*Msp*I Polymorphism at +83 bp in Intron 1 of the Human Apolipoprotein A1 Gene Is Associated With Elevated Levels of HDL Cholesterol and Apolipoprotein A1 in Nondiabetic Subjects but Not in Type 2 Diabetic Patients With Coronary Heart Disease

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OBJECTIVE — Elevated HDL cholesterol and its principal carrier protein apolipoprotein A1 [apo(a1)] are associated with reduced risk of coronary heart disease (CHD). No studies are available on the impact of the −75-bp and/or +83-bp polymorphisms of the apo(a1) gene on HDL cholesterol and apo(a1) levels in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — We determined the prevalence of the −75-bp and +83-bp polymorphisms of the apo(a1) gene by restriction fragment length polymorphism analysis among 308 unrelated nondiabetic subjects with CHD and among 251 unrelated patients with type 2 diabetes with CHD and in randomly selected 82 healthy men (CHD^-).

RESULTS — The rare $M1^-$ and $M2^-$ allele frequencies of the apo(a1) gene were 23 and 1.8%, respectively, among control subjects; 20 and 1.5%, respectively, among nondiabetic subjects with CHD; and 22 and 2.6%, respectively, among patients with type 2 diabetes and CHD (NS). Nonsmoking nondiabetic subjects with CHD having the $M2^{+-}$ genotype had higher HDL cholesterol (1.48 ± 0.19 vs. 1.23 ± 0.02 mmol/l, $P < 0.01$) and apo(a1) (1.43 ± 0.10 vs. 1.36 ± 0.02 g/l, $P < 0.05$) levels than subjects with the $M2^{++}$ genotype, even after adjustment for confounding factors. This association was not found among patients with type 2 diabetes and CHD.

CONCLUSIONS — We conclude that the +83-bp polymorphism of the apo(a1) gene is associated with elevated HDL cholesterol and apo(a1) levels in Finnish nondiabetic subjects but not in patients with type 2 diabetes.

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The protective effect of HDL cholesterol and apolipoprotein A1 [apo(a1)] on the risk of coronary heart disease (CHD) is mediated mainly through the promotion of cholesterol efflux from peripheral cells (1). In addition, both HDL cholesterol and apo(a1) may have antioxidant, antithrombotic, and anti-inflammatory properties, which could have important antiatherogenic effects (1). The level of circulating HDL cholesterol and apo(a1) is dependent on sex, BMI, age (2), smoking (3), alcohol intake, and lipid-lowering medication (4). In addition, different variants in the apo(a1) gene can modify its expression and affect HDL cholesterol and apo(a1) levels (5,6).

Restriction site polymorphisms have been identified at −75 bp in the promoter region and +37 and +83 bp in intron 1 of the apo(a1) gene. Polymorphisms at −75 and +83 bp of the apo(a1) gene have been related to elevated levels of HDL cholesterol and apo(a1) in nondiabetic subjects (5–7), although not confirmed in all studies (8,9). Association of the +83-bp polymorphism with elevated levels of HDL cholesterol has been stronger than that with the −75-bp polymorphism (6). The genotype effect on circulating apo(a1) and HDL cholesterol levels is modulated by sex and environmental factors such as smoking (3,7,10). The differences in HDL cholesterol and apo(a1) levels among various genotypes of the apo(a1) gene are not modified by different diets, suggesting that the possible benefit is independent of fat and cholesterol intake (11).

So far, no studies have been published on the impact of the −75- and/or +83-bp polymorphisms of the apo(a1) gene on HDL cholesterol and apo(a1) levels in patients with type 2 diabetes. To this aim, we screened nondiabetic and type 2 diabetic subjects with CHD for the −75- and +83-bp polymorphisms of the apo(a1) gene.

