Maxwell KN, Soccio RE, Duncan EM, Sehayek E, Breslow JL (2003) Novel putative SREBP and LXR target genes identified by microarray analysis in liver of cholesterol-fed mice. J Lipid Res 44(11):2109–2119

Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M (2003) The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. Proc Natl Acad Sci USA 100:928–933

Shioji K, Kokubo Y, Mannami T, Inamoto N, Morisaki H, Mino Y, Tago N, Yasui N, Iwai N (2004) Association between hypertension and the α-adducin, β1-adrenoreceptor and G-protein β3 subunit genes in the Japanese population; The Suita Study. Hypertens Res (in press)

Takagi S, Iwai N, Yamauchi R, Kojima S, Yasuno S, Baba T, Terashima M, Tsutsumi Y, Suzuki S, Morii I, Hanai S, Ono K, Baba S, Tomoike H, Kawamura A, Miyazaki S, Nonogi H, Goto Y (2002) Aldehyde dehydrogenase 2 gene is a risk factor for myocardial infarction in Japanese men. Hypertens Res 25:677–681

*Original Article*

# Association between Hypertension and the α-Adducin, β1-Adrenoreceptor, and G-Protein β3 Subunit Genes in the Japanese Population; the Suita Study

Keisuke SHIOJI*[1,4,†], Yoshihiro KOKUBO*[2], Toshifumi MANNAMI*[2], Nozomu INAMOTO*[2], Hiroko MORISAKI*[3], Yukari MINO*[1], Naomi TAGO*[1], Naomi YASUI*[1], and Naoharu IWAI*[1,†]

**This study focused on 3 genetic polymorphisms that have previously been implicated in hypertension: the α-adducin (*ADD1*/Gly460Trp), β1-adrenoreceptor (*ADRB1*/Arg389Gly), and G-protein β3 subunit (*GNB3*/C825T) gene polymorphisms. We determined genetic variants using the TaqMan system in a large cohort representing the general population in Japan (867 males, 1,013 females). Logistic analysis indicated that the *ADD1*/G460W polymorphism was associated with hypertension in female subjects. The odds ratio of the WW genotype for hypertension was 1.53 (95%CI, 1.12–2.08) over the WG+GG genotype ($p$=0.0070, $p$ corrected ($p_c$) =0.0420 corrected by the Bonferroni method). The *ADRB1*/R389G polymorphism tended to be associated with hypertensive status in male subjects ($p$=0.0117, $p_c$=0.0702). The odds ratio of the GG genotype for hypertension was 0.38 (95%CI, 0.167–0.780) over the RR+RG genotype. The *GNB3*/C825T polymorphism was not associated with hypertensive status in either male or female subjects. The present results do not agree with those in previous reports. Almost all common variants may have only a modest effect on common diseases, and a single study even employing 1,880 subjects may lack the statistical power to detect a real association. Accordingly, it will be necessary to verify the association between these three genes and hypertension using a larger number of subjects from the Suita cohort or another population.**
**(*Hypertens Res* 2004; 27: 31–37)**

***Key Words*: hypertension, α-adducin, β1-adrenoceptor, G-protein β3 subunit**

## Introduction

Essential hypertension is a multifactorial disorder that is influenced by both genetic and environmental factors. Single nucleotide polymorphisms (SNPs) are mostly biallelic, more stable, and more frequent than microsatellite markers, making them suitable for association studies (*1*). Over the past few years, many SNPs on candidate genes have been separately tested for their association with hypertension, with controversial results, not only due to the inadequate sample sizes but also due to ethnic differences. If a study shows that there are no functional changes for SNPs in candidate genes, the practical implications of such a study depend on the reproducibility of the findings (*2*).

Recently, three specific mutations have been reported to be associated with hypertension: the G460W polymorphism in the α-adducin (*ADD1*) gene; the R389G polymorphism in

From the *[1]Department of Epidemiology, Research Institute, *[2]Department of Preventive Cardiology, and *[3]Department of Bioscience, Research Institute, National Cardiovascular Center, Suita, Japan, and *[4]the Organization for Pharmaceutical Safety and Research of Japan, Tokyo, Japan. †These authors contributed equally to this work.

This study was supported by the Program for the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan.

Address for Reprints: Naoharu Iwai, M.D., Research Institute, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita 565-8565, Japan. E-mail: niwai@res.ncvc.go.jp

Received July 31, 2003; Accepted in revised form October 10, 2003.

**Table 1. Accession Numbers, Nucleotide Sequences, TaqMan Probes and Primers of *ADD1*, *ADRB1* and *GNB3***

| Region | Accession No. | Sequence/primers/probes |
|---|---|---|
| *ADD1*/G460W | IMS-JST010969 | CGGGGCGACGAAGCTTCCGAGGAA[G/T]GGCAGAATGGAAGCAGTCCCAAGT |
| | | 5′-GCTCCCCACTCAGACACAGTTTT-3′ (sense) |
| | | 5′-AGAGACTGCAGCAAGGGTTTCAC-3′ (antisense) |
| | | 5′-VIC-ATTCTGCCATTCCTCGGA-MGB-3′ |
| | | 5′-FAM-TTCTGCCCTTCCTCGG-MGB-3′ |
| *ADRB1*/R389G | rs1801253 | CCCGACTTCCGCAAGGCCTTCCAG[G/C]GACTGCTCTGCTGCGCGCGCAGGG |
| | | 5′-CCGCAGCCCCGACTTC-3′ (sense) |
| | | 5′-GCCGGTCTCCGTGGGT-3′ (antisense) |
| | | 5′-VIC-CTTCCAGGGACTGC-MGB-3′ |
| | | 5′-FAM-TTCCAGCGACTGCT-MGB-3′ |
| *GNB3*/C825T | IMS-JST057355 | AAGCATCATCTGCGGCATCACGTC[C/T]GTGGCCTTCTCCCTCAGTGGCCGC |
| | | 5′-CTCCCACGAGAGCATCATCTG-3′ (sense) |
| | | 5′-TCGTCGTAGCCAGCGAATAGTAG-3′ (antisense) |
| | | 5′-VIC-CACGTCCGTGGCC-MBG-3′ |
| | | 5′-FAM-ACGTCTGTGGCCTT-MGB-3′ |

*ADD1*, α-adducin; *ADRB1*, β1-adrenoceptor; *GNB3*, G-protein β3 subunit, G, glycine; W, tryptophan; C, cysteine; T, threonine.

the β1-adrenoreceptor (*ADRB1*) gene; and the C825T polymorphism in the G-protein β3 subunit (*GNB3*) gene, a substitution of cytosine (C) for thymine (T) at nucleotide position 825 of *GNB3* cDNA. However, there are inconsistencies among the previous association studies (*3–17*). In response to these controversial results, we investigated the associations between hypertension and the *ADD1*/G460W, *ADRB1*/R389G, and *GNB3*/C825T polymorphisms using a large cohort representing the general population in Japan (total, $n=1,880$: 867 males, 1,013 females).

## Methods

### Subjects

The selection criteria and design of the Suita Study have been previously described (*18–20*). The present study 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. The genotypes were determined in 1,880 consecutive subjects, who visited the National Cardiovascular Center between April 2002 and February 2003. All subjects provided their written informed consent.

### DNA Studies

DNA was isolated from peripheral leukocytes according to standard procedures. Polymorphisms were determined by the TaqMan system. The primers and probes are summarized in Table 1. The results were analyzed using an ABI PRISM 7700 Sequence Detection System (PE Biosystems, Foster City, USA) using allelic discrimination software supplied by the manufacturer.

### Statistical Analysis

Values are expressed as the mean±SEM. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc., Cary, USA). Multiple linear regression (blood pressure value) and multiple logistic (presence or absence of hypertension) analyses were performed with other covariates. Subjects were categorized as hypertensive subjects (HTN) when they had a systolic pressure of 140 mmHg or higher and/or a diastolic pressure of 90 mmHg or higher. Subjects who were currently taking hypertensive medication were also categorized as HTN. The effects of polymorphisms on blood pressure and heart rate values were assessed in subjects who were not receiving cardiovascular medications, since HTN with excellent blood pressure control by medication may have normal blood pressure values. We also excluded subjects who were receiving anti-hypertensive treatment, subjects who had had cerebrovascular accidents, subjects with demonstrated ischemic heart disease, and subjects with atrial fibrillation. Differences in numerical data among the groups were calculated by one-way analysis of variance (ANOVA) or the unpaired *t*-test. The difference in genotype or allelic distribution between normotensive subjects (NT) and HTN, and Hardy-Weinberg equilibrium was analyzed by a $\chi^2$ test. In some settings, the probability ($p$) values were corrected ($p_c$) by multiplying 6 ([3 SNPs]×[2 genders], Bonferroni). Values of $p<0.05$ were considered to indicate statistical significance.

## Results

### Subjects

The characteristics of the study population are given in Table

**Table 2. Characteristics of Study Participants**

| Parameter | NT | HTN | *p* | Male | Female | *p* |
|---|---|---|---|---|---|---|
| *n* | 1,105 | 775 | | 867 | 1,013 | |
| Age (years) | 61.9±0.3 | 68.7±0.4 | <0.0001 | 66.3±0.4 | 63.3±0.3 | <0.0001 |
| BMI (kg/m$^2$) | 22.2±0.1 | 23.5±0.1 | <0.0001 | 23.2±0.1 | 22.3±0.1 | <0.0001 |
| SBP (mmHg) | 118.3±0.4 | 146.2±0.5 | <0.0001 | 131.8±0.7 | 128.1±0.6 | <0.0001 |
| DBP (mmHg) | 73.9±0.3 | 83.8±0.3 | <0.0001 | 79.7±0.3 | 76.6±0.3 | <0.0001 |
| PR (beats/min) | 65.3±0.2 | 67.0±0.3 | <0.0001 | 66.0±0.3 | 66.0±0.3 | 0.9334 |
| Creatinine (μmol/l) | 60.8±0.5 | 66.4±0.6 | <0.0001 | 73.9±0.5 | 53.9±0.4 | <0.0001 |
| Total cholesterol (mmol/l) | 5.36±0.02 | 5.39±0.03 | 0.3482 | 5.13±0.03 | 5.58±0.02 | <0.0001 |
| HDL cholesterol (mmol/l) | 1.59±0.01 | 1.52±0.01 | <0.0001 | 1.43±0.01 | 1.68±0.01 | <0.0001 |
| Triglycerides (mmol/l) | 1.14±0.03 | 1.32±0.03 | <0.0001 | 1.38±0.03 | 1.07±0.03 | <0.0001 |
| Blood glucose (mmol/l) | 5.34±0.04 | 5.72±0.04 | <0.0001 | 5.74±0.04 | 5.30±0.04 | <0.0001 |
| %CVA | 1.3 | 4.0 | 0.0001 | 3.6 | 1.4 | 0.0018 |
| %OMI | 0.5 | 1.2 | 0.0014 | 2.1 | 0.5 | 0.0015 |
| %HT | — | — | — | 45.9 | 37.2 | <0.0001 |
| %drinking | 44.7 | 49.8 | 0.0292 | 67.0 | 29.5 | <0.0001 |
| %smoking | 19.0 | 14.6 | 0.0117 | 29.9 | 6.3 | <0.0001 |

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; HDL, high-density lipoprotein; %CVA, percentage of subjects with cerebrovascular accident; %OMI, percentage of subjects with old myocardial infarction; %HT, percentage of subjects with hypertension; %drinking, percentage of subjects who have drinking habit; %smoking, percentage of subjects who have smoking habit; NT, normotensive subjects; HTN, subjects with hypertension. *p* was calculated by unpaired *t*-test.

