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ORIGINAL ARTICLE

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An association analysis between *ApoA1* polymorphisms and the high-density lipoprotein (HDL) cholesterol level and myocardial infarction (MI) in Japanese

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Abstract Association studies were performed to confirm the effect of polymorphisms in apolipoprotein A1 (*ApoA1*) on the high-density lipoprotein cholesterol (HDL-C) level and the incidence of myocardial infarction (MI). A sequence analysis identified nine polymorphisms in *ApoA1*. After considering linkage disequilibrium, four polymorphisms in *ApoA1*and four polymorphisms in the 5′-flanking regions and 3′-flanking regions from the JSNP database were determined in 1,880 subjects recruited from the Suita study, which represents the general population in Japan. Of the eight polymorphisms tested, the *ApoA1* T84C polymorphism had the greatest effect on the levels of HDL-C ($P = 0.0005$, $P_c = 0.0040$ corrected by the Bonferroni method) and triglyceride ($P < 0.0001$, $P_c = 0.0008$). The *ApoA1 Msp*I polymorphism was not associated with HDL-C or triglyceride levels. We confirmed that the *ApoA1* T84C polymorphism was associated with the HDL-C level but not the triglyceride level in patients with MI ($n = 637$). Moreover, this polymorphism was associated with the incidence of MI in male subjects ($P = 0.0326$). A logistic analysis indicated that the frequency of MI in the CC genotype was lower than that in the CT+TT genotype ($P = 0.0145$, OR $= 0.4955$, 95% CI: 0.2746–0.8525). The *ApoA1* T84C polymorphism is an important marker for the HDL-C level and may be a new risk marker for MI in Japanese.

Keywords *ApoA1* · Polymorphisms · HDL cholesterol · Myocardial infarction · Association study

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Introduction

Lipid profiles are well known to play a pivotal role in the progression of coronary artery disease (CAD): a decreased plasma concentration of high-density lipoprotein cholesterol (HDL-C) and an increased plasma concentration of low-density lipoprotein cholesterol (LDL-C) are associated with the development of CAD (Miller and Miller 1975; Kannel et al. 1979). Apolipoprotein A1 (ApoA1), a component of HDL-C, is a major participant in the regulation of reverse cholesterol transport from peripheral tissues to the liver, and this pathway is thought to help protect against atherosclerosis. In fact, epidemiological studies have reported that decreased plasma concentrations of both HDL-C and ApoA1 were associated with premature CAD (Maciejko et al. 1983).

Genetic factors have been reported to influence the distribution of lipids and lipoprotein levels, including the ApoA1 level (Groenendijk et al. 2001a). A rare variant nonsense mutation at codon 84 has been reported to result in ApoA1 deficiency (Matsunaga et al. 1991). Recent epidemiological studies have reported that common *ApoA1* polymorphisms influence the levels of HDL-C and triglycerides (TG) (Ordovas et al. 1986; Jeenah et al. 1990; Pagani et al. 1990; Talmud et al. 1994; Groenendijk et al. 2001b). In addition, several

researchers reported associations between *ApoA1* polymorphisms and CAD (Karathanasis et al. 1983; Ordovas et al. 1986; Reguero et al. 1998), whereas others found no positive association (Ordovas et al. 1991; Marshall et al. 1994; Yamada et al. 2002). One possible reason for the inconsistencies among previous association studies may be that almost all of these studies considered only a few restriction fragment-length polymorphisms instead of every polymorphism in the *ApoA1*gene. Thus, the polymorphism that has the greatest effect on the HDL-C level and the incidence of CAD may have been missed in previous studies.

To evaluate the effects of polymorphisms in *ApoA1* on lipid levels, we sequenced the *ApoA1* gene and conducted an association study using a large cohort (the Suita population $n=1{,}880$), representing the general population in Japan. In addition, we confirmed an association between *ApoA1* polymorphisms and lipid levels. Finally, we investigated the association between the *ApoA1* polymorphism and the incidence of myocardial infarction (MI) using patients with MI ($n=637$).

