Tumor Necrosis Factor-α Gene G-308A and G-238A Polymorphisms are not Associated with Rheumatic Heart Disease in Taiwan

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Purpose. The aim of this study was to test whether tumor necrosis factor (TNF)-α gene polymorphisms could be used as markers of susceptibility or severity of rheumatic heart disease (RHD) among the Chinese population in Taiwan.

Methods. A group of 115 patients with RHD diagnosed by echocardiography, and another group of 103 age and sex-matched normal control subjects were studied. TNF-α gene G-308A and G-238A polymorphisms were identified by polymerase chain reaction-based restriction analysis.

Results. There was no significant difference in the distribution of genotypes and allelic frequencies of the TNF-α gene G-308A and G-238A polymorphisms between RHD patients and controls. Further categorization of RHD patients into mitral valve disease and combined valve disease subgroups also revealed no statistical difference in these gene polymorphisms when compared with controls.

Conclusions. These findings suggest that the TNF-α gene G-308A and G-238A polymorphisms are not suitable genetic markers for RHD in Taiwan Chinese. **(Mid Taiwan J Med 2006;11:149-54)**

Key words

Taiwan, TNF-α gene polymorphisms, rheumatic heart disease

INTRODUCTION

Rheumatic heart disease (RHD) is an autoimmune sequel of beta hemolytic group A streptococcal infection of the pharynx complicated by rheumatic fever (RF) [1]. Autoimmunity induced by antigenic mimicry between the streptococcal glycoprotein and human cardiac myosin and laminin may be responsible for the pathogenesis of rheumatic carditis [2,3]. Some RF patients exhibit progressive fibrosis of the valves and develop chronic valvular disease; however, the factors leading to continued fibrosis and subsequent valve disease remain unclear. It has been shown that TNF-α might be involved in the pathogenesis of RHD. For example, the production of tumor necrosis factor-α (TNF-α) is higher during active rheumatic carditis [4,5]. Furthermore, recent data revealed that RHD was associated with TNF-α gene polymorphisms in a Mexican population [6].

The pro-inflammatory cytokine TNF-α plays an important role in inflammatory processes. The gene for TNF-α is located on chromosome six in the class III region of the major histocompatibility complex [7]. Different polymorphisms have been described, including the TNF-α G-308A polymorphism and the G-238A polymorphism. Both are located within the

Received : 30 March 2006. Revised : 25 April 2006.
Accepted : 4 May 2006.

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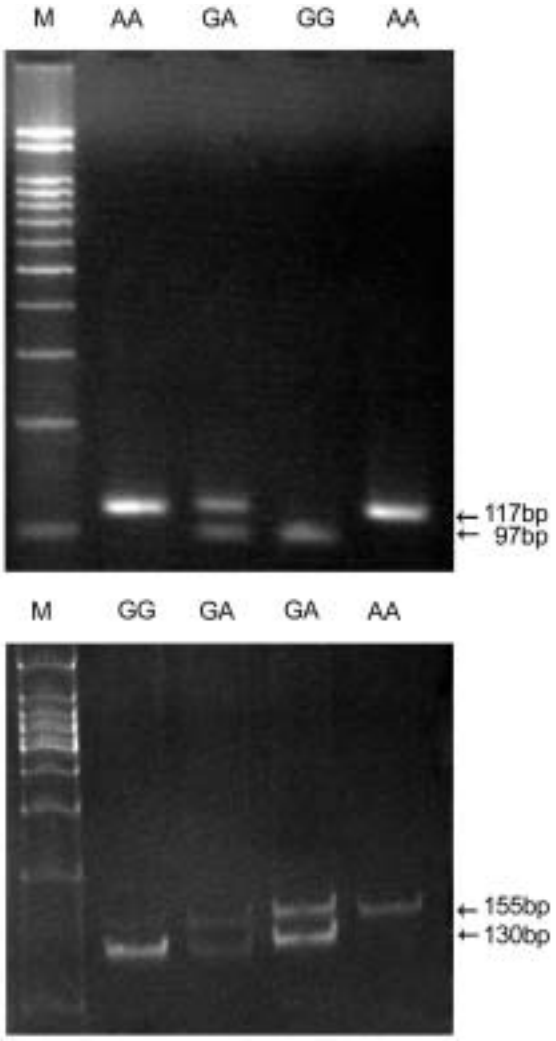