**Table 3. Genotype Distribution of *ADD1*/G460W, *ADRB1*/R389G, and *GNB3*/C825T in NT and HTN**

| Polymorphisms | All subjects | | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|---|---|
| | Genotype frequency (%) | | *p* value | Genotype frequency (%) | | *p* value | Genotype frequency (%) | | *p* value |
| | NT | HTN | ($p_c$) | NT | HTN | ($p_c$) | NT | HTN | ($p_c$) |
| *ADD1*/G460W | GG/GW/WW | GG/GW/WW | | GG/GW/WW | GG/GW/WW | | GG/GW/WW | GG/GW/WW | |
| | 21.8/50.7/27.6 | 20.5/48.6/30.9 | 0.7088 | 20.7/49.2/30.1 | 22.9/50.1/27.0 | 0.2408 | 22.5/51.8/25.7 | 17.9/47.0/35.0 | 0.0238 |
| | | | (1.0000) | | | (1.0000) | | | (0.1428) |
| *ADRB1*/R389G | RR/RG/GG | RR/RG/GG | | RR/RG/GG | RR/RG/GG | | RR/RG/GG | RR/RG/GG | |
| | 63.7/30.8/5.5 | 68.0/28.4/3.7 | 0.0291 | 63.1/31.0/5.9 | 66.3/31.1/2.6 | 0.0398 | 64.0/30.7/5.3 | 69.7/25.4/4.9 | 0.0516 |
| | | | (0.1746) | | | (0.2388) | | | (0.3096) |
| *GNB3*/C825T | CC/CT/TT | CC/CT/TT | | CC/CT/TT | CC/CT/TT | | CC/CT/TT | CC/CT/TT | |
| | 26.0/48.5/25.5 | 22.8/49.7/27.5 | 0.3953 | 24.6/51.0/24.4 | 23.5/48.5/28.0 | 0.4588 | 26.9/46.8/26.3 | 22.1/50.9/27.0 | 0.2897 |
| | | | (1.0000) | | | (1.0000) | | | (1.0000) |

NT, normotensive subjects; HTN, subjects with hypertension; *ADD1*, α-adducin; *ADRB1*, β1-adrenoreceptor; *GNB3*, G-protein β3 subunit; G, glycine; W, tryptophan; R, arginine; C, cysteine; T, threonine; BMI, body mass index. Logistic analysis with age and BMI as covariates was performed. *P*-values were corrected ($p_c$) by multiplying 6 ([3 SNPs]×[2 genders], Bonferroni).

2. The observed genotype and allele frequencies in the Suita population were in accordance with Hardy-Weinberg equilibrium (*ADD1*/G460W, $p=0.9897$; *ADRB1*/R389G, $p=0.2073$; *GNB3*/C825T, $p=0.8307$).

### *ADD1*/G460W

The effects of the three polymorphisms on hypertensive status, blood pressure values, and pulse rate are shown in Tables 3–5. Logistic analysis with age and body mass index (BMI) as covariates indicated that the *ADD1*/G460W polymorphism (WW=1, WG+GG=2) was associated with hypertension only in female subjects ($p=0.0070$, $p_c=0.0420$, Table 4). The WW genotype of the *ADD1*/G460W polymorphism was more frequent in HTN. The odds ratio of the WW genotype for hypertension was 1.53 (95% CI, 1.12–2.08) over the WG+GG genotype.

There were no differences in blood pressure values between NT and HTN (Table 5). In female subjects, the *ADD1*/G460W polymorphism tended to be associated with pulse rate ($p=0.0144$, $p_c=0.0864$). The neglect of subjects receiving anti-hypertensive medication may have obscured

**Table 4. Odds Ratio of the *ADD1*, *ADRB1*, and *GNB3* Genotypes**

| | All subjects | | | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GG+GW | WW | *p* value | ($p_c$) | GG+GW | WW | *p* value | ($p_c$) | GG+GW | WW | *p* value | ($p_c$) |
| *ADD1*/G460W | 1 | 1.1 (0.88–1.36) | 0.4137 | (1.0000) | 1 | 0.77 (0.56–1.05) | 0.0959 | (0.5736) | 1 | 1.53 (1.12–2.08) | 0.0070 | 0.0420 |
| | RR+RG | GG | *p* value | ($p_c$) | RR+RG | GG | *p* value | ($p_c$) | RR+RG | GG | *p* value | ($p_c$) |
| *ADRB1*/R389G | 1 | 0.63 (0.71–1.11) | 0.0651 | (0.3906) | 1 | 0.38 (0.17–0.78) | 0.0117 | (0.0702) | 1 | 1.00 (0.52–1.92) | 0.995 | (1.0000) |
| | CC | CT+TT | *p* value | ($p_c$) | CC | CT+TT | *p* value | ($p_c$) | CC | CT+TT | *p* value | ($p_c$) |
| *GNB3*/C825T | 1 | 0.87 (0.69–1.10) | 0.2368 | (1.0000) | 1 | 0.97 (0.70–1.35) | 0.8599 | (1.0000) | 1 | 0.77 (0.55–1.07) | 0.1247 | 0.7482 |

Odds ratio with its 95% confidential interval is shown. Logistic analysis with age and BMI as covariates was performed. *P* values were corrected ($p_c$) by multiplying 6 ([3 SNPs]×[2 genders], Bonferroni). *ADD1*, α-addcin; *ADRB1*, β1-adrenoceptor; *GNB3*, G-protein β3 subunit; R, arginine; G, glycine; C, cysteine; T, threonine; BMI, body mass index.

the relationship between the polymorphism and blood pressure values.

### *ADRB1*/R389G

Logistic analysis with age and BMI as covariates indicated that the *ADRB1*/R389G polymorphism (GG=1, RR+RG=2) tended to be associated with hypertension only in male subjects ($p=0.0117$, $p_c=0.0702$, Table 4). The odds ratio of the GG genotype for hypertension was 0.38 (95% CI, 0.17–0.78) over the RR+RG genotype. Alcohol consumption is a well-known determinant of blood pressure level, especially in male subjects. The *ADRB1*/R389G polymorphism (GG=1, RR+RG=2) tended to be associated with hypertensive status when also adjusted for alcohol consumption ($p=0.0109$, $p_c=0.0654$, odds ratio=0.37 [95% CI, 0.16–0.48]). There were no differences in blood pressure values between NT and HTN (Table 5). Again, the neglect of subjects receiving anti-hypertensive medication may have obscured the relationship between the polymorphism and blood pressure values.

### *GNB3*/C825T

We did not find any association between the *GNB3*/C825T polymorphism and hypertensive status, systolic blood pressure, or diastolic blood pressure in all subjects, male subjects, or female subjects (Tables 3–5).

## Discussion

In the present study, we investigated the associations between hypertension and three polymorphisms, *ADD1*/G460W, *ADRB1*/R389G, and *GNB3*/C825T, in a population-based sample (the Suita Study) consisting of 1,880 subjects. Our results indicate that the WW genotype of the *ADD1* gene may be involved in hypertension in female subjects.

The *ADD1* gene in humans is highly homologous to that in rats. Known point mutations, one each in the α- and β-adducin subunits, account for up to 50% of the difference in blood pressure between the Milan hypertensive and normotensive rat strains (*21*). Based on initial case-control and linkage analyses, the *ADD1*/G460W polymorphism was implicated in the genetic component of hypertension in Italian and French populations (*3*). In addition, a group of Italian hypertensive subjects with the W allele had lower plasma renin levels and showed a significantly greater fall in blood pressure with sodium restriction or diuretic treatment (*3*). On the other hand, this association was not confirmed by two different studies in Scottish populations (*6*, *8*). In the Japanese population, while Tamaki *et al.* reported that the *ADD1*/G460W polymorphism was involved in hypertension (*5*), Kato *et al.* did not support this association (*7*). Sugimoto *et al.* demonstrated that this polymorphism is associated with low renin hypertension in younger subjects (*22*). The present study indicated that the *ADD1*/G460W polymorphism was influential in female subjects, but the corrected *p* value ($p_c=0.045$) was marginal. Accordingly, additional independent replications in the Japanese population are required to confirm the present association.

In the present study, the *ADD1*/G460W polymorphism was associated with hypertension only in female subjects, but not in male subjects. It remains to be clarified why these polymorphisms do not equally contribute to hypertension in both sexes. Recently, low renin hypertension has been reported to be a significant predictor of systolic sodium sensitivity in females only (*23*). Izawa *et al.* also demonstrated a gender difference in genetic polymorphisms and hypertension (*15*). Accordingly, these results may suggest that sex-based differences should be considered in the association between genetic polymorphisms and hypertension.

Two common polymorphisms, S49G and R389G, were identified in the *ADRB1* gene (*24*). The R389G polymor-

**Table 5. Blood Pressure Values and Pulse Rates among Genotypes of *ADD1*/G460W, *ADRB1*/R389G, and *GNB3*/C825T Polymorphism**

| Variables | *ADD1*/G460W | | | *p* value | ($p_c$) | *ADRB1*/R389G | | | *p* value | ($p_c$) | *GNB3*/C825T | | | *p* value | ($p_c$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GG | GW | WW | | | RR | RG | GG | | | CC | CT | TT | | |
| All subjects | | | | | | | | | | | | | | | |
| *N* | 293 | 663 | 393 | | | 864 | 400 | 69 | | | 347 | 647 | 351 | | |
| PR (beats/min) | 65.7±0.4 | 65.7±0.3 | 64.9±0.4 | 0.1578 | (0.9468) | 65.3±0.3 | 65.7±0.4 | 65.1±0.9 | 0.5977 | (1.0000) | 65.4±0.4 | 65.6±0.3 | 65.2±0.4 | 0.6827 | (1.0000) |
| SBP (mmHg) | 124.8±1.1 | 124.6±0.7 | 126.7±0.9 | 0.1735 | (1.0000) | 125.6±0.6 | 125.4±0.9 | 122.3±2.2 | 0.3774 | (1.0000) | 124.3±1.0 | 125.5±0.7 | 125.6±1.0 | 0.5610 | (1.0000) |
| DBP (mmHg) | 76.2±0.6 | 76.8±0.4 | 76.8±0.5 | 0.6273 | (1.0000) | 76.8±0.3 | 76.9±0.5 | 75.2±1.2 | 0.4277 | (1.0000) | 76.0±0.5 | 77.1±0.4 | 76.7±0.5 | 0.2963 | (1.0000) |
| Res. SBP (mmHg) | −0.5±1.0 | −0.3±0.6 | 0.7±0.8 | 0.5433 | (1.0000) | 0.4±0.6 | −0.2±0.8 | −3.2±2.0 | 0.2209 | (1.0000) | −0.9±0.9 | 0.1±0.7 | 0.4±0.9 | 0.5743 | (1.0000) |
| Res. DBP (mmHg) | −0.5±0.6 | 0.2±0.4 | −0.1±0.5 | 0.6109 | (1.0000) | 0.1±0.3 | 0.0±0.5 | −1.5±0.2 | 0.4350 | (1.0000) | −0.7±0.5 | 0.3±0.4 | 0.0±0.5 | 0.3561 | (1.0000) |
| Male | | | | | | | | | | | | | | | |
| *N* | 126 | 282 | 176 | | | 366 | 185 | 27 | | | 144 | 284 | 156 | | |
| PR (beats/min) | 65.0±0.7 | 65.5±0.5 | 65.5±0.6 | 0.8213 | (1.0000) | 65.5±0.4 | 65.1±0.6 | 64.1±1.5 | 0.6239 | (1.0000) | 65.1±0.6 | 65.7±0.5 | 64.9±0.6 | 0.4875 | (1.0000) |
| SBP (mmHg) | 129.0±1.6 | 126.2±1.1 | 128.0±1.6 | 0.3077 | (1.0000) | 127.3±1.0 | 128.6±1.4 | 121.1±3.5 | 0.1407 | (0.8442) | 125.9±1.5 | 126.9±1.0 | 129.2±1.5 | 0.2610 | (1.0000) |
| DBP (mmHg) | 79.1±0.9 | 78.2±0.6 | 78.5±0.8 | 0.7363 | (1.0000) | 78.6±0.6 | 78.8±0.8 | 75.3±2.0 | 0.2699 | (1.0000) | 78.4±0.9 | 78.4±0.9 | 78.4±0.8 | 0.9992 | (1.0000) |
| Res. SBP (mmHg) | 1.5±1.5 | −0.5±1.0 | −0.3±1.3 | 0.5435 | (1.0000) | −0.1±0.9 | 1.5±1.3 | −7.7±3.3 | 0.0326 | (0.1956) | −1.5±1.4 | −0.5±1.0 | 2.2±1.4 | 0.1469 | (1.0000) |
| Res. DBP (mmHg) | 0.9±0.9 | −0.1±0.6 | −0.4±0.8 | 0.5304 | (1.0000) | 0.0±0.5 | 0.6±0.7 | −2.7±1.9 | 0.2712 | (1.0000) | −0.1±0.8 | 0.0±0.6 | 0.0±0.8 | 0.9907 | (1.0000) |
| Female | | | | | | | | | | | | | | | |
| *N* | 167 | 381 | 217 | | | 498 | 215 | 42 | | | 203 | 363 | 195 | | |
| PR (beats/min) | 66.3±0.6 | 65.9±0.4 | 64.4±0.5 | 0.0144 | (0.0864) | 65.2±0.3 | 66.2±0.5 | 65.8±1.1 | 0.1950 | (1.0000) | 65.6±0.4 | 65.6±0.4 | 65.5±0.5 | 0.9869 | (1.0000) |
| SBP (mmHg) | 121.7±1.4 | 123.3±0.9 | 125.6±1.2 | 0.1000 | (0.6000) | 124.3±0.8 | 122.7±1.3 | 123.1±2.8 | 0.5399 | (1.0000) | 123.2±1.3 | 124.4±1.0 | 122.7±1.3 | 0.5142 | (1.0000) |
| DBP (mmHg) | 74.0±0.7 | 75.9±0.5 | 75.5±0.7 | 0.1213 | (0.7278) | 75.5±0.4 | 75.2±0.7 | 75.1±1.5 | 0.9136 | (1.0000) | 74.3±0.7 | 76.0±0.5 | 75.3±0.7 | 0.1427 | (1.0000) |
| Res. SBP (mmHg) | −1.8±1.3 | −0.2±0.8 | 1.4±1.1 | 0.1508 | (0.9048) | 0.7±0.7 | −1.6±1.1 | 0.0±2.5 | 0.2495 | (1.0000) | −0.4±1.1 | 0.6±0.9 | −1.1±1.2 | 0.4864 | (1.0000) |
| Res. DBP (mmHg) | −1.4±0.7 | 0.4±0.5 | 0.1±0.6 | 0.1051 | (0.6306) | 0.2±0.4 | −0.5±0.6 | −0.2±1.5 | 0.7206 | (1.0000) | −1.0±0.7 | 0.5±0.5 | −0.0±0.7 | 0.1882 | (1.0000) |