Subjects and methods

Subjects

The Suita population The selection criteria and design of the Suita study have been described previously (Mannami et al. 1997; Shioji et al. 2004a). Genotypes were determined in 1,880 consecutive subjects who visited the National Cardiovascular Center between April 2002 and February 2003 (867 men, 1,013 women). The characteristics of this population are shown in Table 1.

Table 1 Characteristics of the Suita population. *P* value was calculated by the Student's *t* test. *BMI* body mass index, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride, *%CVA* percentage of subjects with cerebrovascular accident, *%OMI* percentage of subjects with old myocardial infarction, *%HT* percentage of subjects with hypertension, *%DM* percentage of subjects with diabetes mellitus, *%HLP* percentage of subjects with hyperlipidemia, *%drinking* percentage of subjects with a drinking habit, *%smoking* percentage of subjects with a smoking habit

Parameter	Male	Female	*P* value
n	867	1,013	
Age (year)	66.3 ± 0.4	63.3 ± 0.3	< 0.0001
BMI (kg/m^2)	23.2 ± 0.1	22.3 ± 0.1	< 0.0001
TC (mmol/l)	5.13 ± 0.03	5.58 ± 0.02	< 0.0001
HDL-C (mmol/l)	1.43 ± 0.01	1.68 ± 0.01	< 0.0001
TG (mmol/l)	1.38 ± 0.03	1.07 ± 0.03	< 0.0001
Blood glucose (mmol/l)	5.74 ± 0.04	5.30 ± 0.04	< 0.0001
%CVA	3.6	1.4	0.0018
%OMI	2.1	0.5	0.0015
%HT	45.9	37.2	< 0.0001
%DM	11.4	4.5	< 0.0001
%HLP	14.8	24.0	< 0.0001
%Drinking	67.0	29.5	< 0.0001
%Smoking	29.9	6.3	< 0.0001

When the association between the *ApoA1* T84C polymorphism and the incidence of myocardial infarction was analyzed, subjects with ischemic heart disease were excluded.

The myocardial infarction (MI) group The selection criteria and design of the MI group have been described previously (Takagi et al. 2002). This group consisted of randomly selected inpatients and outpatients with documented MI ($n=637$, 547 men and 90 women) who were enrolled in the Division of Cardiology at the National Cardiovascular Center between May 2001 and April 2003 and met the following criteria: (1) chest pain of ≥30 min duration; (2) electrocardiographic ST segment elevation of ≥0.1 mV in two or more leads in the same vascular territory; and (3) subsequent elevation of creatine phosphokinase levels to more than twice the normal range.

Written informed consent was obtained from every subject after a full explanation of the study, which was approved by the Ethics Committee of the National Cardiovascular Center and by the Committee on Genetic Analysis and Genetic Therapy of the National Cardiovascular Center.

DNA studies

The promoter region (up to −1 kb) and all of the exonic regions in *ApoA1* were sequenced for polymorphisms