RESEARCH DESIGN AND METHODS

Subjects

All unrelated subjects (n = 641: 469 men, 172 women) participating in this study were

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Abbreviations: apo(a1), apolipoprotein A1; CHD, coronary heart disease; MI, myocardial infarction; PCR, polymerase chain reaction; WHO, World Health Organization.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of nondiabetic subjects without CHD (CHD$^-$), nondiabetic subjects with CHD (CHD$^+$), and patients with type 2 diabetes with CHD*

	No diabetes		Type 2 diabetes	
	CHD$^-$	CHD$^+$	CHD$^+$	*P*
n	82	308	251	
Sex (M/F)	82/0	221/87	166/85	
Age (years)	54 ± 1	58 ± 1*	64 ± 1*	<0.001
BMI (kg/m^2)	26.3 ± 0.4	27.9 ± 0.2*	29.8 ± 0.3*	<0.001
Waist-to-hip ratio	0.97 ± 0.01	0.96 ± 0.00	0.99 ± 0.01†	<0.001
Current smokers (%)	34	14*	7*	<0.001
Hypertensives (%)‡	0	89*	94*	<0.001
Users of β-blockers (%)	0	81*	83*	<0.001
Users of lipid-lowering drugs (%)	0	54*	44*	<0.001
Users of estrogen (%)§	0	9	5	0.371
Systolic blood pressure (mmHg)	136 ± 1	139 ± 1†	148 ± 1*	<0.001
Diastolic blood pressure (mmHg)	85 ± 1	81 ± 1*	83 ± 1†	0.003
Fasting plasma glucose (mmol/l)	5.6 ± 0.0	5.6 ± 0.0	9.5 ± 0.2*	<0.001
Fasting plasma insulin (pmol/l)	55.9 ± 4.0	79.5 ± 2.5*	107.4 ± 4.6*	<0.001
Total cholesterol (mmol/l)	5.98 ± 0.12	5.90 ± 0.06	5.72 ± 0.08	0.070
HDL cholesterol (mmol/l)	1.28 ± 0.03	1.21 ± 0.01†	1.08 ± 0.02*	<0.001
Total triglycerides (mmol/l)	1.51 ± 0.12	1.84 ± 0.05†	2.52 ± 0.13*	<0.001
Apo(a1) (g/l)	1.37 ± 0.02	1.34 ± 0.01	1.27 ± 0.02*	<0.001
Apo(b) (g/l)	1.02 ± 0.03	1.18 ± 0.02*	1.19 ± 0.02*	<0.001

Data are *n*, frequencies (%), or means ± SEM. *$P < 0.01$ (CHD$^-$ vs. CHD$^+$ groups); †$P < 0.05$ (CHD$^-$ vs. CHD$^+$ groups); ‡systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥95 mmHg or drug treatment for hypertension; §among female subjects.

Finnish and aged 33–82 years (60 ± 1 years, mean ± SEM). Finland is a genetic isolate (12) and the population of eastern Finland originates from 1 of the 2 groups of settlers that were founders of the present population in Finland (13). Coronary angiogram was performed previously at the University Hospital of Kuopio in 1986–1996 on 308 nondiabetic subjects (87 women, 221 men, aged 58 ± 1 years, BMI 27.9 ± 0.2 kg/m^2) and on 206 patients with type 2 diabetes (141 men, 65 women) who met the diagnostic criteria for type 2 diabetes (14). All of these 514 patients had >50% stenosis in >2 coronary arteries (CHD$^+$). In addition, we screened for the *Msp*I1 and *Msp*I2 polymorphisms of the apo(a1) gene in 45 patients with type 2 diabetes (25 men, 20 women) who had had a definite myocardial infarction (MI) according to the World Health Organization (WHO) criteria (15). All subjects were invited to an outpatient visit, including an interview on smoking history and drug treatment, measurement of height, weight, waist-to-hip ratio, and systolic and diastolic blood pressure. Type 2 diabetes was considered to be present if the diagnosis had been previously made according to the WHO criteria (14). Of the 251 type 2 diabetic patients, 28% were treated with diet only, 44% with oral hypoglycemic drugs, 16% with insulin, and 12% with combination therapy. Altogether, 277 CHD patients were receiving lipid-lowering therapy (statins 91%, fibrates 14%, resins 1%, guar-gum 7%, and combination therapy 10%). All nondiabetic subjects and those whose diabetes diagnosis was uncertain underwent an oral glucose tolerance test. The nondiabetic control group consisted of a random sample of 82 men (age 54 ± 1 years, BMI 26.3 ± 0.4) without a previous history of MI verified at hospital, no current symptoms of CHD by the Rose cardiovascular questionnaire, or ischemic ECG changes on an exercise test (CHD$^-$) (16). Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

Evaluation of clinical characteristics

Weight and height were measured with subjects wearing light clothing without shoes. BMI was computed as kg/m^2. Waist circumference was measured at the level of the umbilicus with the subjects standing and breathing normally. Hip circumference was measured at the level of the greatest hip girth. The waist-to-hip ratio was used as an indicator of body fat distribution. Blood pressure was measured after a 5-min rest on the right arm with subjects in the supine position with a mercury sphygmomanometer. Two readings were taken (1.5-min interval), and the latter reading was used in statistical analyses. In each measurement, blood pressure was read to the nearest 2 mmHg.