PR, pulse rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; Res. SBP and Res. DBP, residuals of SBP and DBP for age and body mass index, respectively. We excluded subjects who were receiving anti-hypertensive or cardiovascular medication, subjects who had had a cerebrovascular accident, subjects with demonstrated ischemic heart diseases, and subjects with atrial fibrillation. *P* was calculated by ANOVA. *P* values were corrected ($p_c$) by multiplying 6 [(3 SNPs)×(2 genders), Bonferroni].

phism is located in the intracellular cytoplasmic tail near the seventh transmembrane region of the receptor, which is the putative Gs-protein binding domain. The R389 variant mediates a higher level of isoproterenol-stimulated adenylate cyclase activity than the G389 variant *in vitro* (*25*). In accordance with this *in vitro* study, Bengtsson *et al.* reported that homozygotes for the *ADRB1*/R389 allele had an increased risk of developing hypertension in a case-control study (*9*). Their genotype-discordant sibling pair analysis demonstrated that siblings who were homozygotes for the R389 allele had significantly higher diastolic pressures and higher heart rates than siblings carrying one or two copies of the G389 allele. However, McCaffery *et al.* reported that subjects carrying any *ADRB1*/G389 allele, but not the *ADRB1*/R389 allele, exhibited elevated systolic and diastolic blood pressure (*10*). The present study, which employed a dominant model, indicated that the GG genotype of the *ADRB1* gene tended to be associated with hypertension in male subjects. However, the frequency of the GG genotype was relatively low, and the sample power was weak (sample power: 0.69, $\alpha$=0.05, two-tailed). A much larger number of subjects may be required to confirm the present association.

The *GNB3*/C825T polymorphism is associated with the occurrence of a splicing variant, *GNB3*-s (encoding G$\beta$3-s), in which nucleotides 498–620 of exon 9 are deleted. This in-frame deletion causes the loss of 41 amino acids and one WD repeat domain of the G$\beta$ subunit (*11*). A significantly higher frequency of the *GNB3*/T825 allele has been reported in subjects with essential hypertension using unselected normotensive control subjects of European origin in three independent studies (*11–13*), but not in a fourth study (*14*). In blacks, the *GNB3*/T825 allele was reported to be a susceptibility factor for the development of hypertension (*17*). In a Japanese population, Izawa *et al.* demonstrated an association between hypertension and the *GNB3*/C825T polymorphism in male subjects (*15*). However, Ishikawa *et al.* reported that the *GNB3*/C825T polymorphism was associated with serum potassium and total cholesterol levels, but not with blood pressure (*16*). The present study revealed that the *GNB3*/T825 allele is not associated with either hypertensive status (*15*, *16*) or the total cholesterol level (all subjects, $p$= 0.9381), contrary to previous reports (*15*, *16*).

As stated above, the present results do not agree with those in previous reports. Almost all common variants may have only a modest effect on common diseases, and a single study may lack the statistical power to detect a real association (*26*, *27*). Recently, it was reported that a meta-analysis of genetic association studies may support the notion that common variants may contribute to a susceptibility to a multifactorial common disease (*27*). It is recommended that a single, nominally significant association should be viewed as tentative until it has been independently replicated at least once, and preferably twice (*27*). Accordingly, it will be necessary to verify the association between these three genes and hypertension using a larger number of subjects from the Suita cohort or another population.

In conclusion, the present tentative results suggest that the WW genotype of the *ADD1* gene may be involved in hypertension in female subjects. The GG genotype of the *ADRB1* gene may play a protective role against hypertension in male subjects.

## Acknowledgements

We would like to express our highest gratitude to Dr. Soichiro Kitamura, President of the National Cardiovascular Center, for his support of our research. We would also like to thank the following people for their continuous support of our population survey in Suita City: Dr. Otosaburo Hishikawa, Dr. Katsuyuki Kawanishi, and Mr. Shigeru Kobayashi. We also thank the members of the Satsuki-Junyukai.

## References

1. SNP attack on complex traits. *Nat Genet* 1998; **20**: 217–218 (Editorial comment).
2. Harrap SB: Private genes, public health. *Lancet* 1997; **349**: 1338–1339.
3. Cusi D, Barlassina C, Azzani T, *et al*: Polymorphisms of $\alpha$-adducin and salt sensitivity in patients with essential hypertension. *Lancet* 1997; **349**: 1353–1357.
4. Melander O, Bengtsson K, Orho-Melander M, *et al*: Role of the Gly460Trp polymorphism of the $\alpha$-adducin gene in primary hypertension in Scandinavians. *J Hum Hypertens* 2000; **14**: 43–46.
5. Tamaki S, Iwai N, Tsujita Y, Nakamura Y, Kinoshita M: Polymorphism of $\alpha$-adducin in Japanese patients with essential hypertension. *Hypertens Res* 1998; **21**: 29–32.
6. Clark CJ, Davies E, Anderson NH, *et al*: $\alpha$-Adducin and angiotensin I-converting enzyme polymorphisms in essential hypertension. *Hypertension* 2000; **36**: 990–994.
7. Kato N, Sugiyama T, Nabika T, *et al*: Lack of association between the $\alpha$-adducin locus and essential hypertension in the Japanese population. *Hypertension* 1998; **31**: 730–733.
8. Kamitani A, Wong ZYH, Fraser R, *et al*: Human $\alpha$-adducin gene, blood pressure, and sodium metabolism. *Hypertension* 1998; **32**: 138–143.
9. Bengtsson K, Melander O, Orho-Melander M, *et al*: Polymorphism in the $\beta$1-adrenergic receptor gene and hypertension. *Circulation* 2001; **104**: 187–190.
10. McCaffery JM, Pogue-Geile MF, Ferrell RE, Petro N, Manuck SB: Variability within $\alpha$- and $\beta$-adrenoreceptor genes as a predictor of cardiovascular function at rest and in response to mental challenge. *J Hypertens* 2002; **20**: 1105–1114.
11. Siffert W, Rosskopf D, Siffert G, *et al*: Association of a human G-protein $\beta$3 subunit variant with hypertension. *Nat Genet* 1998; **18**: 45–48.
12. Benjafield AV, Jeyasingam CL, Nyholt DR, Griffiths LR, Morris BJ: G-protein $\beta$3 subunit gene (*GNB3*) variant in causation of essential hypertension. *Hypertension* 1998; **32**: 1094–1097.
13. Beige J, Hohenbleicher H, Distler A, Sharma AM: G-Protein $\beta$3 subunit C825T variant and ambulatory blood pres-

sure in essential hypertension. *Hypertension* 1999; **33**: 1049–1051.
14. Brand E, Herrmann SM, Nicaud V, *et al*: The 825C/T polymorphism of the G-protein subunit $\beta 3$ is not related to hypertension. *Hypertension* 1999; **33**: 1175–1178.
15. Izawa H, Yamada Y, Okada T, Tanaka M, Hirayama H, Yokota M: Prediction of genetic risk for hypertension. *Hypertension* 2003; **41**: 1035–1040.
16. Ishikawa K, Imai Y, Katsuya T, *et al*: Human G-protein $\beta 3$ subunit variant is associated with serum potassium and total cholesterol levels but not with blood pressure. *Am J Hypertens* 2000; **13**: 140–145.
17. Dong Y, Zhu H, Sagnella GA, Carter ND, Cook DG, Cappuccio FP: Association between the C825T polymorphism of the G protein $\beta 3$-subunit gene and hypertension in blacks. *Hypertension* 1999; **34**: 1193–1196.
18. Mannami T, Konishi M, Baba S, Nishi N, Terao A: Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita study. *Stroke* 1997; **28**: 518–525.
19. Iwai N, Baba S, Mannami T, *et al*: Association of sodium channel $\gamma$-subunit promoter variant with blood pressure. *Hypertension* 2001; **38**: 86–89.
20. Ono K, Mannami T, Baba S, Yasui N, Ogihara T, Iwai N: Lack of association between angiotensin II type 1 receptor gene polymorphism and hypertension in Japanese. *Hypertens Res* 2003; **26**: 131–134.
21. Bianchi G, Tripodi G, Casari G, *et al*: Two point mutations within the adducin genes are involved in blood pressure variation. *Proc Natl Acad Sci USA* 1994; **91**: 3999–4003.
22. Sugimoto K, Hozawa A, Katsuya T, *et al*: $\alpha$-Adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the Ohasama study. *J Hypertens* 2002; **20**: 1779–1784.
23. Hurwitz S, Fisher ND, Ferri C, Hopkins PN, Williams GH, Hollenberg NK: Controlled analysis of blood pressure sensitivity to sodium intake: interaction with hypertension type. *J Hypertens* 2003; **21**: 951–959.
24. Maqbool A, Hall AS, Ball SG, Balmforth AJ: Common polymorphisms of $\beta 1$-adrenoceptor: identification and rapid screening assay. *Lancet* 1999; **353**: 897.
25. Mason DA, Moore JD, Green SA, Liggett SB: A gain-of-function polymorphism in a G-protein coupling domain of the human $\beta 1$-adrenergic receptor. *J Biol Chem* 1999; **274**: 12670–12674.
26. Ioannidis JPA, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG: Replication validity of genetic association studies. *Nat Genet* 2001; **29**: 306–309.
27. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003; **33**: 177–182.