Figure. PCR-based restriction analysis of TNF-α gene G-308A (upper panel) and G-238A (lower panel) polymorphisms shown on 3% agarose electrophoresis.

promoter region [8,9]. It has been demonstrated that these different allelic forms have functional implications. The TNF-α G-308A A allelic form results in two-fold greater transcription than the TNF-α G-308A G allelic form in PMA-stimulated Jurkat T cells and U937 pre-monocytic cells [10]. To test if these polymorphisms might serve as markers of susceptibility or severity of RHD, the distribution of genotypes and allelic frequencies in a normal healthy control population was compared with that in a prospective cohort of 115 patients with RHD.

MATERIALS AND METHODS

Between May 2000 and April 2002, a total of 115 unrelated patients (31 men and 84 women; age range, 28 to 80 yr; mean age, 51.0 ± 12.2 yr) with echocardiographically-documented predominant rheumatic mitral stenosis (MS) were enrolled in this study. Valve lesions were diagnosed by echocardiography, catheterization and cineangiography, or both. The patients were categorized into either a mitral valve disease group (MVD; n = 53; 13 men and 40 women; mean age, 51.1 ± 12.4 yr) or a combined valvular disease group (CVD; n = 62; 18 men and 44 women; mean age, 50.9 ± 12.1 yr). The MVD group consisted of patients with MS (n = 25) and those with MS and mitral regurgitation (n = 28). Patients with predominant mitral regurgitation and those with aortic or tricuspid valvular disease alone were excluded from this study. The control group comprised 103 age- and sex-matched unrelated healthy volunteers (28 men and 75 women; age range, 26 to 79 yr; mean age, 49.7 ± 16.5 yr) who were free of autoimmune diseases, had normal echocardiography findings and no family history of RHD. All participants were ethnic Han Chinese residing in Taiwan.

The study was approved by our hospital's internal review committee, and informed consent was obtained from each participant.

The genomic DNA was prepared from peripheral blood leukocytes using a genomic DNA isolation kit (Blossom, Taipei, Taiwan). Polymerase chain reactions (PCRs) were carried out to a total volume of 50 μL containing genomic DNA, 2-6 pmol of each primer, 1X Taq polymerase buffer (1.5 mmol/L $MgCl_2$), and 0.25 unit of Pro-Taq DNA polymerase (Pro-Tech, Taipei, Taiwan). The TNF-α gene G-308A and G-238A polymorphisms were typed by the restriction fragment length polymorphism (RFLP) method [9,10]. Primers for G-308A polymorphism were (forward) 5'-AGGCAA TAGGTTTTGAGGGCCAT-3' and (backward) 5'-ACACTCCCCATCCTCCCTGCT-3'; those for G-238A polymorphism were (forward) 5'-AAACAG ACCACAGACCTGGTC-3' and (backward) 5'-CTCACACTCCCCATCCTCCCGGATC-3'. The PCR amplifications were performed in a GeneAmp PCR System 2400 programmable thermal cycler (PerKin-Elmer). The cycling

Table 1. Distribution of TNF-α genotypes in patients with rheumatic heart disease (RHD) (n = 115) and healthy control patients (n = 103)

Gene polymorphism	Genotype	RHD patients n (%)	Controls n (%)	χ^2	*p*
G-308A	GG	101 (88)	89 (86)		
	GA	13 (11)	14 (14)		
	AA	1 (1)	0 (0)	1.14	0.57
	G/A	0.93/0.07	0.93/0.07	0.01	0.91
G-238A	GG	112 (97)	100 (97)		
	GA	3 (3)	3 (3)		
	AA	0 (0)	0 (0)	0.02	0.89
	G/A	0.99/0.01	0.99/0.01	0.02	0.89

Genotype frequencies are indicated in absolute values (values in parentheses are percentages). Allelic frequencies are indicated in fractions. TNF-α = tumor necrosis factor-α gene.

