

ATP-binding cassette transporter A1 (ABCA1) R219K (G1051A, rs2230806) polymorphism and serum high-density lipoprotein cholesterol levels in a large Japanese population: cross-sectional data from the Daiko Study

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Abstract. Among polymorphisms in *ATP-binding cassette transporter A1 (ABCA1)* gene, the available evidence demonstrates that the *ABCA1* R219K polymorphism (G1051A, rs2230806) *K* allele is associated with a higher high-density lipoprotein cholesterol (HDL-C) level and may play a protective role against coronary artery disease (CAD) risk in Asians and Caucasians. The findings from many underpowered studies from Asian countries (n=71-597), however, still remain inconsistent. The objective of this study was to overcome the limitations of previous studies in Asia and provide solid epidemiologic evidence. Subjects were participants of a cohort study, who visited the Daiko Medical Center in Nagoya, Japan. The cohort study belongs to the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study). In the Daiko Study, 5,133 participants (1,458 men and 3,675 women) aged 35-69 years enrolled from 2008 through 2010 were eligible for the analyses. The *ABCA1* polymorphism was genotyped by the polymerase chain reaction with confronting two-pair primers (PCR-CTPP) method. Among all the subjects, the genotype frequencies were 23.9% (n=1,225) for *RR*, 49.3% (n=2,532) for *RK*, and 26.8% (n=1,376) for *KK*, which was in Hardy-Weinberg's equilibrium ($P=0.36$). Background characteristics did not significantly differ among the genotypes including alcohol and tobacco use. The mean \pm SD of HDL-C concentration was higher in men and women with *RK* or *KK* genotype than those with *RR*, although the difference between these genotypes was not statistically significant in both sexes ($P=0.31$ in men and 0.26 in women by ANOVA). In the multiple linear regression analysis to estimate the independent effects of the R219K polymorphism on HDL-C level, however, the number of *K* allele was significantly correlated with an increased level of HDL-C (trend $P=0.033$). Those with the *KK* genotype showed a significantly higher HDL-C concentration compared with those with the *RR* genotype by a mean of 1.18 mg/dL. The R219K polymorphism of *ABCA1* independently associated with serum level of HDL-C in a large Japanese population.

Keywords: *ABCA1*, Polymorphism, HDL-C, Coronary artery disease

IN SPITE of the recent decline in the death rate for ischemic heart disease (IHD), it is still one of the biggest causes of mortality worldwide [1]. The mortality rates from IHD greatly vary among populations. The rate is lower in East Asian countries compared with Western countries. For example, the age-adjusted mortality rate (per 100,000 in 2004) was 32.1 in Japan, 40.3 in Korea, and 62.8 in China whereas it was

90.1 in United Kingdom, 89.7 in Germany and 97.6 in United States. The mortality rates, however, were very high in Central and South Asian countries; 400.5 in Kazakhstan, 375.8 in Uzbekistan, 221.6 in Iran, and 207.7 in India [URL:https://apps.who.int/infobase/Comparisons.aspx]. Although such variations in mortality rates may mostly be explained by the difference in lifestyle factors and the levels of medical treatment, some variations can be attributable to genetic factors.

However, considerable part of the pathogenesis of IHD remains yet to be clarified. Atherosclerosis, which is caused by the aggregation of cholesterol in the arterial wall, explains the pathological basis of IHD [2, 3, 4]. This makes the pathways involved in the trans-

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portation and metabolism of cholesterol attractive target for investigation. Accumulated evidence suggested the possible contribution of the interaction between genetic and environmental factors to the genesis of atherosclerosis and subsequent IHD [5].

A reverse relationship between blood high-density lipoprotein cholesterol (HDL-C) and the development or progression of atherosclerosis has been established in epidemiological studies, although there still remains some discussion regarding the role of HDL-C in atherosclerogenesis with respect to its quality and quantity [2, 3, 4]. HDL-C is demonstrated to play important roles in maintaining cholesterol balance and clear excessive cellular cholesterol away from the vessel walls as well. ATP-binding cassette transporter A1 (ABCA1) is shown to play an essential role in the cholesterol efflux in the excessive cellular lipid removal as well as in the formation of HDL-C by promoting the translocation of cholesterol and phospholipids to the plasma membrane and the efflux upon apolipoproteins (apo A-I) [5].

