川崎病におけるCD 14のプロモーター領域の単一塩基多型（C（-260）→ T）解析

C（-260）→T polymorphism in the promoter of CD 14 gene in Kawasaki disease.

竹下誠一郎　　高林　文代　　吉田　直樹

Seiichiro Takeshita　Fumiyo Takabayashi　Naoki Yoshida

静岡県立大学短期大学部
University of Shizuoka, Junior college

Summary

Recent studies have demonstrated that the T allele at position -260 of the CD 14 (lipopolysaccharide receptor) promoter is a risk factor for myocardial infarction. Kawasaki disease (KD) is an infantile systemic vasculitis that might cause angina pectoris and myocardial infarction. To investigate a possible association between the C（-260）→T polymorphism in the promoter of CD 14 gene and the onset of KD, we examined the genotype frequencies in 60 patients with KD and 68 controls, by using polymerase chain reaction (PCR) and restriction fragment assay. The frequencies of T allele and T/T homozygotes tended to be higher (P=0.022 and P=0.046, respectively) in KD patients than in controls. Therefore, there is a weak relationship between C（-260）→T polymorphism in the promoter of CD 14 gene andthe increased susceptibility to KD.

要旨

最近の報告では、lipopolysaccharideの受容体であるCD 14のプロモーター領域の-260番目のT allele（対立遺伝子）が心筋梗塞の危険因子であると報告されている。川崎病（KD）は小児特有の全身性血管炎であり、狭心症や心筋梗塞などの心臓後遺症を残す可能性のある疾患である。CD 14プロモーター領域のC（-260）→Tの単一塩基多型（変異）とKD発症の関連性を検討するために、KD患児群60人とコントロール群68人の遺伝子型をpolymerase chain reaction (PCR)とrestriction fragment assayで決定した。KD群におけるT alleleとT/T homozygotes（ホモ接合体）の頻度は、コントロール群に比較して高い傾向が認められた(P=0.022とP=0.046)。従って、CD 14プロモーター領域のC（-260）→Tの1塩基多型とKD罹患感受性の間には、弱いながらも相関関係があることが判明した。

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Introduction

Recent studies have suggested that inflammation is an important factor in the initiation and development of atherosclerosis [1]. However, the underlying causes of this inflammatory response remain uncertain. Endotoxin (lipopolysaccharide), derived from the cell wall of gram-negative bacteria, is suggested to play possible roles in initiation and development of atherosclerosis [2]. CD 14 is one of the key receptors for endotoxin and expressed on many types of cells. Recent reports revealed that the C (-260) → T polymorphism in the gene encoding the CD 14 receptor is associated with increased risk of myocardial infarction [3-5]. These data are intriguing because microorganism-initiated inflammation has been implicated to playa role in coronary artery disease [6].

Kawasaki disease (KD) is an acute febrile illness that predominantly affects infants and children [7]. This disease is characterized as one type of systemic vasculitis which may have coronary artery involvement [8]. Although the etiology remains unknown, KD is widely believed to be caused by certain infectious agents. The percentages of patients with incidences of KD among siblings and those with recurrences are higher than general nationwide incidence of KD [9], suggesting possible involvement of the hereditary factors in the epidemiology of KD. Linkage of different genetic markers (class I and class II MHC antigens, Immunoglobulin allotypes) with KD has been sought, but only weak associations have been found [10, 11]. The aim of the present study is to investigate a possible association between the C (-260) → T polymorphism in the promoter of CD 14 gene and the onset of KD.

Patients and methods

We studied 60 patients with KD (aged 5 months to 10 years; median 26 months;male/female =37/23) and 68 children (controls, aged 6 months to 14 years; median 23 months; male/female =38/30). Controls included 30 healthy children and 38 patients with other acute febrile diseases. None of controls had the past history of KD. Informed consent was obtained from the parents of all patients and controls.
All KD patients were hospitalized at the National Defense Medical College Hospital. The present study was approved by the institutional ethical review committee. The KD patients were enrolled within 7 days of the onset of illness, with day 1 defined as the first day of fever. All KD patients met the diagnostic criteria for KD established by the Japanese Kawasaki Disease Research Committee and were typical cases which fulfilled requirement of the criteria. All of whole blood samples were
stored at -80℃ until DNA was isolated by standard method.

CD 14 promoter gene-specific primers were prepared: 5' primer
(5'-CTAAGGCACTGAGGATCATCC-3') and 3' primer
(5'-CATGGTCGATAAGTCTTCCG-3'). Polymerase chain reaction (PCR) was performed at a total volume of 50 μl (100-200 ng of genomic DNA, 1 U of Taq

polymerase, 50 pmol of each primer, 200 pmol of each dNTP and 1.5 mmol Mg^{2+}). PCR consisted of 1 cycle of 15 minutes at 85℃ and 4.5 minutes at 94℃; 42 cycles of 30 seconds at 94℃, 1 minute at 55℃, 1 minute at 72℃; and 7 minutes at 72℃. PCR products (4 μℓ) was cleaved in appropriate buffer with 8 U of *HaeIII* restriction enzyme. The DNA fragments were separated by electrophoresis through polyacrylamide gels, and the gels were viewed by UV transillumination. Digestion of the PCR products yielded bands of 418 bp in T/T homozygotes, 263 and 155 bp in C/C homozygotes, and all 3 bands in the C/T heterozygotes (Fig. 1).