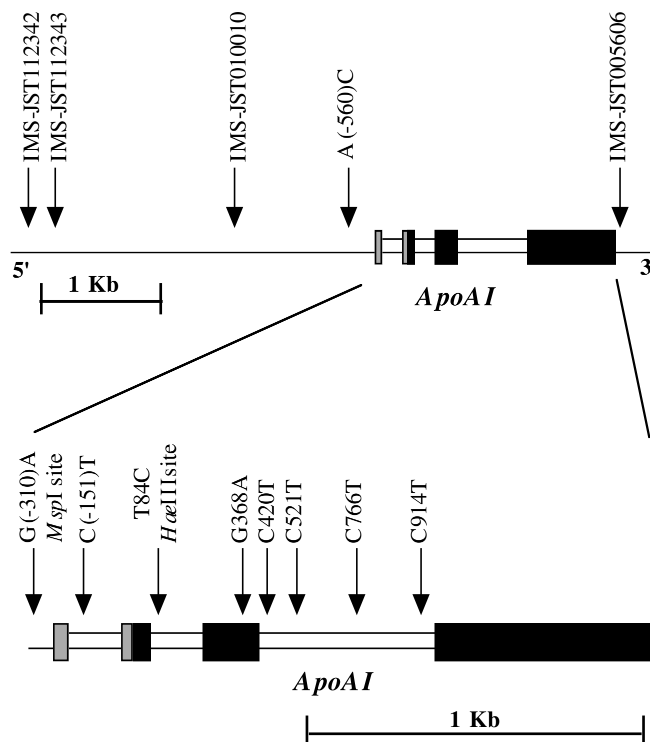

Fig. 1 Schema of the *ApoA1* gene and the positions of the determined polymorphisms. *Gray and black boxes* indicate the 5′-untranslated and coding regions, respectively

in 36 subjects (Fig. 1). For the 5′-flanking regions and 3′-flanking regions, we selected four polymorphisms for genotyping from a public database (JSNP, http://www.snp.ims.u-tokyo.ac.jp, Fig. 1) (Hirakawa et al. 2002). The *ApoE* and ATP-binding cassette transporter A1 (*ABCA1*) G(−273)C polymorphisms were also determined as previously described (Shioji et al. 2004b). *ApoE* polymorphisms were categorized into three genotypes: E2 ($\epsilon2/\epsilon2+\epsilon2/\epsilon3+\epsilon2/\epsilon4$ subjects), E3 ($\epsilon3/\epsilon3$ subjects), E4 ($\epsilon3/\epsilon4+\epsilon4/\epsilon4$ subjects) (Lefevre et al. 1997; Shioji et al. 2004b). All polymorphisms were determined by the TaqMan system. The primer and probe sequences are available on request.

Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM). For TG values, while a logarithmic transformation was applied for the statistical test, untransformed values are shown in the table. LDL-C was calculated by Friedewald's formula [(LDL-C) = (total cholesterol, TC)−(HDL-C)−(TG/5). We excluded those whose HDL-C or TG levels were ≥2.6 mM or 4.53 mM, respectively]. All statistical analyses were performed with the JMP statistical software package (SAS Institute, Inc.). Values of $P < 0.05$ were considered to indicate statistical significance. Multiple linear regression and multiple logistic analyses were performed with other covariates. The residual levels were calculated by adjusting for covariates. Differences in numerical data among the groups were evaluated by Student's t test or one-way analysis of variance (ANOVA). Hardy-Weinberg equilibrium was calculated by a chi-square test. To measure linkage disequilibrium (LD) between polymorphisms, D' and r^2 values were analyzed using the SNPAlyze statistical software package (Dynacom, Inc.). In some settings, the P values were corrected (P_c) by multiplying by 8 (eight polymorphisms, Bonferroni).

Results

Polymorphisms of the promoter and exonic regions in *ApoA1*

We found two polymorphisms in the promoter region, one in intron 1, one in intron 2, one in exon 3, and four in intron 3 (Table 2 and Fig. 1).

LD was evaluated by calculating r^2 values (Table 3). We regarded $r^2 > 0.25$ as tight linkage. Accordingly, we selected four polymorphisms, G(−310)A, T84C, G368A, and C420T, for the following association study. The G(−310)A and T(84)C polymorphisms correspond to the *Msp*I (Pagani et al. 1990; Tuteja et al. 1992) and *Hae*III (Groenendijk et al. 2001b) polymorphisms, respectively. The G368A polymorphism was accompanied by a missense mutation (GCC →

Table 2 Polymorphisms in *ApoA1*. The nucleotide numbers of polymorphisms are given according to the number from ATG

