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The influence of two variants in the adenosine triphosphate–binding cassette transporter 1 gene on plasma lipids and carotid atherosclerosis

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Abstract

Variants in the adenosine triphosphate–binding-cassette transporter 1 (*ABCA1*) gene are known to affect high-density lipoprotein cholesterol and plasma triglycerides and the development of atherosclerosis. We investigated the influence of the R219K and I883M variants in the *ABCA1* gene on plasma lipids and carotid intima media thickness and plaque extent in 688 healthy men (40-60 years old). The R219K variant showed no effect on plasma lipids, but carriers of the K allele displayed a lower intima media thickness ($P = .001$) and a reduced risk of advanced plaque extent (odds ratio [OR], 0.59; 0.39-0.88; $P = .009$) compared with noncarriers. However, this risk reduction was observed in nonsmokers only (OR, 0.47; 0.27-0.80; $P < .001$), but not in smokers (OR, 0.75; 0.41-1.39; $P = .2$). The I883M variant showed no effect on plasma lipids or carotid atherosclerosis. Risk of advanced plaque extent was reduced in subjects carrying the R219K variant alone (OR, 0.59; 0.38-0.94; $P = .025$), but not in subjects carrying both variants. Haplotype distribution did not differ between subjects with and without advanced atherosclerosis irrespective of smoking history. We conclude that smoking abrogates the protective effect of the R219K.

1. Introduction

Reduced high-density lipoprotein cholesterol (HDL-C) is a powerful established risk factor for coronary artery disease (CAD) [1]. Although HDL-C concentration is strongly influenced by environmental factors [2], research has been focused increasingly on genetic causes leading to reduced HDL-C. Recently, the adenosine triphosphate–binding cassette transporter 1 (*ABCA1*) has been identified as the mediator of the initial step of the reverse cholesterol transport (RCT) because it facilitates the efflux of phospholipids and cholesterol from peripheral cells to lipid free apolipoprotein (apo) A-I creating nascent HDL particles [3]. This process counteracts the deposition of cholesterol by oxidized or otherwise modified low-density lipoprotein (LDL) particles in peripheral cells including macrophages within the arterial wall.

Deficiency of *ABCA1* has been identified as the molecular cause of Tangier disease, a rare condition with very low levels of HDL-C, excessive accumulation of cholesteryl esters in tissue macrophages and the reticuloendothelial system, and an increased risk of premature CAD [4]. Several common polymorphisms in the *ABCA1* gene have been identified and were investigated as potential risk factors for atherosclerosis, showing variable effects on HDL-C, plasma triglycerides (TGs), and atherosclerosis [5-11].

Cigarette smoking increases the risk of CAD by 80% in active smokers and by 30% in passive smokers [12]. Increased inflammation in the blood and the vessel wall, impaired vasodilatory function because of decreased nitric oxide availability, and increased coagulability are mechanisms attributed to smoking [13]. Another effect of tobacco use is a modification of the lipid profile such as increased TG

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and decreased HDL levels [14]. However, the exact mechanisms leading to these alterations in lipid profile are not fully understood.

We investigated and report here the role of smoking on the influence of the R219K and I883M variants in the *ABCA1* gene on plasma lipid levels and ultrasonographically quantified intima media thickness (IMT) and severity of atherosclerosis of the carotid artery in a cross-sectional cohort of 688 middle-aged men.

2. Methods

2.1. Study population

Six hundred eighty-eight men, aged 40 to 60 years, were selected from the baseline investigation of the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk population, a population-based prospective study investigating the role of metabolic and genetic factors in the progression of atherosclerosis [15-18]. Exclusion criteria were renal disease, thyroid dysfunction, malignancies, established CAD, heart failure, valvular heart disease, and lipid-lowering medication. Diabetes was diagnosed when fasting blood glucose exceeded 125 mg/dL or when subjects were on hypoglycemic drugs. Smoking history was assessed using standard questionnaires. Subjects were categorized as smokers (current and ex-smokers) and never-smokers. The study protocol was approved by the local ethics committee, and written informed consent was obtained from participants.

