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ORIGINAL ARTICLE

Fas promoter region gene polymorphism is associated with an increased risk for myocardial infarction

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A growing body of evidence has shown that Fas-mediated apoptosis is involved in atherosclerosis progression. Recent studies have revealed that a single nucleotide polymorphism (SNP) in the Fas promoter region (−670G/A) influences Fas expression. Here, we investigated whether −670G/A SNP influences the incidence of myocardial infarction (MI) by examining a comparison between MI patients (n=154) and control subjects (n=462) in a Japanese population. The allele frequency in each group was A 53.6%/G 46.4% in the MI patients, and A 43.9%/G 56.1% in the non-MI subjects (χ^2=8.6; P=0.003). The odds ratio was 2.62 (95% CI: 1.43–4.88). As subjects with the −670AA genotype had a signal transducer and activator of transcription 1 (STAT1)-binding site in the Fas promoter region, STAT-1 activation by interferon-γ may upregulate Fas expression in human vascular smooth muscle cells (VSMCs) of −670AA genotype subjects as described earlier. The Fas upregulation induces excess apoptosis to VSMCs, which leads to unstable plaque formation in atherosclerotic lesions and then potentially to plaque rupture, which can cause MI. Further investigation of hypertensive subjects revealed that the −670AA genotype does not induce hypertension occurrence, supporting that this difference of MI occurrence between the −670AA genotype and the −670GG genotype may be because of plaque rupture followed by excess apoptosis of VSMCs in the atherosclerotic lesion. We conclude that the Fas promoter gene, SNP (−670G/A), may be a risk factor of MI occurrence.
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Keywords: apoptosis; Fas; gene polymorphism; myocardial infarction; VSMCs

INTRODUCTION

In the last 50 years, it has become increasingly recognized that cardiovascular events, including myocardial infarction (MI), are socially crucial problems. Recent studies show that apoptosis plays an important role in atherogenesis. Specifically, the genesis of unstable plaques is deeply correlated with the apoptosis of vascular smooth muscle cells (VSMCs).[1–4]

Fas is a type I transmembrane protein, a member of the tumor necrosis factor/nerve growth factor receptor family, which can transduce the apoptotic signal into susceptible target cells through the binding of the Fas ligand (FasL).[5–7] Experimental studies show that Fas is expressed on the surface of many cell types, such as VSMCs, and mediates apoptosis in these cells.[4,5] Clinical studies have documented the relationship between the abnormal expression of Fas and immune disorders, such as the human autoimmune lymphoproliferative syndrome.[8,9] Furthermore, mutations in the *Fas* gene, resulting in a defective Fas protein expression, have been reported in patients with the autoimmune lymphoproliferative syndrome, which is characterized by generalized hypercellularity of secondary lymphoid organs.[9–11] The *Fas* gene has been mapped to 10q24.1 and its genomic organization, including exons, introns and the promoter region, has been characterized.[12] One of the Fas promoter region polymorphisms is located at the –670 position from the transcription-starting site and results from an ATCCG (G/A) AA substitution.[13] This polymorphism is located on a consensus sequence of the γ-activated sequence, which is a signal transducer and activator of transcription 1 (STAT1)-binding site. Thus, this −670G/A polymorphism may have a function in gene regulation.[14] Recent studies have shown that interferon-γ (IFN-γ) stimulation can upregulate Fas expression through STAT1 activation in monocytes isolated from −670AA genotype subjects but not from subjects with the −670GG genotype.[15,16] Although the Fas/FasL system is strongly correlated with cardiovascular diseases,[17–23] there are few reports about the relationship between *Fas* gene polymorphisms and cardiovascular diseases in humans. Here we investigated whether the *Fas* gene polymorphism influences the development of MI and hypertension in humans.

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METHODS

Study sample

1st panel. Patients with documented evidence of acute MI (acute MI) were identified from the discharge records of the Osaka Medical Center for Cancer and Cardiovascular Disease (Osaka City, Japan). We selected 154 patients (127 males and 27 females, aged 43–84 years) who developed acute MI and who received a percutaneous coronary intervention or coronary artery bypass graft. Age- and sex-matched controls (n=462) without a history of MI were recruited from the outpatients of Osaka University Hospital.