Determination of the polymorphisms in the apo(a1) gene

DNA was extracted from frozen whole-blood samples by the salting-out method (17). The forward primer to amplify the 433-bp fragment at the 5′ end of the apo(a1) gene was 5′-AGGGACAGAGCT GATCCTTGAACTCTTAAG-3′ and the reverse primer 5′-TTAGGGGACACCTAG CCCTCAGGAAGAGCA-3′. Polymerase chain reaction (PCR) was performed in a volume of 20 µl containing 100 ng genomic DNA. The amounts of Mg^{2+}, dNTP, and DNA polymerase (DynaZyme DNA Polymerase; Finnzymes, Espoo, Finland) used in each reaction were 1.5 mmol/l, 25 µmol/l, and 1 U, respectively. The thermal cycles started with 94°C for 4 min and were followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. A total volume of 10 µl containing 20 U *Msp*I was added directly to the PCR product and digested at 37°C overnight. After electrophoresis, the digested products were visualized on a 9% polyacrylamide gel with ethidium bromide staining.

There are 3 *Msp*I restriction sites within the region amplified by PCR located at −75, +37, and +83 bp (6). The genotypes of the −75-bp substitution are GG, GA, and AA (18). Both the G-to-A substitution at −75 bp and T-to-C and/or G-to-A substitutions at +83 bp result in a loss of *Msp*I restriction sites, which are detected simultaneously by a single *Msp*I digestion after PCR. The putative genotypes at −75 bp are defined as M1$^{+/+}$(GG) for the presence of *Msp*I sites in both alleles ($^+$ indicates the presence and $^-$ indicates the absence of the *Msp*I restriction site), M1$^{-/-}$(AA) for the absence of the site in both alleles, and M1$^{+/-}$(GA) for a heterozygote. The putative genotypes at +83 bp are M2$^{+/+}$, M2$^{+/-}$, and M2$^{-/-}$.

Analytical methods

Plasma glucose level in the fasting state was measured by the glucose oxidase method (2300 Stat Plus; YSI, Yellow Springs, OH).

Table 2—*Frequencies (%) of the simultaneous presence (+) or absence (−) of the M1 (*MspI *−75 bp) and M2 (*MspI *+83 bp) polymorphisms of the apo(a1) gene in nondiabetic subjects without CHD (CHD−), in nondiabetic subjects with CHD (CHD+), and in patients with type 2 diabetes with CHD*

Combined genotypes	No diabetes		Type 2 diabetes	
	CHD−	CHD+	CHD+	*P*
n	82	308	251	
$M1^{++}$ $M2^{++}$	47 (57.3)	194 (63.0)	144 (57.4)	NS
$M1^{+-}$ $M2^{++}$	27 (32.9)	90 (29.2)	84 (33.4)	NS
$M1^{+-}$ $M2^{--}$	0	0	1 (0.4)	NS
$M1^{--}$ $M2^{++}$	5 (6.1)	15 (4.9)	12 (4.8)	NS
$M1^{++}$ $M2^{+-}$	2 (2.4)	7 (2.3)	8 (3.2)	NS
$M1^{+-}$ $M2^{+-}$	1 (1.2)	2 (0.6)	1 (0.4)	NS
$M1^{++}$ $M2^{--}$	0	0	1 (0.4)	NS

Data are *n* or *n* (%).

For the determination of plasma insulin, blood was collected in EDTA-containing tubes, and, after immediate centrifugation, the plasma was stored at −20°C (<2 weeks) until the analysis. Plasma insulin concentration was determined by a commercial double-antibody solid-phase radioimmunoassay (Phadeseph Insulin RIA 100; Pharmacia, Uppsala, Sweden). Lipoprotein fractionation was performed by ultracentrifugation and selective precipitation (19) as previously described (20). Cholesterol and triglyceride levels from whole serum and from lipoprotein fractions were assayed by automatized enzymatic methods (Boehringer-Mannheim, Mannheim, Germany). Serum apo(a1) and apo(b) levels were determined from samples stored at −70°C by a commercial immunochemical method (Kone, Helsinki) based on the measurement of immunoprecipitation at 340 nm (21).