2.2. Ultrasound examination of the carotid artery

Intima media thickness of the carotid arteries was measured by high-resolution B-mode ultrasound (HDI 3000 CV from ATL, Miami, FL) according to the Asymptomatic Carotid Artery Plaque Study protocol [19]. Briefly, IMT was measured at the near and far wall of the common carotid artery, the bifurcation, and the internal carotid artery on both sides, resulting in up to 12 measurements; and the mean of all available measurements was calculated (mean IMT). Plaque extent was estimated using color-coded Duplex scanning; and each segment was graded on a 5-point scale ranging from 0 (normal) to 5 (complete luminal obstruction), designated as the *B-score* [20]. The plaque score constitutes the sum of B-scores obtained from all segments investigated. A plaque score of 3 and higher was considered advanced atherosclerosis.

2.3. Laboratory measurements and DNA analysis

Venous blood was drawn after an overnight fast, and plasma was obtained by centrifugation at 3000 rpm at 4°C immediately after blood collection. Samples were either used for measurements immediately or stored frozen at −80°C. Plasma lipids including lipoprotein (a) (Lp[a]) were quantified using standard methods (Roche Diagnostics, Mannheim, Germany). The R219K and I883M status was determined using polymerase chain reaction–based restriction fragment length analysis, as described [5,6]. Apolipoprotein E genotyping was performed as described [21].

2.4. LDL size

Low-density lipoprotein size was estimated using 0.75% to 16% gradient nondenaturing polyacrylamide gel electrophoresis of whole plasma stained with Sudanblack (Labomed, Waldkirch, Germany). Particle size was calculated using a calibration curve created by well-characterized control samples run in parallel on each gel.

2.5. Ambulatory 24-hour blood pressure measurement

The 24-hour blood pressure was measured using the TM-2430 blood pressure monitoring system (Boso, Jungingen, Germany).

Table 1 Clinical and laboratory characteristics

	R219K				I883M			
	RR (350)	RK (274)	KK (64)	RK + KK	II (522)	IM (151)	MM (15)	IM + MM (166)
Age (y)	50.0 (5.2)	49.4 (5.5)	50.9 (5.5)	49.7 (5.5)	50.1 (5.3)	49.2 (5.4)	47.9 (5.7)	49.1 (5.4)
BMI (kg/m^2)	27.2 (3.4)	27.2 (3.8)	26.9 (4.4)	27.2 (3.9)	27.2 (3.9)	27.1 (4.1)	26.3 (4.7)	27.0 (4.1)
SBP (mm Hg)	130.9 (11.8)	129.4 (12.1)	130.9 (13.3)	129.7 (12.3)	130.7 (12.1	129.0 (11.9)	127.9 (12.2)	128.9 (11.9)
DBP (mm Hg)	80.3 (7.4)	79.1 (7.1)	79.7 (6.5)	79.27 (7.0)	79.9 (7.1)	79.5 (7.6)	76.1 (5.2)	79.2 (7.4)
Smoking (%)[†]	49.1	40.5	53.6	46.2	47.5	40.8	50.0	47.2
DM (%)[†]	4.9	4.7	0	3.8	4.4	4.0	6.7	4.2
TC (mg/dL)	230.5 (41.7)	225.5 (40.3)	228.5 (36.0)	226.1 (39.5)	226.9 (40.2)	233.0 (42.5)	234.1 (35.8)	233.1 (41.8)
LDL-C (mg/dL)	148.2 (37.7)	144.2 (37.2)	150.0 (35.7)	145.3 (37.0)	145.2 (37.2)	151.5 (37.9)	155.0 (34.8)	151.8* (37.5)
HDL-C (mg/dL)	54.6 (13.2)	53.4 (12.9)	53.5 (11.4)	53.5 (12.7)	53.9 (13.0)	54.0 (13.0)	57.6 (11.9)	54.3 (12.9)
TGs (mg/dL)	138.4 (92.0)	138.7 (81.0)	132.8 (74.5)	137.6 (79.7)	138.5 (87.2)	138.5 (85.7)	116.2 (45.6)	136.5 (83.0)
Apo A-I (mg/dL)	149.0 (22.2)	148.7 (23.0)	148.8 (20.1)	148.7 (22.4)	148.8 (22.5)	148.7 (21.4)	150.9 (23.8)	148.9 (21.6)
Apo B (mg/dL)	119.7 (25.4)	119.8 (25.5)	120.9 (25.7)	119.2 (25.5)	118.7 (24.8)	122.0 (18.0)	118.9 (19.5)	121.7 (27.3)
LDL size (nm)	26.4 (1.11)	26.2 (1.17)	26.3 (1.28)	26.2 (1.19)	26.3 (1.14)	26.2 (1.20)	26.4 (0.88)	26.3 (1.17)