2nd panel. Participants were recruited from the outpatients of private clinics belonging to the Amagasaki Medical Association. The definition of hypertension was as follows: (a) family history of hypertension, (b) systolic blood pressure $\geqslant$160 mm Hg and/or diastolic blood pressure $\geqslant$100 mm Hg or under chronic antihypertensive medication and (c) onset of hypertension at $<$50 years. Controls of panel 2 were defined as follows: (a) age $>$50 years old; (b) without family history of hypertension; and (c) systolic blood pressure $<$130 mm Hg, diastolic blood pressure $<$85 mm Hg and no history of medication for hypertension.

The definition of hyperlipidemia was as follows: (a) fasting serum total cholesterol concentration $\geqslant$220 mg per 100 ml and (b) under chronic antihyperlipidemic medication. The definition of diabetes mellitus was as follows: (a) fasting blood sugar $\geqslant$140 mg per 100 ml and (b) under chronic antidiabetic medication.

All subjects in this study were Japanese, and gave signed informed consent to participate in the genetic analysis and in all other procedures associated with the study. The study protocol was approved by the Institutional Review Board (IRB) of Osaka University.

Genotype determination using the TaqMan PCR method

Genomic DNA was extracted from 200 µl of buffy coat using a QIAamp DNA Blood Kit (QIAGEN KK, Tokyo, Japan). The G-to-A transversion at the nucleotide position −670 on the promoter region of Fas (Fas −670G/A) was determined by the TaqMan PCR method. The Fas −670G/A polymorphism was detected using the following primers and probes: forward, 5′-CCCTATG GCGCAACATCTGT-3′ and reverse, 5′-TGACTGCGCTGTCCATGTTGT-3′; guanine base (G)-specific probe, 5′-FAM-TGGTTAACTGTCCATTCCAGgAA CGTCTGT-TAMRA-3′; and adenine base (A)-specific probe, 5′-VIC-TGGTTA ACTGTCCATTCCATaAACGTCTGT-TAMRA-3′. PCR proceeded using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles at 92 °C for 15 s and at 60 °C for 60 s. The three genotypes were differentiated by analyzing the fluorescence levels of PCR products using an ABI PRISM 7900HT Sequence Detector (Applied Biosystems).

Statistical analysis

Associations between the polymorphisms and clinical variables were analyzed using the one-way analysis of variance. Differences in genotype or allele distribution were examined by the χ^2 analysis. Multiple regression analysis assessed the contribution of the confounding factors. All numerical values are expressed as the mean ± s.d. Significance is defined as $P<0.05$. All statistical analyses were conducted using JMP software version 3.1.5J for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Relationship between MI and Fas promoter gene polymorphism

To determine whether the Fas promoter region polymorphism contributes to the incidence of MI, 154 patients with MI and 462 control subjects without MI were examined. The clinical characteristics of MI patients and non-MI control subjects are shown in Table 1. The former group had significantly higher number of patients with smoking habits, hypertension, diabetes mellitus and hyperlipidemia compared with the group consisting of control subjects.

Next, we checked the frequency of each genotype in the group with or without MI. As shown in Table 2, the frequency in the control group is 18.0% (AA), 52.0% (GA) and 30.1% (GG), whereas the MI group revealed 29.2% (AA), 48.7% (GA) and 22.1% (GG) (P=0.008 AA *vs.* GA *vs.* GG (additive model), χ^2=3.7; P=0.055 AA+GA *vs.* GG (dominant model), χ^2=8.9; P=0.003 AA *vs.* GA+GG (recessive model)). No significant difference of clinical characteristics (age, gender, hypertension, hyperlipidemia, diabetes mellitus and smoking) was observed with either genotype. Table 3 shows the allele frequency in each group (A: 53.6% and G: 46.4% in the MI patients, A: 43.9% and G: 56.1% in the non-MI subjects, χ^2=8.6; P=0.003) and odds ratio (2.62, 95% CI: 1.43–4.88). Then we selected these factors as the covariates of logistic analysis for MI occurrence and Fas −670G/A polymorphism (Table 4). There was an independent relationship between the genotype of −670G/A polymorphism and MI after adjusting covariates. These data indicate that the Fas promoter region −670G/A polymorphism is independently correlated to the occurrence of MI.