J Hum Genet (2004) 49:141–147
DOI 10.1007/s10038-004-0124-9

ORIGINAL ARTICLE

**Keisuke Shioji · Junko Nishioka · Hiroaki Naraba
Yoshihiro Kokubo · Toshifumi Mannami
Nozomu Inamoto · Kei Kamide · Shin Takiuchi
Masayoshi Yoshii · Yoshikazu Miwa · Yuhei Kawano
Toshiyuki Miyata · Shunichi Miyazaki · Yoichi Goto
Hiroshi Nonogi · Naomi Tago · Naoharu Iwai**

# A promoter variant of the ATP-binding cassette transporter A1 gene alters the HDL cholesterol level in the general Japanese population

Received: 21 November 2003 / Accepted: 24 December 2003 / Published online: 21 February 2004


**Abstract** To investigate the effects of polymorphisms in the ATP-binding cassette transporter A1 (*ABCA1*) gene on the high-density lipoprotein cholesterol (HDL-C) level and the incidence of myocardial infarction (MI), we performed association studies. Sequence analysis identified 14 polymorphisms in the promoter region of *ABCA1*. After considering linkage disequilibrium, three polymorphisms in the promoter region and 11 polymorphisms from the JSNP database were determined in 1,880 subjects recruited from the Suita Study, representing the general population in Japan. We evaluated the association between the *ABCA1* genotype and HDL-C level adjusted not only for standard factors, but also for genetic factors including *ApoA1* and *ApoE* genotypes. Of the 14 polymorphisms tested, the *G(−273)C* ($P=0.0074$), *C(−297)T* ($P=0.0195$), and *IMS-JST071749* ($P=0.0093$) polymorphisms were significantly associated with the HDL-C level in the Suita population. We could reconfirm that the *G(−273)C* genotype was influential in another set of subjects ($P=0.0310$, $n=743$). However, the distribution of the *ABCA1 G(−273)C* genotype in subjects with MI ($n=598$) was not different from that in the control population ($n=801$). These results indicate that *ABCA1 G(−273)C* has a significant effect on the HDL-C level in the general Japanese population, but not on the incidence of MI.

**Keywords** ABCA1 · Polymorphism · Association study · HDL cholesterol · Myocardial infarction

K. Shioji · J. Nishioka · H. Naraba · N. Tago · N. Iwai (✉)
Department of Epidemiology, Research Institute,
National Cardiovascular Center,
5-7-1 Fujishirodai, Suita,
Osaka 565-8565, Japan
E-mail: niwai@res.ncvc.go.jp
Tel.: +81-6-68335012
Fax: +81-6-68352088

Y. Kokubo · T. Mannami · N. Inamoto · T. Miyata
Department of Preventive Cardiology,
National Cardiovascular Center,
5-7-1 Fujishirodai, Suita,
Osaka 565-8565, Japan

K. Kamide · S. Takiuchi · M. Yoshii · Y. Miwa · Y. Kawano
Division of Hypertension and Nephrology,
National Cardiovascular Center,
5-7-1 Fujishirodai, Suita,
Osaka 565-8565, Japan

S. Miyazaki · Y. Goto · H. Nonogi
Division of Cardiology,
National Cardiovascular Center, 5-7-1 Fujishirodai,
Suita, Osaka 565-8565, Japan

## Introduction

The high-density lipoprotein cholesterol (HDL-C) level is inversely correlated with the development of atherosclerosis and is inversely related to the incidence of coronary artery disease (Castelli et al. 1986) and ischemic stroke in the elderly (Sacco et al. 2001). The HDL-C level has been shown to be affected by both genetic and environmental factors, including obesity, smoking, and alcohol consumption. Among genetic factors, the apolipoprotein A1 (*ApoA1*) (Groenendijk et al. 2001a,b) and *ApoE* genotypes (Lefevre et al. 1997; Katsuya et al. 2002) are well known to influence the HDL-C level.

Genetic mutations in the ATP-binding cassette transporter A1 (*ABCA1*) gene have been shown to cause Tangier disease (TD) (Bodzioch et al. 1999; Brooks-Wilson et al. 1999; Rust et al. 1999) and familial HDL deficiency (Marcil et al. 1999). ABCA1 regulates cellular cholesterol efflux and facilitates lipid binding to ApoA1 (Wang and Tall 2003). Patients with TD show characteristic HDL deficiency, defective apolipoprotein-mediated phospholipid and cholesterol efflux from cells, and the accumulation of macrophage foam cells in various tissues, including arteries (Clifton-Bligh et al. 1972). Recent epidemiological studies have reported that *ABCA1* polymorphisms were associated with the HDL-C level

| | | | | | |
|---|---|---|---|---|---|
| -1600 | AAGGGGCCATGCCACCCAGA | GTTATGAGTACCTGGGACTC | CAGAATTCCTTGCCTGGTGG | CCTCCACATGCACTTCCAGG | GCCTGCTTGGGCCTCTTCTA |
| -1500 | TGGGTCTGTCCTGAGTGTTG G(-1498)C | ATAGAACCACTGATGTGAGT | ACCTGGGCTTGAGCCGTGGC | CTGGAGATCCTGTTGACTGT T(-1423)C | AGCATGGAGGGGGCTTGTGC |
| -1400 | AGCTGAATGTCTGTATGCAG T(-1387)C | GTGGTGGGAGTTCTGGAATA | TGATGGAGCTGGAGGTGGGA | AGAGAAGTAGGCTTGGGGCA | GCTCTCTCATGCCACCTCAT |
| -1300 | TCTGGCCAAAACTCAGGTCA | AACTGTGAAGAGTCTAAATG | TGAATCTGCCCTTCAAGGTG | GCTACAAAGGTATCTTTGTC | AAGGTAGGAGACCTTGTGGC |
| -1200 | CTCCACGTGCACTTCCAGGG | CCTGCTTGGGCCTCTTCTAC | GGGTCTGTCCTGAGTCTTCT | ATGAATCTGCCCTTCAGGGC | AGATTCATATTTAGACTCTT |
| -1100 | CACAGTTTGACCTGAGTTTT | GGCCAGAATAAGGTGACATT | TAGTTTGTTGGCTTGATGGA | TGACTTAAATATTTAGACAT | ATGGTGTGTAGGCCTGCATT AT(-1019)(-) |
| -1000 | CCTACTCTTGCCTTTTTTTT (-980)T(10)/T(9)/T(8) | TGCCCCTCCAGTGTTTTGGG | TAGTTTTGCTCCCCTACAGC | CAAAGGCAAACAGATAAGTT G(-926)T | GGAGGTCTGGAGTGGCTACA |
| -900 | TAATTTTACACGACTGCAAT | TCTCTGGCTGCACTTCACAA | ATGTATACAAACTAAATACA | AGTCCTGTGTTTTTATCACA | GGGAGGCTGATCAATATAAT |
| -800 | GAAATTAAAAGGGGGCTGGT G(-790)A | CCATATTGTTCTGTGTTTTT | GTTTGTTT_GTTTCTTTTTTT GTTTTGTT(-752)(-) (I/D #1) | GTTTTTGTGGCCTCCTTCCT | CTCAATTTATGAAGAGAAGC |
| -700 | AGTAAGATGTTCCTCTCGGG | TCCTCTGAGGGACCTGGGGA | GCTCAGGCTGGGAATCTCCA | AGGCAGTAGGTCGCCTATCA | AAAATCAAAGTCCAGGTTTG |
| -600 | TGGGGGGAAAACAAAAGCAG | CCCATTACCCAGAGGACTGT | CCGCCTTCCCCTCACCCCAG C(-559)T | CCTAGGCCTTTGAAAGGAAA | CAAAAGACAAGACAAAATGA |
| -500 | TTGGCGTCCTGAGGGAGATT | CAGCCTAGAGCTCTCTCTCC | CCCAATCCCTCCCTCCGGCT | GAGGAAACTAACAAAGGAAA | AAAAAATTGCGGAAAGCAGG G(-402)C |
| -400 | ATTTAGAGGAAGCAAATTCC | ACTGGTGCCCTTGGCTGCCG | GGAACGTGGACTAGAGAGTC | TGCGGCGCAGCCCCGAGCCC | AGCGCTTCCCGCGCGTCTTA |
| -300 | GGCCGGCGGGCCCGGGCGGG C(-297)T | GGAAGGGGACGCAGACCGCG G(-273)C | GACCCTAAGACACCTGCTGT | ACCCTCCACCCCCA_CCCCAC TGGGG(-226)(-) (I/D #2) | CCACCTCCCCCCAACTCCCT |
| -200 | AGATGTGTCGTGGGCGGCTG | AACGTCGCCCGTTTAAGGGG | CGGGCCCCGGCTCCACGTGC | TTTCTGCTGAGTGACTGAAC | TACATAAACAGAGGCCGGGA |
| -100 | ACGGGGCGGGGAGGAGGGAG G(-99)C | AGCACAGGCTTTGACCGATA | GTAACCTCTGCGCTCGGTGC | AGCCGAATCTATAAAAGGAA | CTAGTCCCGGCAAAAACCCC C(-14)T |
| 1 | *GTAATTGCGAGCGAGAGTGA* | *GTGGGGCCGGGACCCGCAGA* | *GCCGAGCCGAC C CTTCTCTC* C52A | *CCGGGCTGCGGCAGGGCAGG* | *GCGGGGAGCTCCGCGCACCA* |
| 101 | *ACAGAGCCGGTTCTCAGGGC* | *GCTTTGCTCCTTGTTTTTTC* | *CCCGGTTCTGTTTTCTCCCC* | *TTCTCCGGAAGGCTTGTCAA* | *GGGGTAGGAGAAAGAGACGC* |
| 201 | *AAACACAAAAGTGGAAAACA* | *G*GTAAGAGGCTCTCCAGTGA | CTTACTTGGGCGTTATTGTT | TTGTTTCGAGGCCAAGGAGG | CTTCGGGAAGTGCTCGGTTT |
| 301 | CGGGGACTTTGATCCGGAGC T313C | CCCACATCCCCACCACTTGC | AACTCAGATGGGACCGGAGG | CGGTGTTAAATGGGGAGACG G380T | ATGTCCTAGTACGAGCTCTG |
| 401 | GTGACCCCAGGACTCTGCGC | TGCTGCGCTTGGGGCTTGCC | CGACGGTGGAGACCGGGGAG | CATCTCTGGGCGTGGAGACC | CGGGCGCAGTACCCCGGGCT |

**Fig. 1** Nucleotide sequence of the 5'-flanking region and exon 1 of *ABCA1*. The nucleotide sequence in *italics* indicates exon 1

(Wang et al. 2000; Clee et al. 2001; Lutucuta et al. 2001; Harada et al. 2003). However, few of these findings have been replicated, and there are inconsistencies among previous association studies. Accordingly, the associations between *ABCA1* variants and HDL-C are still controversial (Singaraja et al. 2003). One possible reason for these differences may be that the sample sizes in these studies were relatively small and lacked statistical power. Thus, to evaluate the effect of polymorphisms in *ABCA1* on the HDL-C level, we conducted an association study using a large cohort (the Suita population, $n = 1{,}880$), representing the general population in Japan.

## Materials 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. 2004). The genotypes were determined in 1,880 consecutive subjects who visited the National Cardiovascular Center between April 2002 and February 2003 (867 male subjects, 1,013 female subjects).

*The hypertension group* The hypertension (HTN) group consisted of 743 Japanese subjects (422 men and 321 women), aged 18–91 years [65.2 ± 0.4 (mean ± SEM)], who were enrolled in the Division of Hypertension and Nephrology at the National Cardiovascular Center between May 2001 and April 2003.

*The myocardial infarction group* The selection criteria and design of the myocardial infarction (MI) group have been described previously (Takagi et al. 2002). This group consisted of 706 patients with MI (598 men and 108 women, aged 61.3 ± 0.4 years) who were enrolled in the Division of Cardiology at National Cardiovascular Center between May 2001 and April 2003. In the present study, we investigated only males ($n = 598$).