Statistical analysis

The frequency of the alleles and genotypes in both group were compared by chi-square test. A value < 0.05 was considered significant. The relationships between the CD 14 genotype and the clinical inflammatory parameters were tested by 1-way ANOVA.

Results

The distributions and frequencies of alleles and genotypes of polymorphism in the CD 14 promoter in KD patients and controls are shown in Table 1. The frequency of T allele was significantly higher in KD patients than in controls (*62.5%* vs. *47.8%*, P=0.022). The frequency of T/T genotype was also significantly higher in KD patients than in controls (40.0% vs. 20.6%, P=0.046). There was no significant association between the CD 14 genotype and the clinical inflammatory parameters such as Creactive protein (CRP), WBC counts or erythrocyte sedimentation rate (ESR) (data not shown).

Discussion

The present study demonstrated that the frequencies of T allele and T/T homozygotes were tended to be higher (P=0.022 and P=0.046, respectively) in KD patients than in controls. Although both P values were statistically significant (P<0.05) , it does not necessarily mean that the present results indicate a strong as sociation between C (-260) → T polymorphism in the promoter of CD 14 gene and the increased risk for KD onset. The previous reports demonstrated a statistically strong relationship (P<0.001) between C (-260) → T polymorphism in the promoter of CD 14 gene and the increased risk for myocardial infarction in European countries and Japan [3-5]. On the other hand, *Zee et al.* reported that C (-260) → T polymorphism in the promoter of CD 14 gene was not associated with risks for myocardial infarction in the United States [12]. These incompatible results might be induced by ethnic/racial differences.

Reportedly, endotoxin accelerates atherosclerosis in animal models [13]. Furthermore, the promoter polymorphism in the CD 14 gene is associated with

increased carotid atherosclerosis in smokers [14]. Therefore, it is suggested that C (-260) → T polymorphism in the CD 14 promoter is associated not only with myocardial infarction but also with other atherosclerotic diseases. However, C (-260) → T polymorphism in the CD 14 promoter is not associated with an increased risk for ischemic cerebrovascular disease in Japan [15].

The levels of circulating soluble CD 14 are reported to increase in the acute phase of KD [16], and peripheral counts of CD 14-positive monocytes/macrophages and neutrophils increase in KD [17, 18]. In the present study, there was a weak association between C (-260) → T polymorphism in the promoter of CD 14 gene and the increased susceptibility to KD. Therefore, CD 14 receptor may be concerned with the etiology and pathogenesis of KD. To elucidate the role of CD 14 gene in KD, further studies will be needed in the future.

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Fig.1 CD14/-260 genotype analysis by restriction fragment assay.

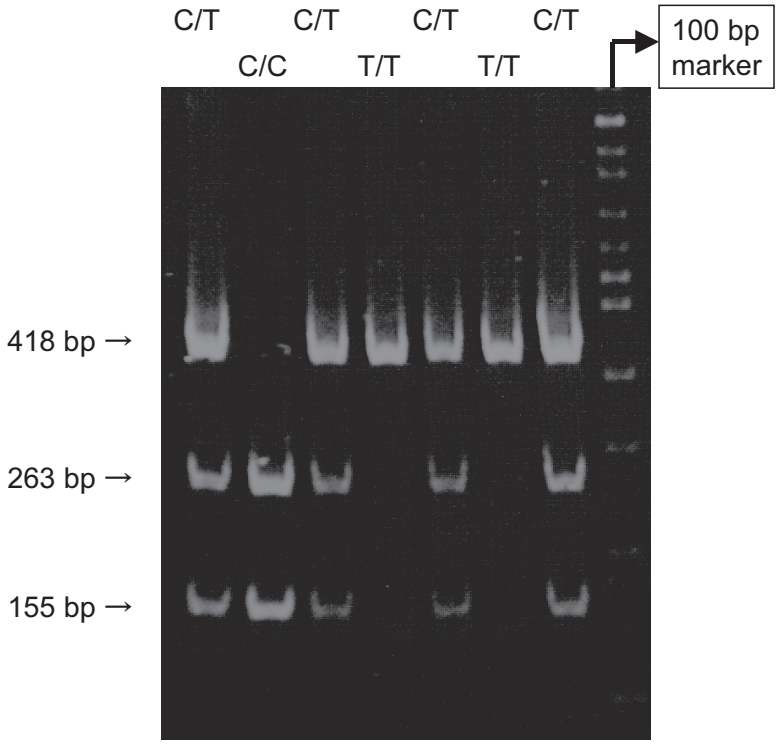

Table 1. Alleles and genotypes of the CD14 promoter polymorphism in KD patients and controls

	KD patients (n=60)	Controls (n=68)	P values
Allele			
T	75 (62.5%)	65 (47.8%)	P=0.022
C	45 (37.5%)	71 (52.2%)	
Genotype			
T/T	24 (40.0%)	14 (20.6%)	P=0.046
C/T	27 (45.0%)	37 (54.4%)	
C/C	9 (15.0%)	17 (25.0%)	