SNP name	dbSNP No.	Minor allele frequency	Amino acid change	Sequence
Polymorphisms detected by sequence				
A(−560)C		0.078	–	GACACTCCCCTCCCGCCCCCACTGA[A/C]CCCTTGACCCCTGCCCTGCAGCCCC
G(−310)A	670	0.156	–	AGGACCAGTGAGCAGCAACAGGGCC[G/A]GGGCTGGGCTTATCAGCCTCCCAGC
C(−151)T	5069	0.078	–	TCAAGGTTCAGGCCTTGCCCCAGGC[C/T]GGGCCTCTGGGTACCTGAGGTCTTC
T84C	5070	0.234	–	CCTAGGGAGCCAACCATCGGGGGGC[T/C]TTCTCCCTAAATCCCCGTGGCCCAC
G368A	–	0.063	Ala → Thr	CTATGTGTCCCAGTTTGAAGGCTCC[G/A]CCTTGGGAAAACAGCTAAAGTAAGG
C420T	2070655	0.375	–	CCCAGCCTGGGGTTGAGGGCAGGGG[C/T]AGGGGGCAGAGGCCTGTGGGATGAT
C521T	5072	0.387	–	CCACAGATGGTCTGGATGGAGAAAC[T/C]GGAATGGGATCTCCAGGCAGGGTCA
C766T	–	0.452	–	TTTGGAGACCAACGTAACTGGGCAC[T/C]AGTCCCAGCTCTGTCTCCTTTTTAG
C914T	5076	0.078	–	CTCCGCGGACAGGTGTCACCCAGGG[C/T]TCACCCCTGATAGGCTGGGGCGCTG
Polymorphisms from JSNP database				
IMS-JST010010		0.219	–	TTCTCCTGGAAGGCCCAGACCTCCC[C/T]CAGCAGGTTACTGATAGGACCTGAG
IMS-JST112343		0.279	–	CACTTTCACAACTTAGAATATCCCT[A/G]TAAGGCTGGAGGCCAGATTTTACCC
IMS-JST112342		0.274	–	CTTGCACCCTTGCGGAGCCTGCAGC[C/T]TTTGCAGTCTGATCAGGGACTTCTC
IMS-JST005606		0.108	–	CGTCGATCTTGGCCCTAAGACGTCC[A/T]GTCTGGGCACGGAGTTGTTGAGATC

Table 3 Linkage disequilibrium among the polymorphisms in *ApoA1*. R^2 values are shown, R^2 values described are based on the genotypes of 36 subjects used for sequence analyses. All values refer to the variant allele indicated in the table

	A(−560)C	G(−310)A	C(−151)T	T84C	G368A	C420T	C521T	C766T	C914T
A(−560)C		0.016	1	0.277	0.006	0.051	0.044	0.084	1
G(−310)A			0.016	0.057	0.004	0.111	0.121	0.158	0.016
C(−151)T				0.277	0.006	0.051	0.044	0.084	1
T84C					0.020	0.184	0.184	0.002	0.277
G368A						0.040	0.044	0.057	0.006
C420T							1	0.767	0.051
C521T								0.767	0.044
C766T									0.084
C914T									

Table 4 Lipid levels among the *ApoA1* T84C genotypes (Suita population). We excluded subjects who were receiving hypolipidemic medication. Values are mean ± SEM, Res. TC, Res. HDL-C, Res. LDL-C, and Res. TG were adjusted for gender, age, BMI, smoking (cigarettes/day), and alcohol consumption (ethanol ml/week). *P* value was calculated by ANOVA. *P* values were corrected (P_c) by multiplying by 8 (eight polymorphisms, Bonferroni). *BMI* body mass index, *%HT* percentage of subjects with hypertension, *%DM* percentage of subjects with diabetes mellitus, *%HLP* percentage of subjects with hyperlipidemia, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride, *Res. TC* residuals of TC; *Res. HDL-C* residuals of HDL-C, *Res. LDL-C* residuals of LDL-C, *Res. TG* residuals of TG; *%drinking* percentage of subjects with a drinking habit; *%smoking* percentage of subjects with a smoking habit