The *ABCA1* gene, consisting of 49 exons, is located on chromosome 9q31.1 [6]. Variations in the *ABCA1* gene may determine plasma HDL-C levels and subsequently influence the risk of IHD. Among polymorphisms in *ABCA1* gene, the available evidence demonstrates that the *ABCA1* R219K polymorphism (G1051A, rs2230806) *K* allele is associated with higher HDL-C level and plays a protective role against IHD risk in Asians and Caucasians [5]. The findings from many small and underpowered studies from Asian countries (n=71-597) [7], however, still remain inconsistent.

The frequency of the *ABCA1* 219K allele has been reported to be higher in East Asian countries than in Western countries. For example, in the HapMap data, the allele frequency was 0.424 in Japanese in Tokyo and 0.419 in Han Chinese in Beijing, whereas 0.208 in Utah residents with Northern and Western European ancestry [URL:[http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2230806\(ss43782758\)](http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2230806(ss43782758))]. This means that the effects of this polymorphism may be more important and should be more clearly revealed in East Asian countries. Although the mortality rate of IHD is relatively low in Japan, the incidence rate of IHD has been increasing in some areas in that country [8]. Further studies with large sample sizes, therefore, will be of significance particularly in East Asian populations.

Therefore, to clarify the association of *ABCA1* R219K polymorphism with serum HDL-C levels in a large Japanese population, we examined this associa-

tion using baseline data of a cohort study.

Materials and Methods

Study subjects

Subjects were participants of a cohort study, who visited the Daiko Medical Center in Nagoya, Japan. The cohort study belongs to the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study), of which study design was described in the previous reports [9-12]. In the Daiko Study, 5,172 men and women aged 35-69 years were enrolled from 2008 through 2010. As of January 2014, 4 participants have withdrawn and 17 were found to be ineligible as to place of residence. In addition, sufficient DNA samples were not available for 16 participants, and the genotyping was unsuccessful for 2 individuals, leaving 5,133 participants (1,458 men and 3,675 women) eligible for the analyses. Written informed consent including genotyping was obtained from all the subjects, and the study protocol was approved by the Ethics Committee of Nagoya University Graduate School of Medicine (approval no. 618).

Lifestyle data and blood samples

Lifestyle exposures were evaluated with a self-administered questionnaire checked by trained staff. The questionnaire included items on smoking and alcohol drinking habits, physical activity and medical history. Smoking and drinking statuses were classified as current, former or never. Total physical activity was assessed using the questionnaire [13]. From the type, frequency, and duration of each daily or leisure time physical activity, the degree of physical activities was elucidated, and converted into metabolic equivalent (MET) hours/day ([MET levels]×[hours of activity/day]). Total physical activity was estimated by summing up the MET hours/day over all the activities. Hypertension was defined as systolic blood pressure \geq 140 mmHg and/or diastolic blood pressure \geq 90 mmHg or self-reported physician-diagnosed hypertension, and history of diabetes mellitus as well as IHD was based on self-report in the questionnaire.

Body mass index was computed by dividing measured body weight (kg) by the square of measured height (m²). The participants donated blood samples for HDL-C measurement and genotyping after overnight fasting. The serum HDL-C level of the participants was measured using an auto-analyzer. Blood

tests were conducted at SRL Co., Ltd., Hachioji, Tokyo.

Genotyping of ABCA1 R219K polymorphism

DNA was extracted from buffy coat kept at -80°C using a Biorobot M48 (QIAGEN Group, Tokyo). The ABCA1 polymorphism was genotyped by the polymerase chain reaction with confronting two-pair primers (PCR-CTPP) method [14]. Each 25 µl reaction tube contained 30-80 ng DNA, 0.12 mM dNTP, 6.25 pmol of each primer, 0.5 U AmpliTaq Gold (Perkin-Elmer, Foster City, CA) and 2.5 µl of 10× PCR buffer including 15 mM MgCl₂. The primers used were F1: 5' TGC AAG GCT ACC AGT TAC ATT TGA C, R1: 5' GCT GCA GCC AGT TTC TCC T, F2: 5' TGA GCT TTG TGG CCT ACC AAG and R2: 5' CAA GTC TAC TCA CCA GGA TTG G. The underlining shows the bases of the SNP (single nucleotide polymorphism). The thermal cycler conditions were 95°C for 10 min denaturing followed by 35 cycles of 95°C for 1 min, 66°C for 1 min and 72°C for 1 min, and 72°C for 5 min for final extension. Each genotype is distinguished as follows: RR genotype (98- and 183-bp bands), RK genotype (98-, 124- and 183-bp bands) and KK genotype (124- and 183-bp bands). The representative gel for the genotyping is shown in Fig. 1.