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.

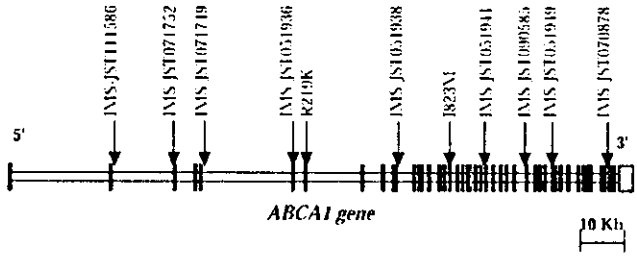


**Fig. 2** Schema of *ABCA1* and the position of the determined polymorphisms. *Grayboxes* indicate exonic regions

### DNA studies

The regions of the promoter and exon 1 in *ABCA1* were sequenced for polymorphisms in 24 subjects (Fig. 1). The primer sequences are available on request. For exonic regions (Fig. 2), we selected nine SNPs for genotyping from the public database (JSNP, http://snp.ims.u-tokyo.ac.jp) (Iida et al. 2001; Hirakawa et al. 2002). Well-known common variants, *ABCA1* R219K and I823M, were also selected (Wang et al. 2000; Clee et al. 2001; Harada et al. 2003).

The preliminary study revealed that *JST-IMS005607* had the greatest effect on the HDL-C level among seven SNPs on the *ApoA1* region, including the promoter region (up to −3Kb).

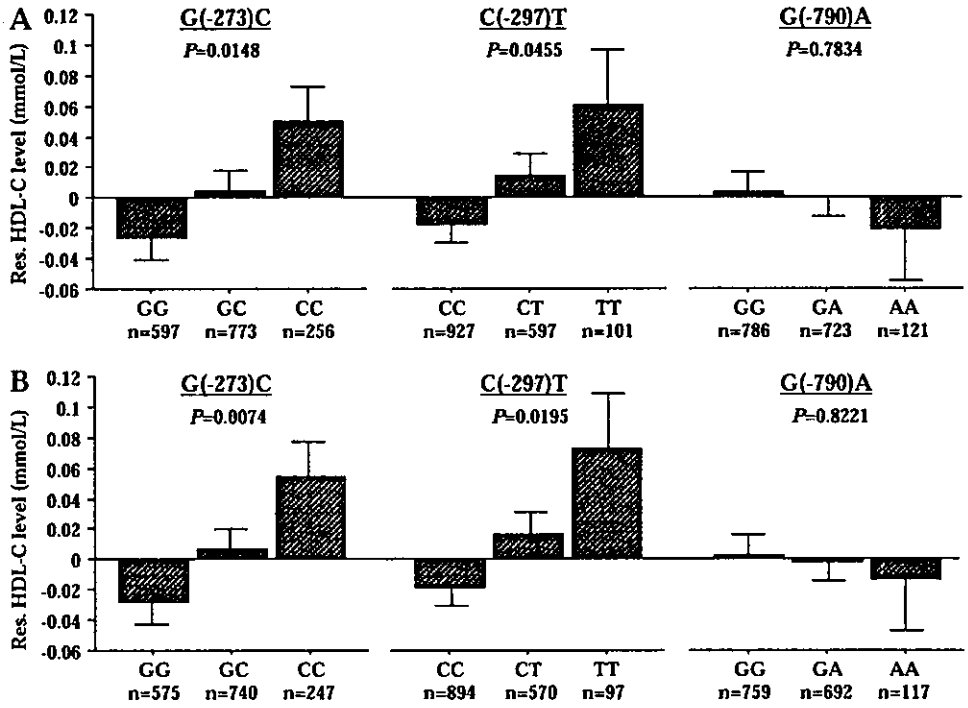


**Fig. 3A, B** Residual HDL cholesterol levels among the *ABCA1 G(−273)C*, *C(−297)T*, and *G(−790)A* genotypes. **A** Residual HDL cholesterol levels adjusted for sex, age, body-mass index, smoking, and alcohol consumption. **B** Residual HDL cholesterol levels adjusted for sex, age, body-mass index, smoking, alcohol consumption, *ApoE* genotype, and *ApoA1* genotype (*JST-IMS005603*)

Thus, we selected *JST-IMS005607* for adjusting HDL-C. The genotyping of *ApoE* was performed according to a previous report (Katsuya et al. 2002). *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). All polymorphisms were determined by the TaqMan System.

### Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM). For triglyceride values, a logarithmic transformation was applied for the statistical test, but untransformed values are shown in the Tables 1 and 2. All statistical analyses were performed with the JMP statistical package (SAS Institute). 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 HDL-C level was calculated by adjusting for sex, age, and body-mass index (BMI), smoking (cigarettes/day) and consumption of alcohol (ethanol, ml/week). For analyses of the effects of the *ABCA1* genotype (in the Suita population), the residual HDL-C level was calculated by adjusting not only for the above five factors, but also for the *ApoA1*(*JST-IMS005603*), and the *ApoE* (*E2*, *E3*, and *E4*) genotypes. Differences in numerical data among the groups were evaluated by one-way analysis of variance (ANOVA). Hardy-Weinberg equilibrium was calculated by a chi-square test (Table 3). To measure linkage disequilibrium (LD) between SNPs, $D'$ and $r^2$ values were analyzed using the SNPAlyze statistical package (Dynacom).

## Results

### Polymorphisms of the 5′-flanking region and exon 1 of the *ABCA1* gene

We found 14 polymorphisms in the promoter region, 1 polymorphism in exon 1 (5′-untranslated region), and 2 polymorphisms in intron 1 (Fig. 1).

LD was evaluated by calculating $r^2$ values (Table 1). We regarded $r^2>0.5$ as tight linkage. The minor allele frequency of the *T(−1423)C* and *G52A* polymorphisms was low (4% each), and these SNPs were neglected in further analyses. The frequencies of *T*(10), *T*(9), and *T*(8) were 4, 92, and 4%, respectively, in the (−980)*T*(10)/*T*(9)/*T*(8) polymorphism, and this polymorphism was also neglected because this is not suitable for TaqMan genotyping. Accordingly, we selected three polymorphisms, *G(−790)A*, *C(−297)T*, and *G(−273)C*, for the following association study.

### Association study of *ApoA1* and *ApoE*

To observe the effect of *ABCA1*polymorphisms on the HDL-C level more clearly, the HDL-C level should be adjusted by various well-known influential factors.

The *ApoA1 IMS-JST005603* polymorphism was associated with the levels of HDL-C and triglyceride [HDL-C: TT 1.54 ± 0.001 mmol/l, TC 1.59 ± 0.02, CC 1.68 ± 0.04, $P=0.0002$ (residual); triglyceride: TT 1.26 ± 0.03 mmol/l, TC 1.15 ± 0.04, CC 0.95 ± 0.09, $P<0.0001$ (residual)]. *IMS-JST005603* corresponds to the *Hae*III (*C317T*) polymorphism described in a previous paper (Groenendijk et al. 2001b).

The *ApoE* polymorphism was also strongly associated with the levels of total cholesterol and HDL-C [total cholesterol: *E2* 5.13 ± 0.06 mmol/l, *E3* 5.37 ± 0.02, *E4* 5.41 ± 0.05, $P=0.0002$ (residual); HDL-C: E2 1s.67 ± 0.03 mmol/l, *E3* 1.56 ± 0.01, *E4* 1.52 ± 0.02, $P<0.0001$ (residual)].

Accordingly, we evaluated the effect of the *ABCA1* polymorphisms on the HDL-C level adjusted for the

**Table 1** Linkage disequilibrium between SNPs in the 5′-flanking region and exon 1 of the *ABCA1* gene. *I/D#1 GTTTTGTTTT(−752)*

| Genotype | *G(−1498)C* | *T(−1423)C* | *T(−1387)C* | *AT(−1019)(−)* | *G(−926)T* | *G(−790)A* | I/D#1 | *C(−559)T* |
|---|---|---|---|---|---|---|---|---|
| *G(−1498)C* | | 0.01976 | 0.41818*** | **1*** ** | 0.41818*** | 0.00047 | **0.67347*** ** | 0.22034** |
| *T(−1423)C* | | | 0.04726 | 0.01976 | 0.04726 | 0.01003 | 0 | 0.06087 |
| *T(−1387)C* | | | | 0.41818*** | **1*** ** | 0.00111 | **0.67347*** ** | **0.65714*** ** |
| *AT(−1019)(−)* | | | | | 0.41818*** | 0.00047 | **0.67347*** ** | 0.22034** |
| *G(−926)T* | | | | | | 0.00111 | **0.67347*** ** | **0.65714*** ** |
| *G(−790)A* | | | | | | | 0.14667* | 0.16483** |
| I/D#1 | | | | | | | | **1*** ** |
| *C(−559)T* | | | | | | | | |
| *G(−402)C* | | | | | | | | |
| *C(−297)T* | | | | | | | | |
| *G(−273)C* | | | | | | | | |
| I/D#2 | | | | | | | | |
| *G(−99)C* | | | | | | | | |
| *C(−14)T* | | | | | | | | |
| *C52A* | | | | | | | | |
| *T313C* | | | | | | | | |
| *G380T* | | | | | | | | |

$R^2$ values are shown in the *upper right*, and *bolded values* indicate $r^2 > 0.5$. Absolute *D′*-values are shown in the *lower left*, and *bolded*
Significance levels: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

*ApoA1 IMS-JST005603* and *ApoE*polymorphisms in addition to standard factors, including sex, age, BMI, smoking, and consumption of alcohol.

Association study of *ABCA1* (Suita population)

The association between the *G(−273)C* polymorphism and the lipid level in the Suita population is presented in Table 2. The genotype frequency of the *G(−273)C* polymorphism in the Suita population was not deviated from the Hardy-Weinberg equilibrium. The HDL-C level adjusted for age, sex, BMI, smoking, and consumption of alcohol was significantly associated with the *G(−273)C* polymorphism ($P = 0.0148$). The *G(−273)C* polymorphism was even more tightly associated with the HDL-C level when adjusted for the *ApoE* and *ApoA1(IMS-JST005603)* genotypes in addition to the standard factors ($P = 0.0074$). The *C(−297)T* polymorphism was also associated with the HDL-C level ($P = 0.0455$ adjusted for age, sex, BMI, smoking, and consumption of alcohol; $P = 0.0195$ when also adjusted for the *ApoE* and *ApoA1* genotypes). The effect of the *C(−297)T* polymorphism on the HDL-C level may be, at least in part, explained by its linkage with the *G(−273)C* polymorphism ($r^2 = 0.46667$, $D'$ value $= 1$, $P < 0.0001$). *G(−790)A* was not associated with the lipid levels. Among the polymorphisms selected from JSNPs, including R219K and I823M, only the *IMS-JST071749* polymorphism was associated with the HDL-C level ($P = 0.0060$ adjusted for age, sex, BMI, smoking, and consumption of alcohol; $P = 0.0093$ when also adjusted for the *ApoE* and *ApoA1 (IMS-JST005603)* genotypes). The *R219K* and *I823M* polymorphisms were not associated with the HDL-C level [$P = 0.3877$ (*R219K*) and $P = 0.2286$ (*I823M*) adjusted for age, sex, BMI, smoking and consumption of alcohol; $P = 0.1926$ (*R219K*) and $P = 0.1209$ (*I823M*) when also adjusted for the *ApoE* and *ApoA1* genotypes].

Association study of *ABCA1* (HTN group)

To reconfirm the association between the *G(−273)C*, *C(−297)T*, and *IMS-JST071749* polymorphisms and the HDL-C level, we determined the genotypes in the HTN group. As shown in Table 3, the *G(−273)C* polymorphism was associated with the residual HDL-C level ($P = 0.0310$). The genotype frequency of the *G(−273)C* polymorphism in the HTN group was in accordance with Hardy-Weinberg equilibrium and did not differ from that of the Suita population ($P = 0.2953$). The *C(−297)T*($P = 0.1829$) and *IMS-JST071749*($P = 0.4130$) polymorphisms were not associated with the residual HDL-C level. Thus, a positive association was observed between *G(−273)C* and the HDL-C level in two groups: the Suita population and the HTN group.