ApoA1 T84C genotype	TT	TC	CC	*P* value	P_c value
Number (males/females)	469/487	279/310	48/42	0.5378	
Age (years)	63.8 ± 0.4	63.9 ± 0.5	65.5 ± 1.2	0.3890	
BMI (kg/m^2)	22.7 ± 0.1	22.6 ± 0.1	22.4 ± 0.3	0.7253	
%HT	37.6	37.2	48.9	0.0977	
%DM	6.5	7.3	10	0.4527	
%HLP	41.6	42.8	33.3	0.2307	
TC (mmol/l)	5.32 ± 0.03	5.39 ± 0.03	5.27 ± 0.09	0.2325	1
HDL-C (mmol/l)	1.54 ± 0.01	1.59 ± 0.02	1.68 ± 0.04	0.0005	0.0040
LDL-C (mmol/l)[a]	3.24 ± 0.02	3.29 ± 0.03	3.16 ± 0.08	0.2357	1
TG (mmol/l)[b]	1.26 ± 0.03	1.15 ± 0.04	0.95 ± 0.09	< 0.0001	0.0008
Res. TC (mmol/l)	−0.02 ± 0.03	0.03 ± 0.03	−0.05 ± 0.08	0.3332	1
Res. HDL-C (mmol/l)	−0.03 ± 0.01	0.02 ± 0.01	0.12 ± 0.04	0.0002	0.0016
Res. LDL-C (mmol/l)[a]	−0.01 ± 0.02	0.03 ± 0.03	−0.07 ± 0.08	0.3235	1
Res. TG (mmol/l)[b]	0.05 ± 0.03	−0.05 ± 0.03	−0.24 ± 0.09	< 0.0001	0.0008
%Drinking	47.7	47.9	55.6	0.3550	
%Smoking	18.3	20.0	13.3	0.2688	

[a]The formula for calculating LDL-C is described in "Subjects and methods", and we excluded subjects whose HDL-C or TG levels were ≥2.6 mM or 4.53 mM, respectively (TT, *n*(male/female) = 457/478: TC, *n* = 274/301: CC, *n* = 48/41)
[b]Test performed on log-transformed values

ACC, Ala → Thr) at codon 61 in exon 4 (Matsunaga et al. 1991).

Association study of *ApoA1* (Suita population)

The T84C polymorphism had the greatest effect on the levels of HDL-C and TG, but not the levels of TC and LDL-C, among the eight polymorphisms (sample power = 0.96, αvalue = 0.05, two-tailed, Table 4). The IMS-JST112342 and IMS-JST112343 polymorphisms were associated with the levels of HDL-C and TG (residuals of HDL-C, $P = 0.0059$, $P_c = 0.0472$, each: residuals of TG, $P = 0.0002$, $P_c = 0.0016$, each). The other polymorphisms were not associated with HDL-C or TG levels. The IMS-JST112342 polymorphism was in almost complete linkage with the IMS-JST112343 polymorphism ($r^2 = 0.98157$, D' value = 1, $P < 0.0001$). The IMS-JST112342 and IMS-JST112343 polymorphisms were in tight linkage with the T84C polymorphism ($r^2 = 0.41365$, D' value = 0.71155, $P < 0.0001$, each). Accordingly, the effects of the IMS-JST112342 and IMS-JST112343 polymorphisms may be mainly explained by their linkage with the T84C polymorphism. We previously reported that the *ApoE* genotype and the *ABCA1* G(−273)C effect the HDL-C level (Shioji et al. 2004b). Accordingly, we performed the multiple logistic analysis, which included gender, age, body mass index (BMI), smoking, alcohol consumption, *ApoE* genotype, *ABCA1* G(−273)C, and *ApoA1* T84C. As shown in Table 5, the multiple logistic analysis indicated that *ApoE* genotype, *ApoA1* T84C, and *ABCA1* G(−273)C were independent factors significantly associated with the HDL-C level. No significant deviation from the Hardy-Weinberg equilibrium was observed in the T84C polymorphism ($P = 0.8075$). Thus, we selected the T84C polymorphism for the following association study.