Statistical analysis

Accordance with the Hardy-Weinberg equilibrium, which indicates an absence of discrepancy between genotype and allele frequency, was checked using the χ^2 test. The difference in the serum HDL-C level between the ABCA1 R219K genotypes by sex was tested by the analysis of variance (ANOVA). For comparisons of the clinical profiles between the genotypes, the χ^2 test or ANOVA was applied where appropriate.

To consider potential confounders, the HDL-C level was also analyzed with multiple linear regression analysis, in which we included the genotype, sex, age (continuous variable), body mass index (continuous variable), smoking and drinking statuses (current or others), total physical activity (MET hours/day, continuous variable), hypertension and diabetes mellitus (yes or no) as independent variables, and HDL-C concentration as a dependent variables. A two-tailed value of $P < 0.05$ was considered significant unless otherwise indicated. Trend analyses for genotypes were done with the number of K allele as an independent variable in the multiple regression analysis. We evaluated the drinking status by qualitative and quantita-

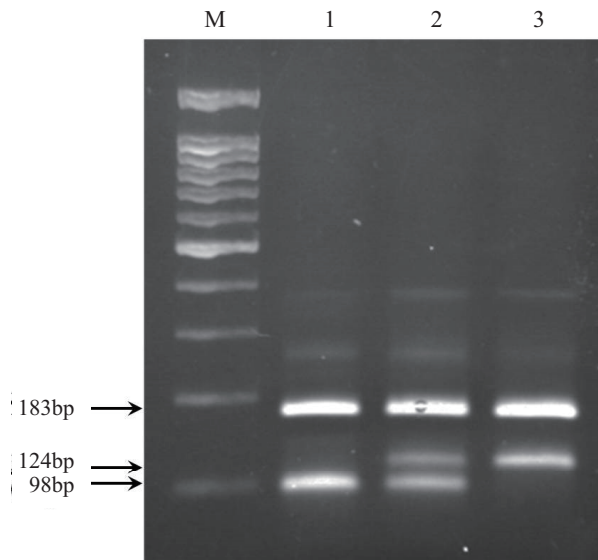


Fig. 1 Representative gel for ABCA1 rs2230806 polymorphism Lane M, a 100-bp ladder; lane 1, a RR homozygote with fragments of 98- and 183- bp; lane 2, a RK heterozygote with fragments of 98-, 124- and 183- bp; and lane 3, a KK homozygote with fragments of 124- and 183- bp bands. The logic of PCR-CTPP for R219K polymorphism (corresponding base change from A to G) can be explained as follows: as described in elsewhere [14], confronting pairs of primers are used; one pair is for the K allele and the other is for the R allele. One primer for the G allele (antisense primer 1 R) is set to include C (antisense of G) at the 3' end, with the counterpart (sense primer 1 F) upstream. A sense primer including A (primer 2 F) and an antisense primer (primer 2 R) downstream of primer 2 F are set for the A allele. The sizes of the DNA products to be amplified specifically for each allele are 98 bps and 124 bps, respectively. The DNA sequence between primers 1 F and 2 R is also amplified (which is called the "common band"), which can be observed as the 183-bp bands.

tive combined; to explain in detail, we first defined the 5 categories of drinking status as follows: nonhabitual drinker, former habitual drinker, or habitual drinker of ethanol at < 23, 23 - < 46, and ≥ 46 g per day (equivalent to < 1 gou, 1-2 gou and > 2 gou of Japanese sake, respectively; where 1 gou [a unit for Japanese sake] = 180ml) per day, and then converted each category into indicator variables. The statistical analyses were conducted using the STATA version 11.1 (Stata Corp., College Station, TX) and Statistical Analysis System version 9.1 (SAS Institute, Cary, NC).

Results

Clinical profiles of subjects by *ABCA1* R219K genotype were shown in Table 1. Among all the 5,133 subjects, the genotype frequencies were 23.9% (n=1,225) for *RR*, 49.3% (n=2,532) for *RK*, and 26.8% (n=1,376) for *KK*, which was in Hardy-Weinberg's equilibrium ($P=0.36$). As demonstrated in Table 1, background characteristics did not significantly differ among the genotypes including alcohol and tobacco use.

Table 2 shows the mean serum HDL-C level by sex and genotype. The mean HDL-C concentration was higher in men and women with *RK* or *KK* genotype than those with *RR*, although the difference between

genotypes was not statistically significant in both sexes ($P=0.31$ in men and 0.26 in women by ANOVA).

In the multiple linear regression analysis to estimate the independent effects of the R219K polymorphism on HDL-C level (Table 3), the number of *K* allele was significantly associated with an increased level of HDL-C (trend $P=0.033$). Those with the *KK* genotype showed a significantly higher HDL-C concentration compared with those with the *RR* genotype by a mean of 1.18 mg/dL.

