains among the highest in the world, at 4.3 per 100,000 population for initial presentation, and 0.2 per 100,000 for recurrence, currently almost exclusively due to occurrence among individuals of Māori and Pacific ancestry [1,2]. From 1993 to 2009, in the age group at highest risk (5–14 year olds), Pacific people have had the highest rates of initial attacks (81.2 per 100,000), followed by Māori (40.2 per 100,000)

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[3]. Social and economic factors are believed to be involved in this distinctive susceptibility, but genetic predisposition for RF in Māori and Pacific people is suggested by epidemiological evidence and HLA genetic studies [4].

Rheumatic fever (RF) is an autoimmune disease caused by cross-reactive immune responses between Group A β-hemolytic Streptococcus (GAS) and host tissue antigens. Arthritis, Sydenham's chorea and the initiation of acute carditis are triggered by antibodies, and thus are mediated by the Th2 type immune response. Carditis is the major cause of mortality and morbidity of RF and is affected by Th1 and Th17 type cellular immune reactions [4].

Interleukin-6 (IL-6) has a wide influence on both humoral and cellular immune responses [5], and polymorphisms within the genes involved in the IL-6 regulation have been associated with RF [1,6,7]. Among them, the rs1800797 SNP is predicted to be functional (SNP Web Info selection tools, http://www.niehs.nih.gov/snpinfo) [8] and was significantly associated with increased IL-6 mRNA expression in primary human bronchial epithelial cells, lymphoblastic cells, and fibroblasts [9].

Interleukin-1 (IL-1) is a classic proinflammatory Th1 type cytokine. An augmented production of IL-1 by peripheral blood mononuclear cells (PBMC) was proposed to be the initial event of the cytokine dyscrasias seen in RF [6]. The IL-1 receptor antagonist (IL-1RA) competitively inhibits IL-1 α and β, promoting termination of the inflammatory response [6]. A polymorphism in the 86-bp variation number tandem repeat (VNTR) in the second intron of IL1RN was previously associated with RHD [1,6,7]. The IL1RN rs447713 A allele corresponds to the VNTR long genotypes and the G allele to the short genotype.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is a protein receptor expressed on the surface of CD4+ T cells, which are the main effectors cells of chronic valve lesions in RHD [4]. CTLA-4 negatively regulates T-cell function by transmitting an inhibitory signal and by competing with the stimulatory CD28 protein for the CD80 and CD86 surface proteins on antigen-presenting cells. T-cells are the major effectors of RHD [4]. Polymorphisms within the CTLA-4 gene also have been associated with this condition [1,6,7]. The CTLA-4 rs3087243 (CT60 A/G) SNP is associated with other autoimmune diseases [10] including T1DM [11] and Rheumatoid Arthritis [12].

Morbidity and mortality in RF are largely determined by the extent of the cardiac involvement in the chronic phase which, in turn, is highly influenced by the aggressiveness of the initial acute carditis. Yet, no treatments have been proven to effectively alter the natural history of the acute carditis [13]. New immunomodulators have been approved and successfully used in other refractory autoimmune conditions but have not yet been trialed in RF. Some of these medications target IL-6, IL-1 and CTLA-4, proteins directly involved in RF pathophysiology, and whose genes have previously been associated with RF [6].

In this study we investigate functional variants of IL1RN (rs447713), IL-6 (rs1800797) and CTLA4 (rs3087243), in a cohort of 204 NZ RHD patients and 116 controls with self-reported Māori and Pacific ancestry.

2. Material and methods

2.1. Participants

2.1.1. Patients

The RF patients were all recruited from the Auckland (NZ) urban area. Among the 208 initially included, 102 were approached after routine medical consultation in RF clinics in Central, South and West Auckland, 55 were approached at secondary prevention clinics at the time of the penicillin shots, and 51 were contacted by phone and later visited at home.

The inclusion criteria comprised RHD confirmed by echocardiography and the existence of at least one grandparent with Māori and Pacific ancestry and at least one year of disease when clinical data were reviewed.

2.1.2. Controls

The 116 controls were selected from the controls of a previous genetic study on rheumatoid arthritis [14]. The inclusion criteria encompassed the absence of any autoimmune condition and Polynesian ancestry.

2.1.3. Ancestry

Ancestry of participants was assessed by the self-reported ancestry of the eight great-grandparents. When information about great-grandparents was not available, information about grandparents was used. In a similar way, when information about grandparents was not available, the self-reported ancestry of the parents was used. Patients and controls were classified by the degree of Māori and Pacific ancestry, where each Māori/Pacific great-grandparent contributed 12.5% of Polynesian descent.

2.2. Ethics/agreements

Patients or their legal representatives signed informed consent after being fully informed about the study, both verbally and with written information. Controls had consented for participation in studies in any complex disease. The study was ethically approved by the New Zealand Health and Disability Ethics Committee (HDEC) and, in every district involved in the project, reviewed and supported by local and Māori and Pacific research advisory committees.

2.3. Clinical data

Clinical data were collected by interview of patients and family and by careful review of medical files by a physician specialist in RF. Cases were subdivided into different clinical categories. Carditis was classified as mild, moderated or severe according to the 2012 World Heart Federation guideline for the echocardiographic diagnosis of RHD [1].

2.4. Selection of genetic variants

2.4.1. IL-6 rs1800797

The rs1800795 (−174G/C) SNP was the only IL-6 polymorphism previously associated to RF [15,16]. Pairwise LD analysis indicated that all the most commonly investigated IL-6 polymorphisms (rs2069827, rs1800797, rs1800795, rs1554606, rs2069849, rs2069861 and rs1818879) were in strong LD [17] and rs1800795 (−174G/C) was in 98% LD ($r^2 = 0.98$) with the IL-6 promoter rs1800797 (−597G/A) SNP over all 1000 Genomes populations. As mentioned in the Introduction, the rs1800797 SNP is predicted to be functional.

2.4.2. IL1RN rs447713

Although many polymorphisms have been described for the IL-1RA gene (IL1RN), the majority are in LD such that a single polymorphism, the 86-bp variation number tandem repeat (VNTR) in the second intron of IL1RN, has been frequently used to evaluate the allelic variation of the gene [7]. Five alleles have been reported, which have been further divided into two categories: long genotype (including alleles 1, 3, 4, and 5) and short genotype (allele 2 only) [18]. The number of repeats affects the cellular production and the plasma concentration of IL-1RA [19]. The majority of studies associate allele 2 (A2) with an exacerbated inflammatory activity and allele 1 (A1) (and the other long genotypes) with a