In addition, to investigate whether this contribution of the −670G/A polymorphism is specific for MI occurrence, we examined 330 patients with hypertension and 219 control subjects without hypertension. Table 5 compares the clinical characteristics of these two groups. Age, gender and blood pressure were significantly higher in the hypertensive patients than in the control subjects. We checked the

Table 1 Clinical characteristics of patients

Variable	*MI (+)*	*MI (−)*	P-*value*
N	154	462	
Age (years)	63.8 ± 8.7	63.7 ± 8.7	NS
Gender (% males)	127 (82%)	381 (82%)	NS
Smoking habits (%)	91 (61%)	176 (38%)	<0.0001
Hypertension (%)	78 (52%)	185 (40%)	0.008
Diabetes mellitus (%)	53 (36%)	39 (8%)	<0.0001
Hyperlipidemia (%)	73 (48%)	104 (22%)	<0.0001

Abbreviations: MI, myocardial infarction; NS, nonsignificant.

Table 2 Frequency of Fas polymorphism

	MI (+)	*MI (−)*
Fas	*n* (%)	*n* (%)
Genotype		
AA	45 (29.2)	83 (18.0)
GA	75 (48.7)	240 (52.0)
GG	34 (22.1)	139 (30.1)

Abbreviation: MI, myocardial infarction.
Additive model: AA *vs* GA *vs.* GG, P=0.008.
Dominant model: AA+GA *vs.* GG, χ^2=3.7, P=0.055.
Recessive model: AA *vs* GA+GG, χ^2=8.9, P=0.003.

Table 3 Allele frequency and odds ratio

	MI (+)	*MI (−)*
Allele	*n* (%)	*n* (%)
A	165 (53.6)	406 (43.9)
G	143 (46.4)	518 (56.1)

Abbreviations: CI, confidence interval; MI, myocardial infarction.
χ^2=8.6, P=0.003.
Odds ratio=2.62 (95% CI; 1.43–4.88).

Table 4 Logistic analysis

	Wald χ^2	P-*value*
Diabetes mellitus	42.9	<0.0001
Smoking habits	29.9	<0.0001
Hyperlipidemia	26.6	<0.0001
Hypertension	17.2	<0.0001
A allele	9.56	0.002
Gender	1.68	0.19
Age (years)	0.23	0.62

Table 5 Clinical characteristics of patients

Variable	*HT (+)*	*HT (−)*	P-*value*
N	330	219	
Age (years)	60.0 ± 10.8	63.9 ± 9.1	<0.0001
Gender (% males)	158 (48%)	86 (39%)	0.047
SBP (mm Hg)	142 ± 18.8	117 ± 10.7	<0.0001
DBP (mm Hg)	83.7 ± 12.4	69.6 ± 8.1	<0.0001
Smoking habits (%)	136 (41%)	76 (35%)	NS
Diabetes mellitus (%)	60 (18%)	32 (15%)	NS
Hyperlipidemia (%)	148 (46%)	82 (38%)	NS

Abbreviations: DBP, diastolic blood pressure; HT, hypertension; NS, nonsignificant; SBP, systolic blood pressure.

Table 6 Frequency of Fas polymorphism

	HT (+)	*HT (−)*
Fas	*n (%)*	*n (%)*
Genotype		
AA	77 (23.3)	61 (27.9)
GA	157 (47.6)	110 (50.2)
GG	96 (29.1)	48 (21.9)

Abbreviation: HT, hypertension.
Additive model: AA *vs.* GA *vs.* GG, P=0.146.
Dominant model: AA+GA *vs.* GG, χ^2=3.5, P=0.061.
Recessive model: AA *vs.* GA+GG, χ^2=1.4, P=0.232.