Values are means (SD). SBP indicates systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; TC, total cholesterol.

* $P = .048$ vs II adjusted for age and smoking.

[†] Not significant using the χ^2 test.

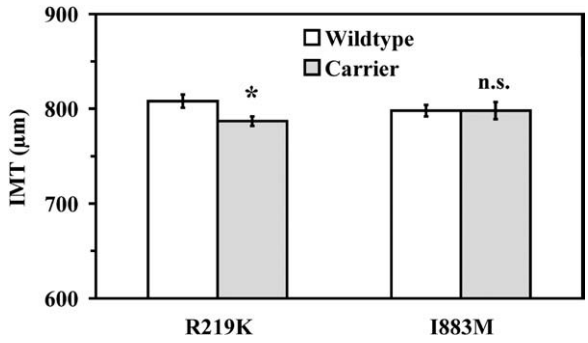

Fig. 1. The R219K and I883M variants and IMT. Values are means (SE); white bars = wild type; black bars = carriers of the variant; *$P < .01$ adjusted for age and smoking.

2.6. Statistical analysis

Continuous variables were expressed as means ± SD unless indicated otherwise. Data for IMT are given as means ± SEM. Normally distributed variables were compared between genotypes using the Student *t* test. Body mass index (BMI), HDL-C, and TGs were not normally distributed and naturally log-transformed. Distribution of categorical variables among genotypes was compared using the Pearson χ^2 test. Analysis of variance was performed to compare continuous variables between more than 2 groups. Age and smoking history were used as covariates for analysis of covariance where indicated. Odds ratios (ORs) were calculated using binary logistic regression models. Presence or absence of *advanced atherosclerosis*, defined as a plaque score of 3 and higher, was entered as dependent variable; and age, BMI, blood pressure, LDL cholesterol (LDL-C), diabetes mellitus, HDL-C, and TGs were entered as independent variables. Odds ratios are reported with 95% confidence intervals (95% CIs). A *P* value less than .05 was considered statistically significant. All statistical analyses were performed using the statistical software SPSS for Windows (Version 11.0; SPSS, Chicago, IL). The standardized pairwise linkage disequilibrium statistic (*D'*) and haplotype frequencies were estimated according to Terwilliger and Ott [22].

3. Results

3.1. Frequencies of the R219K and I883M variants

Allele frequency was 29.2% for the K allele at position 219 and 13.1% for the M allele at position 883; carrier frequency was 49.1% for the K allele and 21.9% for the M allele. Distribution of genotypes was in Hardy-Weinberg equilibrium ($P = .30$ and $P = .33$, for R219K and I883M, respectively). The 2 variants were in a weak, but statistically significant, linkage disequilibrium ($D/D_{max} = 0.1587$, $P < .01$). The calculated haplotype frequencies were 63.0% for the wild-type haplotype 219 R and 883 I, 23.9% for 219 K and 883 I, 7.8% for 219 R and 883 M, and 5.3% for the variant haplotype 219 K and 883 M.