Association among *ApoA1* T84C and lipid profile (the MI group)

To confirm the association between the *ApoA1* T84C polymorphism and the levels of HDL-C and TG, we

Table 5 Sum of square and *F* value of high-density lipoprotein cholesterol (HDL-C) from multiple logistic analyses. *BMI* body mass index, *ABCA1* ATP-binding cassette transporter A1 gene

Source	Sum of squares	*F* value	Probability > *F*
BMI	31,815	171.5	< 0.0001
Gender	18,881	101.7	< 0.0001
Alcohol consumption (ethanol ml/week)	13,588	73.2	< 0.0001
ApoE genotype	4,360	11.7	< 0.0001
ApoA1 T84C	2,981	8.0	0.0003
Smoking (cigarettes/day)	1,972	10.6	0.0011
Age	1,761	9.5	0.0021
ABCA1 G(−273)C	1,475	4.0	0.0190

Table 6 Lipid levels among the *ApoA1* T84C genotypes [miocardial infarction (MI) group]. Values are expressed as the mean ± SEM. *P* value was calculated by ANOVA. *BMI* body mass index, *%HT* percentage of subjects with hypertension, *%DM* percentage of subjects with diabetes mellitus, *%HLP* percentage of subjects with hyperlipidemia, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride

ApoA1 T84C genotype	TT	TC	CC	*P* value
Number (males/females)	326/61	204/27	17/2	0.3264
Age (years)	62.1 ± 0.5	62.4 ± 0.7	60.2 ± 2.4	0.6632
BMI (kg/m^2)	23.7 ± 0.2	23.7 ± 0.2	24.7 ± 0.7	0.3780
%HT	55.8	55.2	42.1	0.5076
%DM	47.1	37.2	31.6	0.0439
%HLP	55.5	60.2	52.6	0.4844
TC (mmol/l)	5.18 ± 0.06	5.30 ± 0.07	5.21 ± 0.24	0.2752[a]
HDL-C (mmol/l)	1.09 ± 0.02	1.11 ± 0.03	1.35 ± 0.08	0.0050[a]
LDL-C (mmol/l)	3.34 ± 0.06	3.47 ± 0.07	3.57 ± 0.25	0.2252[a,b]
TG (mmol/l)	1.48 ± 0.05	1.53 ± 0.06	1.21 ± 0.21	0.2872[c]

[a]Test performed on residual values adjusted for gender, age, and BMI
[b]The formula for calculating LDL-C is described in "Subjects and methods", and we excluded subjects whose HDL-C or TG levels were ≥2.6 mM or 4.53 mM, respectively [TT, *n*(male/female) = 322/61: TC, *n* = 202/27: CC, *n* = 16/2]
[c]Test performed on log-transformed residual values adjusted for gender, age, and BMI

determined the genotypes in the MI group. The T84C polymorphism was associated with the HDL-C level but not the TG level (Table 6). The T84C polymorphism also affected the prevalence of diabetes mellitus (DM, *P* = 0.0439). No significant deviation from the Hardy-Weinberg equilibrium was observed in the MI group (*P* = 0.2403). Thus, a positive association was observed between the T84C polymorphism and the HDL-C level in two groups: the Suita population and the MI group.