We also conducted the logistic regression analysis to estimate the risk of (history of) IHD (n = 130), which revealed the reduced point estimates of OR, although statistically not significant (data not shown).

Table 1 Clinical Profiles of subjects by *ABCA1* R219K (rs2230806) genotype (n=5,133)

	<i>KK</i> (26.8%, n = 1,376)	<i>RK</i> (49.3%, n = 2,532)	<i>RR</i> (23.9%, n = 1,225)	<i>P</i>
Female	988 (71.8%)	1,818 (71.8%)	869 (70.9%)	0.84
Age (years)	52.6 ± 10.4	52.5 ± 10.3	52.5 ± 10.3	0.98
Body mass index (kg/m ²)	21.8 ± 3.3	21.7 ± 3.1	21.7 ± 3.1	0.65
Current smokers	163 (11.8%)	298 (11.8%)	146 (11.9%)	0.99
Current drinkers	757 (55.0%)	1,357 (53.6%)	663 (54.1%)	0.70
Hypertension	226 (16.4%)	363 (14.3%)	192 (15.7%)	0.19
Diabetes mellitus	54 (3.9%)	89 (3.5%)	42 (3.4%)	0.75

Values are expressed as means ± standard deviation, or n (%).

Table 2 Mean ± standard deviation of HDL-C level (mg/dl) by sex and *ABCA1* R219K (rs2230806) genotype and sex

	<i>KK</i> (n = 1,376)	<i>RK</i> (n = 2,532)	<i>RR</i> (n = 1,225)	<i>P</i> *
Male (n=1,458)	57.3 ± 15.0	56.4 ± 13.7	55.7 ± 14.0	0.31
Female (n=3,675)	68.7 ± 14.5	68.7 ± 14.9	67.7 ± 14.9	0.26

* By analysis of variance (ANOVA).

Table 3 β -coefficients and SE for HDL-C level (mg/dL) from multiple linear regression analysis

	β -coefficient	SE	<i>P</i>
Intercept	81.5	2.08	<0.001
<i>R/K</i> 219, <i>RK</i> *	0.82	0.48	0.089
<i>R/K</i> 219, <i>KK</i> *	1.18	0.54	0.031
Sex	8.72	0.46	<0.001
Age	-0.06	0.02	0.002
Current smoking	-1.52	0.61	0.012
Current drinking	1.12	0.39	0.004
Body mass index	-1.44	0.07	<0.001
Hypertension	1.28	0.57	0.024
Diabetes mellitus	-1.89	1.06	0.074
Total physical activity (METs hour/day)	0.12	0.02	<0.001

*: *P* value for trend = 0.033.

The analysis results of the association between *ABCA1* R219K polymorphism and HDL-C level adjusted by drinking status and in males were substantially in the same direction compared to that of overall, with the β -coefficient of 0.840 for *RK* genotype, 1.818 for *KK* genotype, 0.911 with the number of *K* allele, with the *P*-values of 0.321, 0.058 and 0.057, respectively. The association between R219K of *ABCA1* and triglyceride (TG) levels was neither statistically significant (data not shown).

Discussion

We found a significant association between the R219K polymorphism *K* allele of *ABCA1* and a higher serum HDL-C level in a large Japanese population.

Previous studies have reported that the R219K polymorphism was associated with increased circulating HDL-C. Considerable efforts have also been made to examine the relationship between the *ABCA1* polymorphism and the risk of CAD.

It was reported by Clee *et al.* that the R219K *K* allele was associated with a higher level of HDL-C and a lower level of TG [15]. Additional studies in Caucasian, Asian and multi-ethnic populations have also shown the association between the *ABCA1* R219K polymorphism and the HDL-C level [6]. Our data based on a Japanese population revealed consistent elevations in blood HDL-C levels associated with the *K* allele of *ABCA1* R219K polymorphism. In the present study, the mean HDL-C level was 1.6 mg/dL higher in *KK* genotype than in *RR* in men, and 1.0 mg/dL higher in *KK* than in *RR* in women. The mean difference between *KK* and *RR* genotypes varied from -0.4 to 19.0 mg/dL in Asian populations and from -15 to 3.1 mg/dL in Western populations [16, 17]. In other representative studies in Asians, the differences were 2.32 and 1.16 mg/dL in Li *et al.* [18], 1.16 and 1.55 mg/dL in Li *et al.* [19], and 4.65 and 1.55 mg/dL by Zhao and Xiao, respectively [20], while 0.77 and 0.39 mg/dL in Porchay-Balderelli *et al.* in Caucasians [21]. In Japan, the HDL-C level was 0.5 mg/dL lower in *KK* than in *RR*, and 0.4 mg/dL lower in *RK* than in *RR* by Harada *et al.* [22]. Generally, there was a significant correlation between the *K* allele and a higher serum HDL-C level as summarized in a meta-analysis [5]. The difference of the HDL-C level among genotypes, however, was relatively small in Caucasian populations.