3.2. Effect of the R219K and I883M variants on plasma lipids and IMT

Table 1 illustrates the clinical characteristics and laboratory parameters of the subjects according to the genotypes. Carriers of the I883M variant had a higher LDL-C compared with men homozygous for the wild-type allele. Carriers of the R219K variant had a lower IMT compared with noncarriers (781 ± 6 vs 802 ± 7 μm, $P < .01$), whereas the I883M variant had no effect on IMT (798 ± 9 vs 798 ± 6 μm, not significant [NS]) (Fig. 1).

3.3. Effect of the R219K and I883M variants in combination on plasma lipids and IMT

Furthermore, plasma lipids, apolipoproteins, and LDL size did not differ between the groups according to the combined carrier status of the 2 variants (Table 2).

Table 2
Clinical and laboratory characteristics according to carrier status

	R/R, I/I (n = 277)	Carrier M (n = 73)	Carrier K + M (n = 93)	Carrier K (n = 245)
Age (y)	50.1 (5.3)	49.6 (4.9)	48.7 (5.7)	50.1 (5.4)
BMI (kg/m^2)	27.2 (3.9)	26.9 (4.0)	27.1 (4.2)	27.2 (3.8)
SBP (mm Hg)	130.9 (11.8)	131.0 (12.1)	127.5 (11.6)	130.5 (12.5)
DBP (mm Hg)	80.2 (7.3)	80.6 (8.0)	78.1 (6.9)	79.6 (7.0)
Smoking (%)	49.1	41.7	51.1	44.4
DM (%)	4.3	6.9	2.1	4.5
TC (mg/dL)	228.8 (40.6)	228.8 (40.6)	229.8 (38.7)	224.7 (29.8)
LDL-C (mg/dL)	146.5 (36.8)	154.9 (40.7)	149.4 (34.7)	143.7 (37.7)
HDL-C (mg/dL)	54.5 (13.1)	54.8 (13.7)	54.0 (12.2)	53.2 (12.8)
TGs (mg/dL)	138.6 (92.0)	137.7 (92.9)	135.1 (74.7)	138.6 (81.7)
Apo A-I (mg/dL)	149.5 (22.4)	146.8 (21.6)	150.5 (21.5)	148.0 (22.8)
Apo B (mg/dL)	118.7 (24.5)	123.6 (28.4)	120.2 (26.3)	118.8 (25.2)
LDL size (nm)	26.4 (1.12)	26.3 (1.07)	26.2 (1.24)	26.3 (1.17)

Values are means (SD). R/R, I/I indicates homozygous for wild-type allele at position 219 and 883; carrier M, homozygous for wild-type R allele at position 219 and carrying the variant M allele at position 883; carrier M + K, carrying the variant K allele at position 219 and the variant M allele at position 883; carrier K, homozygous for wild-type M allele at position 883 and carrying the variant K allele at position 219.

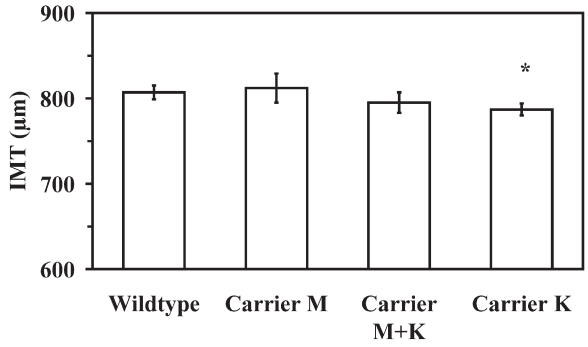

Fig. 2. Combined carrier status and IMT. R/R, I/I indicates homozygous for wild-type allele at position 219 and 883; carrier M, homozygous for wild-type R allele at position 219 and carrying the variant M allele at position 883; carrier M + K, carrying the variant K allele at position 219 and the variant M allele at position 883; carrier K, homozygous for wild-type M allele at position 883 and carrying the variant K allele at position 219; values are means (SE); *$P < .05$ vs wild type, adjusted for age and smoking.