Association between *ABCA1 G(−273)C* and incidence of MI

We next evaluated whether the *ABCA1 G(−273)C* polymorphism was associated with the incidence of MI. The HDL-C level in the male MI group ($1.09 \pm 0.01$, $P < 0.0001$) was significantly lower than that in the male Suita subjects ($1.44 \pm 0.02$). The effects of this genotype on the HDL-C level were not observed in this group, probably because a substantial proportion of this group had dyslipidemia and had been treated with hypolipidemic drugs.

No significant association was observed between the *ABCA1 G(−273)C* polymorphism and the incidence of MI [the MI group: *GG* $n = 212$ (38.6%), *GC* $n = 289$ (45.2%), *CC* $n = 130$ (16.2%); the Suita population: *GG* $n = 309$ (35.5%), *GC* $n = 362$ (48.3%), *CC* $n = 130$ (16.2%), $P = 0.4443$].

(−), *I/D#2T GGGG(−226)(−)*

| *G(−402)C* | *C(−297)T* | *G(−273)C* | I/D#2 | *G(−99)C* | *C(−14)T* | *C52A* | *T313C* | *G380T* |
|---|---|---|---|---|---|---|---|---|
| 0.22034** | **0.73333*** | 0.22034** | **0.73333*** | 0.29781*** | **0.55012*** | 0.01524 | **0.55012*** | **0.52781*** |
| 0.06087 | 0.01449 | 0.06087 | 0.01449 | 0.06636 | 0.10559* | 0.21726*** | 0.10559* | 0.11538* |
| **0.65714*** | 0.30667*** | **0.65714*** | 0.30667*** | **0.71214*** | 0.37882*** | 0.04726 | 0.37882*** | 0.36111*** |
| 0.22034** | **0.73333*** | 0.22034** | **0.73333*** | 0.29781*** | **0.55012*** | 0.01524 | **0.55012*** | **0.52781*** |
| **0.65714*** | 0.30667*** | **0.65714*** | 0.30667*** | **0.71214*** | 0.37882*** | 0.04726 | 0.37882*** | 0.36111*** |
| 0.16483** | 0.07692 | 0.16483** | 0.07692 | 0.15119** | 0.09502* | 0.01003 | 0.09502* | 0.09582* |
| 1*** | 1*** | 1*** | 1*** | 0.40741*** | 1*** | 0.06158 | 1*** | 1*** |
| 1*** | 0.46667*** | 1*** | 0.46667*** | 0.46798*** | **0.57647*** | 0.06087 | **0.57647*** | **0.55981*** |
| | 0.46667*** | 1*** | 0.46667*** | 0.46798*** | **0.57647*** | 0.06087 | **0.57647*** | **0.55981*** |
| | | 0.46667*** | 1*** | 0.21839** | **0.80952*** | 0 | **0.80952*** | **0.7978*** |
| | | | 0.46667*** | 0.46798*** | **0.57647*** | 0.06087 | **0.57647*** | **0.55981*** |
| | | | | 0.21839** | **0.80952*** | 0 | **0.80952*** | **0.7978*** |
| | | | | | 0.26978*** | 0.06636 | 0.26978*** | 0.25325*** |
| | | | | | | 0.10559* | 1*** | 1*** |
| | | | | | | | 0.10559* | 0.11538* |
| | | | | | | | | 1*** |

*values* indicate $D' > 0.5$. All values refer to the variant allele indicated in the table

**Table 2** Lipid levels in the *ABCA1 G(−273)*C genotypes (Suita population). Subjects who were receiving anti-hyperlipidemic medication were excluded. Values are mean ± SEM. *P*-values calculated by ANOVA

| Factors | *GG* | *GC* | *CC* | *P*-value |
|---|---|---|---|---|
| *n* (male/female) | 306/291 | 358/415 | 127/129 | |
| Age (y) | 64.1 ± 0.5 | 63.7 ± 0.4 | 63.9 ± 0.7 | 0.7934 |
| BMI (kg/m$^2$)[a] | 22.7 ± 0.1 | 22.4 ± 0.1 | 22.9 ± 0.2 | 0.0607 |
| Smoking (cigarettes/day) | 9.2 ± 0.5 | 8.5 ± 0.5 | 8.6 ± 0.8 | 0.5806 |
| Alcohol consumption (ml/week) | 85.7 ± 5.5 | 80.1 ± 4.9 | 71.3 ± 8.5 | 0.3597 |
| Total cholesterol (mmol/l) | 5.31 ± 0.03 | 5.36 ± 0.03 | 5.38 ± 0.05 | 0.3559 |
| HDL[b]cholesterol (mmol/l) | 1.53 ± 0.02 | 1.58 ± 0.01 | 1.60 ± 0.03 | 0.0258 |
| Triglycerides (mmol/l)[c] | 1.25 ± 0.04 | 1.15 ± 0.03 | 1.18 ± 0.05 | 0.2583 |
| Residual HDL cholesterol (mmol/l)[d] | −0.03 ± 0.01 | 0.00 ± 0.01 | 0.05 ± 0.02 | 0.0148 |
| Residual HDL cholesterol (mmol/l)[e] | −0.03 ± 0.01 | 0.01 ± 0.01 | 0.05 ± 0.02 | 0.0074 |

[a]Body-mass index
[b]High-density lipoprotein
[c]Test performed on log-transformed values
[d]Residual HDL cholesterol was adjusted for sex, age, body-mass index, smoking, and alcohol consumption
[e]Residual HDL cholesterol was adjusted for sex, age, BMI, smoking, alcohol consumption, *ApoE*genotype, and *ApoA1*genotype (*JST-IMS005603*)

**Table 3** Lipid levels in the *ABCA1 G(−273)*Cgenotypes (hypertension group). Values are mean ± SEM. *P*-values calculated by ANOVA

| Factors | *GG* | *GC* | *CC* | *P*-value |
|---|---|---|---|---|
| *n* (male/female) | 165/128 | 196/141 | 58/47 | |
| Age (y) | 64.5 ± 0.6 | 65.6 ± 0.6 | 65.3 ± 1.1 | 0.4561 |
| BMI (kg/m$^2$) | 24.1 ± 0.3 | 23.8 ± 0.3 | 23.3 ± 0.4 | 0.2766 |
| Smoking (cigarettes/day) | 11.6 ± 0.9 | 10.9 ± 0.9 | 12.1 ± 1.6 | 0.7828 |
| Drinking habit (I/II)[a] | 117/170 | 154/180 | 41/60 | 0.3460 |
| Total cholesterol (mmol/l) | 5.18 ± 0.05 | 5.28 ± 0.05 | 5.33 ± 0.09 | 0.2316 |
| HDL cholesterol (mmol/l) | 1.31 ± 0.02 | 1.36 ± 0.02 | 1.44 ± 0.04 | 0.0259 |
| Triglycerides (mmol/l)[b] | 1.54 ± 0.07 | 1.52 ± 0.07 | 1.64 ± 0.12 | 0.9429 |
| Residual HDL cholesterol (mmol/l)[c] | −0.04 ± 0.02 | 0.02 ± 0.02 | 0.07 ± 0.04 | 0.0310 |

[a]Drinking habit: *I* subjects with drinking habit, *II* subjects without drinking habit
[b]Test performed on log-transformed values
[c]Residual HDL cholesterol was adjusted for sex, age, BMI, smoking, and drinking habit

## Discussion

In the present study, we evaluated the effects of polymorphisms in *ABCA1* on the HDL-C level using a large cohort representing the general population in Japan (the Suita Study). To evaluate the genetic influence of *ABCA1*polymorphisms on HDL-C level, the HDL-C level was adjusted not only for standard

factors but also for other important genetic factors including the *ApoA1* and *ApoE* polymorphisms. Moreover, we reconfirmed the effects of *ABCA1 G(−273)C* polymorphism on HDL-C in the HTN group. We next investigated the association between the *ABCA1 G(−273)C* and the incidence of MI, but did not observe any association.

The present study is distinguished by three main features: (1) an association study using a large cohort study (the Suita population), (2) taking into account of the influence of the *ApoA1* and *ApoE* polymorphisms, and (3) a confirmation of the association using another set of subjects (the HTN group).

We found that three SNPs were associated with the HDL-C level in 14 SNPs of the *ABCA1*gene in the Suita population. However, if we applied Bonferroni's correction for multiple tests, three SNPs might not be considered significantly associated with the HDL-C level [*G(−273)C*, $P=0.1036$: *C(−297)T*, $P=0.273$: *IMS-JST071749*, $P=0.1302$, $P$ values are corrected by multiplying with 14 (14 SNPs)]. Thus, we verified this positive association in another set of subjects (the HTN group). This association study revealed that *G(−273)C*, but not *C(−297)T* or *IMS-JST071749*, was associated with the HDL-C level. Thus, it is highly likely that *ABCA1 G(−273)C* was truly associated with the HDL-C level.

Since the *ABCA1 G(−273)C* polymorphism is in the promoter region, it is likely that this polymorphism may alter the expression level of ABCA1. However, this polymorphic site had no consensus sequence for transcriptional factors. The *TGGGG(−226)(−)* insertion-deletion polymorphism, which is one of the polymorphisms in LD with the *G(−273)C* polymorphism ($r^2=0.46667$), was in the middle of the consensus sequence of the ZNF202 binding site (GnT repeat)(Porsch-Ozcurumez et al. 2001). The insertion allele, which mainly corresponds to the *(−273)C* allele, should disrupt this binding site and may be associated with higher transcriptional activity of the *ABCA1* gene, which may lead to higher HDL cholesterol levels. However, the *C(−297)T*polymorphism, which was in more tight LD with the *TGGGG(−226)(−)* insertion-deletion polymorphism, appeared to have less effect on the HDL cholesterol level than the *G(−273)C* polymorphism. It remains to be determined whether this discrepancy merely reflects a statistical error or if the *G(−273)C* polymorphism might have additional functional significance. A more detailed promoter analysis will be needed to determine which polymorphisms are functionally important.

The present study revealed that the *ABCA1I823M* polymorphism was not associated with the HDL-C level, inconsistent with a previous report (Harada et al. 2003). This discrepancy may be due to the study design, since a small-scale association study has relatively weak statistical power. In the present study, the sample power was 0.77 for the distribution, sample size, frequencies of the alleles, and $\alpha$ value (0.05, two-tailed). The sample size in the previous study ($n=410$) does not seem to be sufficient to give adequate statistical power. Moreover, the frequency of the *I823* allele in the previous study (allele frequency 0.492) was different from that in the Suita population (0.36) and JSNP information (0.38). Thus, the subjects in the previous study did not seem to be representative of the general Japanese population, as noted by Harada et al. (2003).

Recently, the polymorphisms in the promoter region of *ABCA1*, which corresponds to *C(−559)T* in the present study and seems to be in tight linkage with *G(−273)C* ($r^2=1$, $D'$-value$=1$), was found to be modestly, but not significantly ($P=0.09$), associated with the HDL-C level using LCAS subjects (Lutucuta et al. 2001). The effect of the *ABCA1 G(−273)C* polymorphism on the HDL-C level was significant, but still relatively weak ($r^2=0.0050$). Accordingly, the sample size ($n=372$) in the previous study (Lutucuta et al. 2001) seems to have been too small to detect the effect of polymorphisms on the HDL-C level clearly.