conditions were set as follows: one cycle at 94˚C for 5 minutes, 35 cycles at 94˚C for 30 seconds, 60˚C for 30 seconds, 72˚C for 45 seconds, and one final cycle of extension at 72˚C for 7 minutes. The G-308A polymorphism in the promoter region of the TNF-α gene was categorized by digestion of PCR products with *Nco*I restriction enzyme followed by 3% agarose gel electrophoresis. The non-digested fragment was a single band of 117 bp (AA); the homozygous G allele was digested into a 97 bp and a 20 bp band (GG); heterozygotes (GA) showed three fragments of 117, 97 and 20 bp (Figure, upper panel). The homozygous A allele of the TNF-α G-238A polymorphism appeared as a single 155 bp band; the G allele was digested by *Bam*HI restriction enzyme, resulting in a 130 bp and a 25 bp band (Figure, lower panel).

Differences in genotype distribution between patients with RHD and control subjects were tested by the χ^2 test with 2 degrees of freedom (df). For statistical analysis of the allelic frequency distribution in the polymorphisms, the two groups were compared by the χ^2 test with 1 df. Allelic frequencies were calculated from genotype frequencies in patients with RHD and control patients. The differences among the MVD, CVD and control groups were estimated by the χ^2 test with 4 df. All statistical analyses were performed by NCSS 2000 Software (Kaysville, Utah, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

The χ^2 test confirmed that the genotype proportions of TNF-α polymorphisms G-308A and G-238A fit the Hardy-Weinberg equilibrium. The distribution of the TNF-α genotypes and allelic frequencies among patients with RHD and control patients are shown in Table 1. There was no significant difference in the distribution of the G-308A polymorphism ($\chi^2 = 1.14$, $p = 0.57$) between patients and controls. The allelic frequencies of the TNF-α G-308A polymorphism did not differ significantly between patients and controls ($\chi^2 = 0.01$, $p = 0.91$).

There was no statistically significant difference in genotype distribution of the TNF-α G-238A polymorphism between patients with RHD and control patients ($\chi^2 = 0.02$, $p = 0.89$). The allelic frequency of the TNF-α G-238A polymorphism did not differ significantly between patients and controls ($\chi^2 = 0.02$, $p = 0.89$).

No statistically significant difference in the G-308A ($\chi^2 = 3.37$, $p = 0.50$) polymorphism was observed among the MVD and CVD subgroups and the control group. Likewise, there was no significant difference in the distribution of G-238A ($\chi^2 = 0.52$, $p = 0.77$) polymorphism among the MVD and CVD subgroups and the control group (Table 2).

DISCUSSION

Several studies have suggested that genetic susceptibility to RF and RHD is linked to HLA class II alleles/haplotypes (HLA-DR2 in African-Americans, HLA-DR4 in American Caucasians, HLA-DQA1 in Chinese southern Hans, HLA-DQA1*0104 and DQB1*05031 in Japanese, and

Table 2. Distribution of TNF-α genotypes in patients with mitral valve disease (MVD) (n = 53) and combined valvular disease (CVD) (n = 62), and healthy control patients (n = 103)

Gene polymorphism	Genotype	MVD n (%)	CVD n (%)	Controls n (%)
G-308A	GG	46 (87)	55 (89)	89 (86)
	GA	6 (11)	7 (11)	14 (14)
	AA	1 (2)	0 (0)	0 (0)
Significance*		$\chi^2 = 3.37$, $p = 0.50$		
G-238A	GG	51 (96)	61 (98)	100 (97)
	GA	2 (4)	1 (2)	3 (3)
	AA	0 (0)	0 (0)	0 (0)
Significance*		$\chi^2 = 0.52$, $p = 0.77$		0.99/0.01

Genotype frequencies are indicated in absolute values (values in parentheses are percentages). *Comparison among "MVD", "CVD" and "control" groups. TNF-α = tumor necrosis factor-α gene.