However, the combined carrier status did show a significant effect on IMT. Only subjects carrying the K variant alone (787 ± 8 μm, $P < .05$), but not the M variant in combination with K variant (795 ± 7 μm) or the M variant alone (812 ± 12 μm), had a significantly reduced IMT compared with subjects homozygous for both wild-type variants (807 ± 7 μm) (Fig. 2).

3.4. Effect of the R219K and I883M variants on risk of atherosclerosis

The risk of advanced atherosclerosis—defined as a plaque score of 3 and higher—was significantly reduced in carriers of the K allele (Table 3). The unadjusted relative risk in men carrying the K allele was reduced by 12.5% (model 1, Table 3; $P < .01$) compared with men homozygous for the R allele. The risk remained significantly reduced after adjustment for age, BMI, LDL-C, smoking history, diabetes mellitus, and systolic blood pressure (model 2, Table 3; OR, 0.61; $P < .05$). Further adjustment for HDL-C and TGs did not affect the risk reduction observed (model 3, Table 3; OR, 0.59; $P < .01$). Lipoprotein (a) concentration and apo E genotype did not influence these results (data not shown). The I883M variant had no effect on the risk of carotid atherosclerosis (Table 3).

3.5. Effect of the R219K and I883M variants in combination on risk of atherosclerosis

The reduced risk of advanced atherosclerosis observed in carriers of the K allele at position 219 was attenuated by the carrier status at position 883. The unadjusted relative risk was 0.85 (95% CI, 0.72-1.00; $P = .065$) for carriers of the K allele alone, 1.04 (95% CI, 0.92-1.16; NS) for carriers of the M variant alone, and 0.87 (95% CI, 0.78-0.98; $P < .05$) for carriers of both variants, that is, R219K and I883M. After adjustment for age, BMI, LDL-C, blood pressure, smoking history, and diabetes, the risk reduction was nearly significant in subjects carrying the K allele alone (OR, 0.64; 95% CI, 0.41-1.01; $P = .054$), lost significance in carriers of both variants (OR, 0.70; 95% CI, 0.36-1.34; $P = .2$), and was unchanged in carriers of the M variant alone (OR, 1.29; 95% CI, 0.69-2.51; NS). After further adjustment for HDL-C and TG concentration, the risk of advanced atherosclerosis was reduced significantly only in carriers of the R allele alone (OR, 0.60; 95% CI, 0.38-0395; $P < .05$) but not in men carrying both variants (OR, 0.71; 95% CI, 0.37-1.36; $P = .2$) and was not influenced in subjects carrying the M variant alone (OR, 1.28, 95% CI, 0.65-2.52; NS.).

The calculated haplotype frequencies were 63.0%, 23.9%, 7.8%, and 5.3% (for the haplotypes R219-I883, K219-I883, R219-M883, and K219-M883, respectively) in the whole study population; 62.0%, 24.8%, 7.4%, and 5.8% in subjects without advanced plaque extent; and 65.5%, 21.4%, 8.7%, and 4.4% in subjects with advanced plaque extent. Distribution of the calculated haplotype frequencies did not differ between subjects with and without advanced plaque extent ($\chi^2 = 1.77$, $P = .6$).

3.6. Influence of smoking history on IMT and risk of atherosclerosis

Lipid parameters according to smoking history are given in Table 4. A positive smoking history abolished the protective effect of the R219K variant. The IMT was lower

Table 3
Risk of advanced atherosclerosis

Carrier status	Smoking status	Model 1[a]	Model 2[b]	Model 3[c]
219K	All	0.88 (0.79-0.96)*	0.61 (0.41-0.91)†	0.59 (0.39-0.88)*
	Nonsmokers	0.81 (0.71-0.93)*	0.51 (0.30-0.86)*	0.47 (0.27-0.80)*
	Smokers	0.92 (0.80-1.06)	0.76 (0.41-1.39)	0.75 (0.41-1.39)
883M	All	0.98 (0.88-1.10)	1.18 (0.75-1.87)	1.19 (0.75-1.91)
	Nonsmokers	1.04 (0.91-1.18)	1.39 (0.76-2.53)	1.33 (0.72-2.45)
	Smokers	0.92 (0.81-1.04)	0.82 (0.39-1.72)	0.85 (0.40-1.80)