While the *ABCA1 G(−273)C* polymorphism was associated with HDL-C level, it was not found to be associated with the incidence of MI. The *ApoE* polymorphism (*E2*, *E3*, and *E4*) had the greatest influence on the HDL-C level among the three polymorphisms, *ABCA1 G(−273)C* ($r^2=0.0050$), *ApoA1 JST-IMS005603* (0.0100), and *ApoE*(0.0118). However, the *ApoE* polymorphism was only weakly associated with the incidence of MI ($P=0.0840$). Thus, *ABCA1 G(−273)C* may have too weak an influence on the HDL-C level to alter the incidence of MI through a reduction of the HDL-C level. More large numbers of MI subjects might be necessary to detect the influence of the *ABCA1 G(−273)C* polymorphism on MI incidence.

In summary, the present study provides the first evidence that the common *ABCA1 G(−273)C* polymorphism in the promoter region is significantly associated with the level of HDL cholesterol in the Japanese.

**Acknowledgements** This study was supported by the Program for the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan. We would also like to thank Dr. Otosaburo Hishikawa, Dr. Katsuyuki Kawanishi, and Mr. Shigeru Kobayashi for their continuous support of our population survey in Suita City. We also thank the members of the Satsuki-Junyukai. Finally, we express our gratitude to Dr. Soichiro Kitamura, President of the National Cardiovascular Center, and to Dr. Hitonobu Tomoike, Direktor General of the National Cardiovascular Center Hospital, for their support of our study.

## References

Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 22:347–351

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, et al (1999) Mutations in *ABC1* in Tangier

disease and familial high-density lipoprotein deficiency. Nat Genet 22:336–345
Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB (1986) Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 256:2835–2838
Clee SM, Zwinderman AH, Engert JC, Zwarts KY, Molhuizen HOF, Roomp K, Jukema JW, van Wijland M, van Dam M, Hudson TJ, Brooks-Wilson A, Genest J Jr, Kastelein JJ, Hayden MR (2001) Common genetic variation in *ABCA1*is associated with altered lipoprotein levels and a modified risk for coronary artery disease. Circulation 103:1198–1205
Clifton-Bligh P, Nestel PJ, Whyte HM (1972) Tangier disease. Report of a case and studies of lipid metabolism. N Engl J Med 286:567–571
Groenendijk M, Cantor RM, de Bruin TW, Dallinga-Thie GM (2001a) The *apoAI-CIII-AIV* gene cluster. Atherosclerosis 157:1–11
Groenendijk M, Cantor RM, De Bruin TWA, Dallinga-Thie GM (2001b) New genetic variants in the *apoA-I* and *apoC-III* genes and familial combined hyperlipidemia. J Lipid Res 42:188–194
Harada T, Imai Y, Nojiri T, Morita H, Hayashi D, Maemura K, Fukino K, Kawanami D, Nishimura G, Tsushima K, Monzen K, Yamazaki T, Mitsuyama S, Shintani T, Watanabe N, Seto K, Sugiyama T, Nakamura F, Ohno M, Hirata Y, Yamazaki T, Nagai R (2003) A common Ile 823 Met variant of ATP-binding cassette transporter A1 gene (*ABCA1*) alters high-density lipoprotein cholesterol level in Japanese population. Atherosclerosis 169:105–112
Hirakawa M, Tanaka T, Hashimoto Y, Kuroda M, Takagi T, Nakamura Y (2002) JSNP: a database of common gene variations in the Japanese population. Nucl Acids Res 30:158–162
Iida A, Saito S, Sekine A, Kitamura Y, Kondo K, Mishima C, Osawa S, Harigae S, Nakamura Y (2001) High-density single-nucleotide polymorphism (SNP) map of the 150-kb region corresponding to the human ATP-binding cassette transporter A1 (*ABCA1*) gene. J Hum Genet 46:522–528
Katsuya T, Baba S, Ishikawa K, Mannami T, Fu Y, Inamoto N, Asai T, Fukuda M, Higaki J, Ogata J, Ogihara T (2002) Epsilon 4 allele of apolipoprotein E gene associates with lower blood pressure in young Japanese subjects: the Suita Study. J Hypertens 20:2017–2021
Lefevre M, Ginsberg HN, Kris-Etherton PM, Elmer PJ, Stewart PW, Ershow A, Pearson TA, Roheim PS, Ramakrishnan R, Derr J, Gordon DJ, Reed R (1997) *ApoE* genotype does not predict lipid response to changes in dietary saturated fatty acids in a heterogeneous normolipidemic population. The DELTA Research Group. Dietary effects on lipoproteins and thrombogenic activity. Arterioscler Thromb Vasc Biol 17:2914–2923
Lutucuta S, Ballantyne CM, Elghannam H, Gotto AM Jr, Marian AJ (2001) Novel polymorphisms in promoter region of ATP binding cassette transporter gene and plasma lipids, severity, progression, and regression of coronary atherosclerosis and response to therapy. Circ Res 88:969–973
Mannami T, Konishi M, Baba S, Nishi N, Terao A (1997) Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita Study. Stroke 28:518–525
Marcil M, Brooks-Wilson A, Clee SM, Roomp K, Zhang LH, Yu L, Collins JA, van Dam M, Molhuizen HO, Loubster O, Ouellette BF, Sensen CW, Fichter K, Mott S, Denis M, Boucher B, Pimstone S, Genest J Jr, Kastelein JJ, Hayden MR (1999) Mutations in the *ABC1* gene in familial HDL deficiency with defective cholesterol efflux. Lancet 354:1341–1346
Porsch-Ozcurumez M, Langmann T, Heimerl S, Borsukova H, Kaminski WE, Drobnik W, Horner C, Schumacher C, Schmitz G (2001) The Zinc finger protein 202 (ZNF202) is a transcriptional repressor of ATP-binding cassette transporter A1 (ABCA1) and *ABCG1* gene expression and a modulator of cellular lipid efflux. J Biol Chem 276:12427–12433
Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, Assmann G (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 22:352–355
Sacco RL, Benson RT, Kargman DE, Boden-Albala B, Tuck C, Lin IF, Cheng JF, Paik MC, Shea S, Berglund L (2001) High-density lipoprotein cholesterol and ischemic stroke in the elderly: the Northern Manhattan Stroke Study. JAMA 285:2729–2735
Shioji K, Kokubo Y, Mannami T, Inamoto N, Morisaki H, Mino Y, Tago N, Yasui N, Iwai N (2004) Association between hypertension and the α-adducin, β1-adrenoreceptor and G-protein β3 subunit genes in the Japanese population: the Suita Study. Hypertens Res (in press)
Singaraja RR, Brunham LR, Visscher H, Kastelein JJP, Hayden MR (2003) Efflux and atherosclerosis. The clinical and biochemical impact of variation in the *ABCA1* gene. Arterioscler Thromb Vasc Biol 23:1322–1332
Takagi S, Iwai N, Yamauchi R, Kojima S, Yasuno S, Baba T, Terashima M, Tsutsumi Y, Suzuki S, Morii I, Hanai S, Ono K, Baba S, Tomoike H, Kawamura A, Miyazaki S, Nonogi H, Goto Y (2002) Aldehyde dehydrogenase 2 gene is a risk factor for myocardial infarction in Japanese men. Hypertens Res 25:677–681
Wang N, Tall AR (2003) Regulation and mechanisms of ATP-binding cassette transporter A1-mediated cellular cholesterol efflux. Arterioscler Thromb Vasc Biol 23:1178–1184
Wang J, Burnett JR, Near S, Young K, Zinman B, Hanley AJ, Connelly PW, Harris SB, Hegele RA (2000) Common and rare *ABCA1* variants affecting plasma HDL cholesterol. Arterioscler Thromb Vasc Biol 20:1983–1989

# Protein C and antithrombin deficiency are important risk factors for deep vein thrombosis in Japanese

T. SAKATA, A. OKAMOTO, T. MANNAMI,* H. MATSUO† and T. MIYATA‡

*Laboratory of Clinical Chemistry, *Department of Preventive Cardiology, †Department of Cardiology and ‡Research Institute, National Cardiovascular Center, Fujishirodai, Suita, Osaka, Japan*

**To cite this article:** Sakata T, Okamoto A, Mannami T, Matsuo H, Miyata T. Protein C and antithrombin deficiency are important risk factors for deep vein thrombosis in Japanese. *J Thromb Haemost* 2004; **2**: 528–30.

Correspondence: T. Sakata, Laboratory of Clinical Chemistry, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan.
Tel.: +81 6 6833 5012 (ext 2296); fax: +81 6 6835 1176; e-mail: tsakata@hsp.ncvc.go.jp

Received 6 November 2003, accepted 6 November 2003

The frequency of factor (F)V Leiden mutation is relatively high among individuals of Caucasian descent, being from 2 to 15% in the general population and up to 50% in selected patients with thromboembolism [1]. The risk of the first episodes of thromboembolism as estimated in a large case–control study is 7-fold for heterozygous FV Leiden carriers [2]. Although the frequency of deficiencies of natural anticoagulants, protein C or antithrombin in the general population is low, prospective studies indicate that low levels of protein C and antithrombin

are associated with increased incidence of venous thrombosis [3,4]. We tried to elucidate the association between the deficiency of protein C or antithrombin and deep vein thrombosis in Japanese, since the FV Leiden is not in the Japanese population [5] and there have been no reports assessing the prevalence of protein C or antithrombin deficiency in Asian populations.

The reference group was a population randomly selected from the residents of Suita, a city located in the second largest urban area in Japan (Osaka area). The sample comprised 12 200 men and women aged 32–89 years. The subjects have been visiting the National Cardiovascular Center every 2 years since 1989 for regular health checkups [6]. In the present study, 4517 blood donors (2090 male, 2427 female) aged 32–89 years were enrolled for measurement of protein C and antithrombin activity. As a study population for the patient group, 108 consecutive outpatients between April 1994 and March 1998 (54 men, 54 women; mean age ± SD 57.8 ± 17.2 years and 49.6 ± 18.0 years, respectively) with deep vein thrombosis admitted to the Department of Cardiology at the National Cardiovascular Center were enrolled. The diagnoses of deep vein thrombosis were based on radioisotope venography and/or contrast venography. Protein C amidolytic activity was measured using S-2366 as a substrate and Protac derived from *Agkistrodon contortrix* venom as the activator. Antithrombin activity was measured as a heparin cofactor activity using a chromogenic substrate S-2238.

In the identification of protein C deficiency, the ratio of protein C to factor (F)X was recommended by the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis [7]. In our study, we calculated the ratio of protein C activity to antithrombin activity (AT/PC ratio), because the antithrombin activity assay was easier and more reliable than the FX antigen assay. Using the criteria of an AT/PC ratio > 3 SD (1.27) and protein C activity < 3 SD (59.3%), six and seven individuals were identified as a heterozygous protein C deficiency from the reference group and patient group, respectively. Furthermore, by the same criteria, seven and six individuals were identified as an antithrombin deficiency from the reference group and patient group, respectively (Table 1).

The prevalence of protein C deficiency (0.13%) obtained by use of the combined criteria of protein C activity and the antithrombin/protein C ratio agreed with the results previously reported in Scotland (0.20%) [8]. Also, the prevalence (0.15%) of antithrombin deficiency obtained in our study was very similar to the result previously reported in Scotland (0.17%) [9].

We obtained the prevalence of protein C (6.5%) and antithrombin (5.6%) deficiencies in patients with deep vein thrombosis. The prevalence in the deep vein thrombosis group was statistically higher than that in the general population, indicating that each deficiency is a severe risk factor for deep vein thrombosis in the Japanese population (Table 1). The prevalence of protein C deficiency in the deep vein thrombosis group in Europe or the USA was reported to be 1.1% and 2.4% in unselected and selected patients, respectively, and antithrombin deficiency was 3.2% and 3.8% in unselected and selected patients, respectively [10]. Therefore, the prevalence of each deficiency in the Japanese deep vein thrombosis group seemed to be higher than that in Westerners with deep vein thrombosis. One possible reason is that the FV Leiden mutation and the prothrombin G20210A mutation are present only in the Caucasian population [1,11] and not in the Japanese population [5,12].