Association between *ApoA1* T84C and incidence of MI

We next evaluated whether the *ApoA1* T84C polymorphism was associated with the incidence of MI. Since the MI group and the Suita population were not matched for gender, we investigated only males. The T84C polymorphism was significantly associated with the incidence of MI (Table 7). Logistic analysis indicated that the frequency of MI in the CC genotype was lower than that in the CT + TT genotype [*P* = 0.0145, OR = 0.4955, 95% CI: 0.2746–0.8525, sample power = 0.75 (α value = 0.05, two-tailed)]. Accordingly, subjects with the CC genotype had higher levels of HDL-C and were less susceptible to MI. However, multiple logistic analysis, which included hypertension (HT), DM, hyperlipidemia (HLP), smoking, and the T84C polymorphism, indicated that smoking (*P* < 0.0001), DM (*P* < 0.0001), HLP (*P* = 0.0003), and HT (*P* = 0.0339) were predictors of incidence of MI but that the T84C polymorphism was not a predictor (*P* = 0.0175).

Table 7 Association between the *ApoA1* T84C polymorphism and the incidence of myocardial infarction (MI). All subjects are male. Values are expressed as the mean ± SEM. *Control* Suita subjects without ischemic heart disease, *MI* patients with myocardial infarction, *BMI* body mass index, *%HT* percentage of subjects with hypertension, *%DM* percentage of subjects with diabetes mellitus, *%HLP* percentage of subjects with hyperlipidemia, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TG* triglyceride

	Control	MI group	*P* value
Number	806	547	
Age (years)	65.8 ± 0.4	60.8 ± 0.4	
BMI (kg/m^2)	23.3 ± 0.1	23.8 ± 0.1	0.0003[a]
%HT	44.7	54.3	0.0003[a]
%DM	11.1	41.6	< 0.0001[a]
%HLP	40.6	57.9	< 0.0001[a]
TC (mmol/l)	5.14 ± 0.03	5.16 ± 0.04	0.3168[b]
HDL-C (mmol/l)	1.43 ± 0.01	1.08 ± 0.02	< 0.0001[b]
LDL-C (mmol/l)	3.10 ± 0.03	3.34 ± 0.04	< 0.0001[b,c]
TG (mmol/l)	1.40 ± 0.04	1.54 ± 0.05	0.0641[d]
ApoA1 T84C			
TT/TC/CC	477/280/49 59.2%/34.7%/6.1%	326/204/17 59.6%/37.3%/3.1%	0.0326[a]

[a]The distributions in the Suita population and patients with MI were compared by the chi-square test
[b]Student's *t*-test was performed on residual values adjusted for age and BMI
[c]The formula for calculating LDL-C is described in "Subjects and methods", and we excluded subjects whose HDL-C or TG levels were ≥2.6 mM or 4.53 mM, respectively (Control, *n* = 794: MI group, *n* = 403)
[d]Student's *t* test was performed on log-transformed residual values adjusted for age and BMI

Discussion

In the present study, we conducted a sequence analysis and detected nine polymorphisms in *ApoA1*. We evaluated the effects of eight polymorphisms, including four selected from the JSNP database, on the lipid profile using a large cohort representing the general population in Japan. We next confirmed the effects of the *ApoA1*

T84C polymorphism on the HDL-C level in the MI group. Finally, we found a positive association between the *ApoA1* T84C polymorphism and the incidence of MI. However, this polymorphism was not an independent predictor when we performed the multiple logistic analysis, which included the established risk factors of smoking, DM, HLP, and HT.

The present study can be distinguished by three main features: an association study using a large cohort study in the general population (the Suita population), confirmation of the association using another set of subjects (the MI group), and the detection of a new protective marker for MI in the *ApoA1* gene.

As described previously (Zaman et al. 1997), the *ApoA1 Msp*I and *Sst*I polymorphisms were not associated with the levels of total cholesterol and HDL-C in the Shibata study, which represented the Japanese rural population. In the present study, we also did not observe an association between the *ApoA1Msp*I [G(−310)A] polymorphism and HDL-C or TG levels. Since the *ApoA1Msp*I polymorphism has only weak linkage with the *ApoA1* T84C polymorphism ($r^2=0.0567$, $P=0.0568$), the *ApoA1* T84C polymorphism may have the greatest effect on the HDL-C level in the Japanese population.