[a] Unadjusted.
[b] Adjusted for age, BMI, blood pressure, LDL-C, and diabetes mellitus.
[c] Adjusted for age, BMI, blood pressure, LDL-C, diabetes mellitus, HDL-C, and TGs.
* $P < .01$.
† $P < .05$.

Table 4
Smoking status and lipid parameters

	Nonsmokers	Smokers	P^a
TC (mg/dL)	224.6 (39.8)	232.5 (41.4)	.012
LDL-C (mg/dL)	144.6 (36.9)	149.1 (37.7)	.116
HDL-C (mg/dL)	55.7 (12.8)	52.1 (13.2)	<.001
TGs (mg/dL)	123.7 (77.7)	155.0 (93.0)	<.001
Apo B (mg/dL)	116.5 (25.1)	122.7 (25.5)	.002
Apo A-I (mg/dL)	149.3 (21.5)	148.4 (23.5)	.576
LDL size (nm)	26.5 (11.2)	26.1 (11.6)	<.001

[a] Student *t* test.

in nonsmokers (765 ± 8 vs 806 ± 11 μm, $P < .001$), but not in carriers of the K allele with a positive smoking history (797 ± 11 vs 797 ± 11 μm, NS), compared with subjects homozygous for the wild-type R allele.

The unadjusted relative risk of increased plaque score was 0.81 ($P < .01$; model 1, Table 3) after adjustment for age, BMI, blood pressure, LDL-C, and diabetes 0.51 ($P < .01$; model 2, Table 3) and, after further adjustment for HDL and TGs, 0.47 ($P < .01$; model 3, Table 3) for carriers of the K variant without smoking history. In carriers with positive smoking history, the risk reduction did not reach statistical significance (Table 3). The I883M variant had no influence irrespective of smoking history (Table 3). Haplotype distribution did not differ significantly between subjects with a plaque score of 3 and higher and those with a plaque score less than 3 irrespective of smoking history (data not shown).

4. Discussion

Ultrasound examinations of the carotid arteries to measure IMT and to assess plaque extent are validated surrogate markers of atherosclerosis including that of coronary arteries and correlate well with clinical end points [23]. We therefore used this imaging technique to investigate the influence of the R219K and I883M variants in the *ABCA1* gene on the extent of carotid atherosclerosis and plasma lipids in 688 middle-aged men. Our study confirms previous observations that variation in the *ABCA1* gene can influence the risk of atherosclerosis. Men carrying the R219K variant had a reduced risk of carotid atherosclerosis and lower IMT values. However, we could not detect differences in plasma lipids between carriers and noncarriers.

In patients heterozygous for Tangier disease, a wide range of HDL-C from markedly reduced levels up to normal ones was reported [24]. Our data clearly demonstrate a protective effect of the K variant from atherosclerosis of the carotid arteries. This finding is in accordance with a previous report showing, for the R219K allele, less severe CAD and slower progression of CAD in carriers of the K allele [6]; but although Clee et al [6] demonstrated increased HDL-C and reduced TG levels in carriers, we observed no effect of the K allele on plasma lipids. However, the present study included only healthy subjects with higher HDL-C and lower TG levels compared with the study by Clee et al investigating subjects with established CAD. Our results on lipids are in agreement with recent studies reporting an influence of *ABCA1* variants on coronary heart disease without affecting HDL-C and TG concentrations [7,9].