**Table 1** Comparison of prevalence of protein C and antithrombin deficiencies between the deep vein thrombosis group and the general population

| | Number of heterozygote (prevalence, %) | | | |
|---|---|---|---|---|
| | Patients with DVT ($n$ = 108) | General population ($n$ = 4517) | Odds ratio (95% CI) (vs. general population) | $P$-value |
| Protein C | 7 (6.48%) | 6 (0.13%) | 52.1 (17.2, 157.9) | <0.0001 |
| Antithrombin | 6 (5.56%) | 7 (0.15%) | 37.9 (12.5, 114.8) | ≤0.0001 |

CI, Confidence interval; DVT, deep vein thrombosis.

## Acknowledgements

We would like to express our gratitude to O. Hishikawa, the President, and K. Kawanishi, a committee in chief of Suita City Medical Association, for their continuous support of our population survey in this area. We also express our gratitude to S. Baba and H. Tomoike for sample collection, steering and discussion. This study was supported by Health Sciences Research Grants for Research on Specific Diseases, Blood Coagulation Disorders from the Ministry of Health Labor and Welfare, Japan, the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research (of Japan), and Special Coordination Funds for Promoting Science and Technology, the Japanese Government.

## References

1 Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. *Lancet* 1995; **346**: 1133–4.
2 Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to a poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet* 1993; **342**: 1503–6.
3 Sanson BJ, Simioni P, Tormene D, Moia M, Friederich PW, Huisman MV, Prandoni P, Bura A, Rejto L, Wells P, Mannucci PM, Girolami A, Buller HR, Prins MH. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. *Blood* 1999; **94**: 3702–6.
4 Folsom AR, Aleksic N, Wang L, Cushman M, Wu KK, White RH. Protein C, antithrombin, and venous thromboembolism incidence: a prospective population-based study. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1018–22.
5 Fujimura H, Kambayashi J, Monden M, Kato H, Miyata T. Coagulation factor V Leiden mutation may have a racial background. *Thromb Haemost* 1995; **74**: 1381–2.

6 Mannami T, Baba S, Ogata J. Potential of carotid enlargement as a useful indicator affected by high blood pressure in a large general population of a Japanese city: the Suita study. *Stroke* 2000; **31**: 2958–65.
7 Pabinger I, Allaart CF, Hermans J, Briet E, Bertina RM. Hereditary protein C-deficiency: laboratory values in transmitters and guidelines for the diagnostic procedure. Report on a study of the SSC Subcommittee on Protein C and Protein S. Protein C Transmitter Study Group. *Thromb Haemost* 1992; **68**: 470–4.
8 Tait RC, Walker ID, Reitsma PH, Islam SI, McCall F, Poort SR, Conkie JA, Bertina RM. Prevalence of protein C deficiency in the healthy population. *Thromb Haemost* 1995; **73**: 87–93.
9 Tait RC, Walker ID, Perry DJ, Islam SI, Daly ME, McCall F, Conkie JA, Carrell RW. Prevalence of antithrombin deficiency in the healthy population. *Br J Haematol* 1994; **87**: 106–12.
10 De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. *Blood* 1996; **87**: 3531–44.
11 Rosendaal FR, Doggen CJM, Zivelin A, Arruda VR, Aiach M, Siscovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH. Geographic distribution of the 20210 G to A prothrombin variant. *Thromb Haemost* 1998; **79**: 706–8.
12 Miyata T, Kawasaki T, Fujimura H, Uchida K, Tsushima M, Kato H. The prothrombin gene G20210A mutation is not found among Japanese patients with deep vein thrombosis and healthy individuals. *Blood Coagul Fibrinolysis* 1998; **9**: 451–2.

シンポジウムⅠ　総　説

# 日本人における凝固制御因子の異常と静脈血栓症との関連

阪田　敏幸[1]　松尾　汎[2]　岡本　章[1]　万波　俊文[3]

●要　約：プロトロンビンG20210Aや第V因子Leidenなどの遺伝子変異は欧米人に見られる遺伝子多型で，静脈血栓症発症に対する極めて重要なリスクファクターである．しかし，日本人ではこれらの遺伝子多型が見られず，日本人の先天性血栓性素因は明らかにされていない．今回我々は，一般住民4505人と深部静脈血栓症患者108人におけるプロテインCおよびアンチトロンビン欠乏症の頻度を比較することにより，日本人における両欠乏症の先天性血栓性素因としての重要性を検討した．その結果，一般住民におけるプロテインCおよびアンチトロンビン欠乏症の頻度はそれぞれ0.20%，0.18%であった．また，深部静脈血栓症患者におけるプロテインCおよびアンチトロンビン欠乏症の頻度は，それぞれ6.5%，5.6%であった．このことから両欠乏症は共に日本人の静脈血栓症の重要なリスクファクターであることが示唆された．

●索引用語：プロテインC欠乏症，アンチトロンビン欠乏症，深部静脈血栓症，リスクファクター

静脈学，14(5)：339-345，2003

## はじめに

血管内で血液の流動性を保つため，血管内皮細胞上に2つの異なった抗凝固機構が存在する(**Fig. 1**)．一つは，ヘパラン硫酸というヘパリン様糖鎖構造をもつプロテオグリカンに，プロテアーゼインヒビターであるアンチトロンビン(AT)および外因系凝固インヒビター(TFPI；tissue factor pathway inhibitor)が結合し，凝固反応の過程で生じるトロンビンやXa因子，VIIa因子－組織因子複合体を阻害する機構である．もう一つは，血管内皮細胞上のプロテインC(PC)レセプター(EPCR：endothelial cell protein C receptor)に結合したPCが，トロンボモジュリンに結合したトロンビンで活性化されて活性化PC(APC)となり，これがプロテインS(PS)を補助因子として活性型第Ⅴ因子と活性型第Ⅷ因子を失活させ，凝固反応を抑制する機構である．ここに関わる因子の量的および質的低下は，しばしば血栓症を起こす原因となり先天性血栓性素因と呼ばれる．欧米人においてはプロトロンビンG20210A[1]や第Ⅴ因子Leiden[2]などの遺伝子多型が存在し，静脈血栓症発症に対する極めて重要なリスクファクターである．また，PCやATなどの血液凝固制御因子の先天性欠乏症も血栓性素因とされている[3,4]．しかし，日本人ではプロトロンビンG20210A[5]や第V因子Leiden[6]などの遺伝子多型は報告されておらず，PCやAT欠乏症と静脈血栓症との関連についても明らかではない．今回我々

1　国立循環器病センター臨床検査部
2　同　　心臓血管内科
3　同　　集団検診部
受付：2003年5月19日

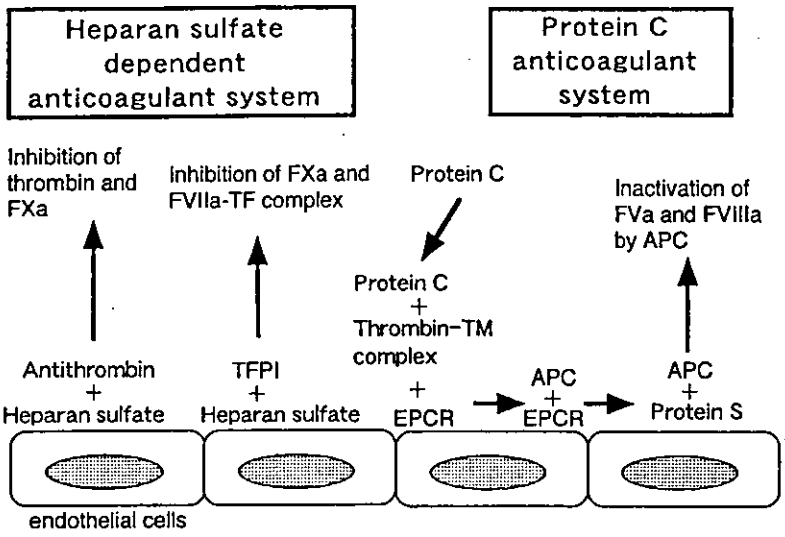


**Fig. 1**　Anticoagulant mechanisms on the endothelial cell surface.
TFPI: tissue factor pathway inhibitor, TF: tissue factor, EPCR: endothelial cell protein C receptor, APC: activated protein C, TM: thrombomodulin.

は，日本人一般住民および深部静脈血栓症(DVT)患者におけるPCおよびAT欠乏症の頻度を求め，DVT発症との関連について述べる．さらに，後天性のリスクファクターとして，最近第V因子Leiden非依存性のAPCレジスタンスの報告があり，日本人における第V因子Leiden非依存性APCレジスタンスについても概説する．

## 1．PC欠乏症

1998年から2000年までの約2年間に当センターで一般住民検診を受診した4505名(吹田studyより)と，1994年から1998年までの5年間に当センターを受診しDVTと診断された患者108名を対象とした．DVTの診断はRI，Echo，Venography，CTの何れかで行った．PCおよびATのアミド活性を測定し，AT/PC比を求めた．今回，AT/PC活性比>1.57をPC欠乏症のcriteriaとし，9名(9/4505，0.2%)をPC欠乏症とした．**Fig. 2**に一般住民検診受診者4505名におけるPC正常群(n=4496)およびPC欠乏症群(n=9)のPC活性(**Fig. 2A**)およびAT/PC活性比(**Fig. 2B**)を示した．PC欠乏症群のPC活性レンジは47.9%～78.6%で，PC正常群のPC活性レンジと重複が見られた．しかし，AT/PC活性比ではPC正常群とPC欠乏症群との間で重なりは見られなかった．PC欠乏症のcriteriaとしてAT/PC活性比>1.57の正当性を検討するた

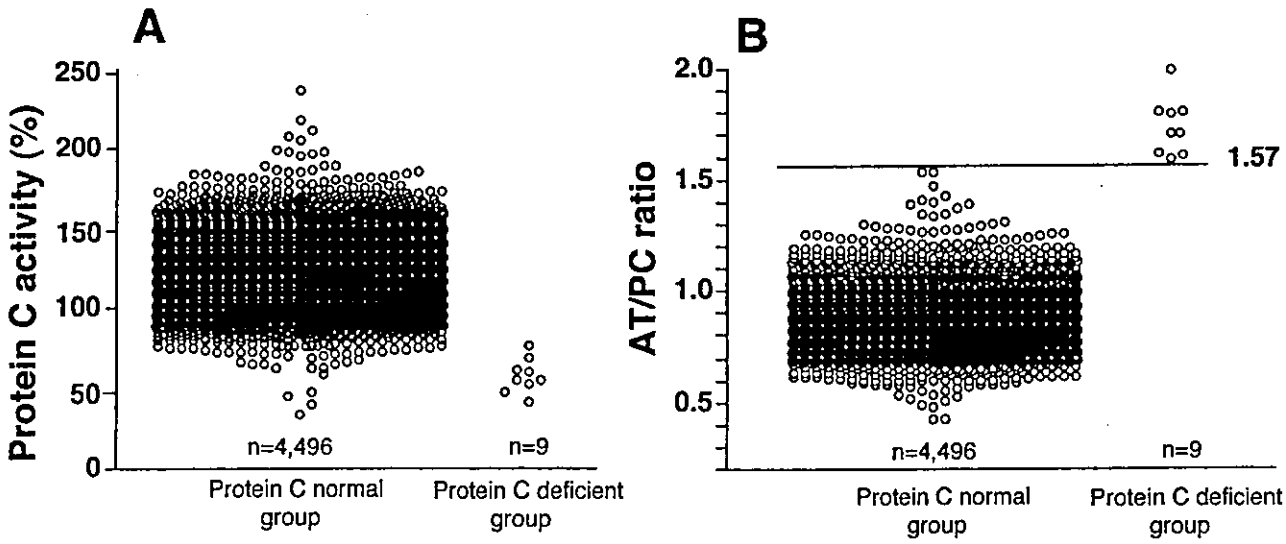


**Fig. 2**　Distribution of protein C activity (A) and antithrombin/protein C ratio (B) in the Japanese general population.
AT/PC ratio: antithrombin activity/protein C activity ratio.