The present study provides evidence that the *ApoA1* T84C polymorphism is associated with the incidence of MI. The *ApoA1* T84C polymorphism may act in a proatherogenic or antiatherogenic fashion via the modulation of the HDL-C level because the *ApoA1* polymorphisms have effects on the HDL-C level but not the LDL-C level. Yamada et al. reported that *ApoA1* polymorphisms were weakly associated with the incidence of MI (Yamada et al. 2002), but they did not investigate the *ApoA1* T84C polymorphism. Since the *ApoA1* T84C polymorphism may have the greatest effect on the HDL-C level in the Japanese population, it is possible that the *ApoA1* T84C polymorphism may also most strongly influence the risk of MI in the Japanese population. Since the sample power was 0.75 (α value=0.05, two-tailed), this study has adequate statistical power. However, the present association ($P=0.0145$) was marginal. After adjustment of risk factors—smoking, DM, HLP, and HT—the significant association between the *ApoA1* T84C polymorphism and the incidence of MI was not observed. Contradictory results often occur in association studies due to ethnic differences or variations, including covariates such as gender and environmental factors. It has been recommended that a single nominally significant association should be viewed as tentative until it has been independently replicated at least once and preferably twice (Lohmueller et al. 2003). Accordingly, it will be necessary to verify the association between the *ApoA1* T84C polymorphism and the incidence of MI using another set of subjects.

The *ApoA1* T84C polymorphism, an *Hae*III restriction site, has recently been reported, and the frequency of the T allele of *ApoA1* T84C in familial combined hyperlipidemia probands has been reported to be higher than in their spouses (Groenendijk et al. 2001b). It has been reported that individuals homozygous for the T84C allele had higher TG and ApoC-III levels but not higher ApoA1 levels (Groenendijk et al. 2001b). Thus, it may be possible that this polymorphism could be in tight linkage with unknown polymorphisms located in exon or promoter regions in *ApoA1*or another genes.

We tried haplotype and diplotype analyses in the *ApoA1* gene. Since all of the polymorphisms were located on one haplotype block and the *ApoA1* T84C polymorphism strongly influenced the levels of HDL and TG, we found no useful haplotype combination that was more influential than the *ApoA1* T84C polymorphism.

The *ApoA1* T84C polymorphism was associated with the TG level in the Suita population but not in the MI group. One possible reason for the different results between the Suita population and the MI group may be that a substantial proportion of the MI group had dyslipidemia and had been treated with hypolipidemic drugs. We could not retrospectively research who was treated with hypolipidemic drugs and what kind of hypolipidemic drug was used in patients with MI, because we made DNA sample anonymous. Accordingly, we did not have the ability to investigate the relationship between *ApoA1* T84C and the lipid levels according to the drugs used drugs, such as statin or fibrate. Thus, another study is needed to confirm the genotype/drug interaction.

Our results indicated that three polymorphisms, *ApoE* genotype, *ApoA1* T84C, and *ABCA1* G(−273)C, were independently associated with the HDL-C level. Multiple components have been proposed to regulate the HDL-C level, including cholesterol ester transfer protein (CETP) (Barter et al. 2003), phospholipid transfer protein (Huuskonen et al. 2001), hepatic lipase (Deeb et al. 2003), lecithin cholesterol acyltransferase (Zhang et al. 2004), scavenger receptor class B type 1 (Hsu et al. 2003), endothelial lipase (Ma et al. 2003), ABC transporters, apolipoproteins, and several transcriptional factors. In Japanese, several polymorphisms such as *CETP* (Inazu et al. 1990) and *ApoE* (Zaman et al. 1997) were reported to be associated with the HDL-C level. Accordingly, a prospective study should be required to establish the contribution of the *ApoA1* T84C polymorphism on the HDL-C level and the incidence of MI.

In conclusion, the present results suggest that the *ApoA1* T84C polymorphism significantly affects the HDL-C level in the general Japanese population and that this polymorphism may be a new risk marker for MI in Japanese.

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