frequency of each genotype in the group with or without hypertension. As shown in Table 6, the frequency in the control group is 27.9% (AA), 50.2% (GA) and 21.9% (GG), whereas the hypertensive group revealed 23.3% (AA), 47.6% (GA) and 29.1% (GG) (P=0.146 AA *vs.* GA *vs.* GG (additive model), χ^2=3.5; P=0.061 AA+GA *vs.* GG (dominant model), χ^2=1.4; P=0.232 AA *vs.* GA+GG (recessive model)). We then selected age and gender as the covariates of logistic analysis for hypertension occurrence and Fas −670G/A polymorphism. We found a weak but positive effect of the −670GG genotype on hypertension (P=0.044) after adjusting covariates. These data indicate that the effect of Fas promoter region −670G/A polymorphism on hypertension and MI is contradictory, supporting the notion that the occurrence of MI in the Fas −670AA subjects is not associated with hypertension.

DISCUSSION

In this study, we have shown that the G/A polymorphism in the *Fas* gene is associated with an increased occurrence of MI. These findings suggest that the A allele of G/A polymorphism in the promoter region (−670G/A) of the *Fas* gene may be a new genetic risk factor for MI.

Recent studies have shown that excess apoptosis of VSMCs promotes unstable plaque formation in atherosclerosis.[24,25] In this study, we showed for the first time that a Fas promoter polymorphism may be a risk factor for MI occurrence. This may be dependent on the upregulation of Fas on VSMCs by IFN-γ through STAT1 activation.[15,16]

Interferon-γ is known to be a macrophage-activating factor produced by lymphocytes.[26] Many reports have shown that IFN-γ is associated with the incidence of cardiovascular disease in *in vivo* experiments.[27–30] In atherosclerotic lesions, IFN-γ is predominantly secreted from type 1 helper T cells.[26] In addition, IFN-γ can activate the JAK (Janus kinase)/STAT1 pathway, following STAT1 binding to the γ-activated sequence.[31] Moreover, it is reported that the Fas receptor expression is regulated by STAT1, and the oligonucleotide with −670A in the Fas promoter region has a 3.5-fold higher binding ability to STAT1 than that with −670G.[15,16] As Fas receptor upregulation in VSMCs induces Fas-mediated apoptosis and subsequent promotion of unstable plaques,[24,32,33] this Fas promoter gene AA genotype can cause a two-fold increase in MI occurrence compared with the GG genotype.

To confirm whether this −670G/A polymorphism is uniquely associated with MI, we examined the contribution of Fas promoter region polymorphism to the incidence of hypertension. We observed that the Fas promoter region −670AA genotype does not induce hypertension. These data support that the Fas −670AA genotype is specifically associated with MI occurrence because of plaque rupture followed by the excess of VSMC apoptosis. After the adjustment of age and gender, however, the Fas −670GG genotype is weakly but significantly correlated to the incidence of hypertension (P=0.044). In this regard, we have reported earlier that Fas signaling can activate endothelial nitric oxide synthase activation in endothelial cells, and can thereby regulate blood pressure.[34] Endothelial cells are highly resistant to Fas-mediated apoptosis owing to FLIP (Fas-associated death domain-like interleukin-1β-converting enzyme inhibitory protein) expression. Earlier we have shown that FasL stimulation can induce endothelial nitric oxide synthase activation in endothelial cells instead of apoptosis, and Fas-mutated *lpr* mice have higher blood pressure as a result of impaired endothelial function compared with wild-type mice.[34] Thus, we predict that the −670AA subjects may have lower blood pressure compared with −670GG subjects, because of the Fas receptor upregulation on intact endothelial cells after endothelial nitric oxide synthase activation with FasL stimulation.

In conclusion, we show that a polymorphism in the promoter region of the *Fas* gene (−670G/A) is associated with an increased risk for MI in Japanese subjects.

Study limitations

We predict that the upregulation of the Fas receptor on VSMCs induces excess VSMC apoptosis by the Fas–FasL system, after the generation of unstable plaques in the atherosclerotic lesion and promotion of plaque rupture. However, a limitation of this study was that we could not check plaque conditions in the atherosclerotic lesion of each genotype subjects. Further investigations are necessary to elucidate this association between the Fas −670G/A polymorphism and the formation of vulnerable atherosclerotic plaques.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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