Although efficient cholesterol efflux from peripheral cells is crucial for assembling HDL particles, the availability of apo A-I and steps further downward the RCT cascade, such as cholesterol esterification and cholesterol transfer, also influence HDL-C concentration [25]. The HDL-mediated protection from atherosclerosis presumably results—at least in part—from efficient removal of cholesterol from macrophages. Even in the absence of a detectable increase of HDL-C, a minute but significant increase in removal of excess cholesterol from macrophages in the arterial wall could conceivably result in atheroprotection. Hence, HDL-C concentration may not be sensitive enough to reflect the activity of *ABCA1*-mediated cholesterol efflux from macrophages and the level of atheroprotection. This notion finds support from recent studies using *ABCA1* knockout mice, which were bone marrow transplant recipients from wild-type donor mice; this model demonstrated that expression of *ABCA1* in macrophages plays an important role in plaque development without significantly raising HDL-C concentration [26,27].

Previous reports suggest that the R219K variant influences HDL-C concentration only in special subpopulations, such as smokers [8] or subjects with elevated Lp(a) or apo E-3 [28], indicating a gene-environment or gene-gene interaction. However, in our population, the R219K variant was protective independent of Lp(a) concentration and apo E genotype (data not shown).

In contrast to previous reports, the R219K variant was protective in nonsmokers only, suggesting that the "genetic advantage" of carrying the K variant is abolished by the established risk factor of smoking. The exact mechanisms influencing the cardiovascular risk by cigarette smoke are not fully elucidated. Alterations in activities of enzymes of the RCT such as lecithin:cholesterol acyl transferase, cholesteryl ester transfer protein, and phospholipids transfer protein and decreased insulin sensitivity may be involved in the decrease of HDL-C in smokers [29-31].

Recent reports suggest a role for *ABCA1* in the regulation of inflammatory responses of macrophages and macrophage recruitment into the arterial wall [32-35]. Because atherosclerosis is considered to be an inflammatory process, variants in the *ABCA1* gene might also influence inflammation in atherosclerotic lesions independent of that on RCT. Our data would be coping with this notion because no plasma lipid parameter including LDL size differed between carriers and noncarriers. In contrast, the erythrocyte sedimentation rate was significantly lower in carriers of the K variant than in noncarriers in our population, even after adjustment for age and BMI (9.92 vs 8.64 mm/[L h], $P < .05$). Again, this effect could be observed in nonsmokers

only, strengthening the notion that smoking might influence *ABCA1* activity. However, this possible effect of the R219K variant needs further investigation.

The I883M variant had no effect on plasma lipids or carotid atherosclerosis in our study population. This observation contrasts with previous studies reporting either an increased progression of CAD and increased risk of cardiac events without an effect on plasma lipids [6] or an increased HDL-C [5,36] in subjects homozygous for the I883M variant. Further analyses of this variant in different ethnic populations are needed to clarify the role of this variant [37].

To further investigate if the R219K variant is the causative variant, we combined the R219K carrier status with the I883M carrier status. This approach revealed that the K219 variant is protective only in subjects homozygous for the I883 variant (Fig. 2, Table 3). This observation could be attributed to a proatherogenic effect of the M883 variant [6,38]. However, in our population, the I883M variant itself had no effect on carotid atherosclerosis. Therefore, we suggest that the R219K variant is possibly not the causative variant. In fact, the R219K variant may be in linkage disequilibrium with another variant responsible for the observed risk reduction. This observation is further supported by our haplotype analysis showing no difference in haplotype distribution between subjects with advanced carotid atherosclerosis and controls.

We conclude from this study that the R219K variant in the *ABCA1* gene can alter risk of atherosclerosis independently from HDL-C and TG plasma concentration in nonsmoking, healthy, middle-aged men. Because smoking abolished the protective effect of the R219K variant, we hypothesize that smoking may influence *ABCA1* efficacy. Considering the functions of *ABCA1* other than lipid transport, the fact that the protective effect of the R219K is not dependent on plasma HDL-C or TG concentration suggests to us that another function of *ABCA1* may be implicated.

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