HLA-DRB1*1602 allele and HLA-DRB1*1602-DQA1*0501-DQB1*0301 haplotype in Mexicans) [11-14]. However, the results were inconsistent as well as discrepant among the different populations studied. It has been suggested that a persistent inflammatory process occurs in the heart tissue of RHD patients, in the absence of infectious agents. Rheumatic involvement is present in 99% of stenotic mitral valves excised at the time of mitral valve replacement [15]. Guedez et al [16] reported that MVD patients had 5-fold fewer episodes of acute recurrent RF compared with multivalvular lesion patients; the mean rates of RF recurrence in MVD patients was 0.7 while that of multivalvular lesion patients was 3.3. The extent of original inflammation and recurrence of RF are not the only predictors of the crippling process. Ultimately, the deformed valve is subject to nonspecific fibrosis and calcification. The anatomic changes in severe MS and aortic stenosis may result from the combined effects of a smoldering rheumatic process and constant trauma to the mitral vlave or aortic valve by turbulent flow [17]. A recent study has reported that the ACE DD genotype is associated with an increased risk of subsequent heart valve damage in patients with RF [18]. However, we found an association between the ACE II genotype and RHD. An increased risk of RHD has been reported to be associated with ACE I allele. The difference might be caused by ethnic factors [19]. We also found that patients with RHD had a lower frequency of transforming growth factor-β1 (TGF-β1) C-509T CC genotype and a higher frequency of T869C T allele, which supported the role of the TGF-β1 gene C-509T and T869C polymorphisms in determining the risk/protection of RHD in Taiwan Han Chinese [20]. Previous studies have shown a high production of interleukin (IL)-1, IL-2 and TNF-α by peripheral and mitral valve-infiltrating T cells in patients with RF and RHD [5,6,21], suggesting these inflammatory cytokines may play a role in the pathogenesis of these diseases. We did not find an association between IL-1β, IL-1receptor antagonist, IL-4 or IL-10 gene polymorphisms and RHD in our population [22]. An association between TNF-α G-238A polymorphism and RHD in a Mexican population has been reported. The G allele and GG genotype frequencies were both significantly increased in RHD patients when compared with healthy controls [7]. However, we did not find an association between TNF-α G-238A polymorphism and RHD in our population. The difference might be caused by ethnic factors.

In this case-controlled study, no association was found between the TNF-α gene G-308A and G-238A polymorphisms and RHD. Our results show no evidence of an association between these polymorphisms and the pattern of valve damage seen in RHD patients. Other single nucleotide polymorphisms on the TNF-α gene at positions −1031, −863, −857, −376, and +489 need to be studied to clarify the relationship with RHD [23,24].

We conclude that the TNF-α gene G-308A and G-238A polymorphisms are not suitable genetic markers for RHD among the Han Chinese

population in Taiwan. Further studies are needed to clarify the pathogenesis of progression of RHD.

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甲型腫瘤壞死因子基因G-308A及G-238A之多型性與風濕性心臟病無相關性

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目的　風濕性心臟病是以慢性發炎過程爲其表徵，細胞激素甲型腫瘤壞死因子在慢性發炎反應上擔任重要角色。本研究的目的在探討甲型腫瘤壞死因子基因多型性是否能當作台灣人風濕性心臟病病患感受性或危險性的標記。

方法　以115位經心臟超音波診斷爲風濕性心臟病病患及103位年齡性別相配的正常人爲對象，用聚合酶連鎖反應限制酶分析法研究風濕性心臟病患及正常人的甲型腫瘤壞死因子基因G-308A及G-238A之多型性。

結果　風濕性心臟病患及正常人間的甲型腫瘤壞死因子基因G-308A及G-238A之多型性基因型及對偶基因頻率分佈並無差異。將風濕性心臟病病患分成二尖瓣疾病及多瓣膜疾病兩組，也未發現兩組間的甲型腫瘤壞死因子基因G-308A及G-238A之多型性基因型及對偶基因頻率分佈有統計學上的差異。

結論　甲型腫瘤壞死因子基因G-308A及G-238A多型性不適合當作台灣人風濕性心臟病的標記。（**中台灣醫誌 2006;11:149-54**）

關鍵詞

台灣，甲型腫瘤壞死因子基因多型性，風濕性心臟病

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收文日期：2006年3月30日　　修改日期：2006年4月25日

接受日期：2006年5月4日