There are several possible speculations for the dif-

ferences between populations noted above. Some differences may be at least partially attributed to social, environmental or genetic variations. There seems to be a great difference in the SNP genotype frequencies between different ethnic groups. Concerning *ABCA1* R219K polymorphism, the frequency of *K* allele was reported to be 0.488 in Japanese [22] while that in the Caucasian population ranged between 0.25 and 0.46 [21]. The *K* allele frequency was 0.515 in this research, which was similar to those in other studies in Japan (Harada *et al.*: 0.479, n=265 [22] and Yamakawa *et al.*: 0.541, n=327 [23]). Moreover, there is a great variation and diversity in the effects of dietary and other lifestyle factors on lipid levels between ethnicities or study populations, which may also lead to the different interaction with the effect of this *ABCA1* R219K polymorphism. Different effects of the same SNP on serum lipid levels also reportedly exist between distinct races or ethnicities [24].

Several studies have been already reported about SNPs in *ABCA1* among Caucasian and Asian populations [15, 25]. To date, epidemiological studies have reported that genetic alterations within the coding region of *ABCA1* influence the occurrence and development of CAD by determining the plasma HDL-C level in the general population [5].

In addition to *ABCA1*, a lot of components that regulate HDL-C have been proposed. They include ABC transporters, apolipoproteins, cholesteryl ester transfer protein (CETP) and lecithin cholesterol acyltransferase (LCAT) [18]. Thus, further studies considering gene-gene interactions with these factors may be required to understand the role of *ABCA1* polymorphisms in the regulation of HDL-C and cardiovascular risk.

R219K polymorphism is located in the two major extracellular loops of *ABCA1* protein, which is important for the interaction with apoA-I and for cholesterol efflux. Therefore, it is likely that the R219K variant is a functional mutation to modulate HDL-C level. A lot of supposed glycosylated sites have been reported within this loop, and a lot of potential losses in the variants that induce the function determining the level of HDL-C have been suggested [22]. Meanwhile, some other studies even suggest that *ABCA1* 219K allele modified the risk of CHD without important modification of plasma HDL-C level [26]. Either way, given that based on the functional analysis using SIFT and PolyPhen, this R219K variation of *ABCA1* is suggested not to be functional in itself, it could rather be causal

through the linkage disequilibrium (LD) with other functional variants [5]. In addition, we could only analyze HDL cholesterol levels and not efflux capacity in the present study, which is considered to be an important limitation of this study, because cholesterol efflux capacity has been associated with carotid intima-media thickness and the risk of cardiovascular independent of HDL-C levels [27, 28].

The strength of this study includes the large sample size (n=5,133) and consideration of potential confounding factors, namely, sex, age, smoking, alcohol intake, BMI, physical activity, hypertension and diabetes. The effect of the *K* variant in the *ABCA1* R219K polymorphism examined was in the same direction compared to that in the recent meta-analysis in the Asian populations [29], and in the opposite direction to that in the Japanese subjects [22]. The forementioned recent meta-analysis of HDL-C and *ABCA1* R219K consisted of mixture of cohort studies and hospital-based case-control studies; the population of which consisted of Caucasians, Chinese, Japanese and Iraqis, most of the Asian populations of which were Chinese. The only Japanese study included is a hospital-based case-control study that consisted of IHD patients who underwent coronary interventions, which might potentially cause bias to the estimates. The sample size of the above-mentioned study is smaller than the present

study. Given the considerably large sample size, the present study may have a significant impact by providing the first robust evidence for the protective effect of the *K* variant in the *ABCA1* R219K polymorphism in Japanese.

However, because we focused on *ABCA1* R219K polymorphism, we could not evaluate the influence of other polymorphisms of *ABCA1* gene. Further studies that include the effects of other *ABCA1* polymorphisms will be required.

In summary, the R219K polymorphism of *ABCA1* was independently associated with serum level of HDL-C in a large Japanese population. Further epidemiological and biological studies are required to understand the relationships between this polymorphism and serum HDL-C and/or the risk of CAD, especially in countries where the *K* allele is common.

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