Statistical analysis

All calculations were performed using SPSS, version 7.5.1 (SPSS, Chicago). Data are presented as means ± SEM. Statistical significance between the 2 groups was evaluated with the χ^2 test or Student's *t* test when appropriate. In comparison of the 2 groups, the adjustment for confounding factors (BMI, age, sex, lipid-lowering drugs, and smoking) was done with the analysis of covariance (1-way analysis). The normality of the distribution of variables was tested with the Kolmogorov-Smirnov test. Because plasma glucose, insulin, and serum triglycerides were not normally distributed, they were logarithmically transformed in statistical analyses.

RESULTS — The characteristics of the study groups by the presence of CHD are given in Table 1. The groups differed from each other with respect to all clinical characteristics and biochemical measurements. Subjects with type 2 diabetes were more obese and had higher levels of systolic blood pressure, glucose, insulin, and total triglycerides, and lower levels of HDL cholesterol, than nondiabetic subjects.

The frequencies of the hetero- and homozygous subjects for the *Msp*I polymorphisms at −75 and +83 bp of the apo(a1) gene were in Hardy-Weinberg equilibrium. The frequencies of the combined genotypes were not in linkage disequilibrium between the 2 polymorphic sites. $M1^-$ and $M2^-$ allele frequencies of the apo(a1) gene were 23 and 1.8%, respectively, in control subjects; 20 and 1.5%, respectively, in nondiabetic patients with CHD; and 22 and 2.6%, respectively, in type 2 diabetes patients with CHD (NS between groups). The frequencies of the combined genotypes (haplotypes) of the *Msp*I1 and *Msp*I2 polymorphisms did not differ among the study groups (Table 2).

The $M2^-$ allele of the apo(a1) gene was associated with elevated HDL cholesterol levels in nondiabetic CHD subjects but not in patients with type 2 diabetes

Table 3—*Comparison of clinical characteristics, HDL cholesterol, apo(a1), serum triglycerides, fasting plasma glucose, and insulin levels among subjects with different* MspI*2 (+83 bp) genotypes of the apo(a1) gene in nondiabetic subjects without CHD (CHD−), in nondiabetic subjects with CHD (CHD+), and in patients with type 2 diabetes with CHD*

	No diabetes				Type 2 diabetes	
	CHD−		CHD+		CHD+	
	$M2^{++}$	$M2^{+-}$	$M2^{++}$	$M2^{+-}$	$M2^{++}$	$M2^{+-/--}$*
n	79	3	299	9	240	11
Sex (M/F)	79/0	3/0	214/85	7/2	159/81	7/4
Age (years)	54 ± 1	50 ± 3	58 ± 1	56 ± 3	64 ± 1	62 ± 2
BMI (kg/m^2)	26.3 ± 0.4	27.7 ± 2.3	27.9 ± 0.2	28.8 ± 1.5	29.8 ± 0.3	29.6 ± 1.1
HDL cholesterol (mmol/l)	1.27 ± 0.03	1.39 ± 0.18	1.20 ± 0.01	1.38 ± 0.11†§	1.08 ± 0.02	1.02 ± 0.06
Nonsmokers	1.28 ± 0.04	1.38;1.70‖	1.23 ± 0.02	1.48 ± 0.19†	1.15 ± 0.03	0.90 ± 0.06
Apo(a1) (g/l)	1.37 ± 0.02	1.46 ± 0.18	1.34 ± 0.01	1.35 ± 0.07	1.27 ± 0.02	1.21 ± 0.07
Nonsmokers	1.37 ± 0.03	1.46;1.78‖	1.36 ± 0.02	1.43 ± 0.10‡	1.32 ± 0.02	1.10 ± 0.09‡
Tg (mmol/l)	1.52 ± 0.13	1.35 ± 0.20	1.86 ± 0.05	1.27 ± 0.11†§	2.52 ± 0.14	2.65 ± 0.50
Insulin (pmol/l)	55.1 ± 4.0	76.4 ± 27.2	79.9 ± 2.6	68.0 ± 7.6	107 ± 4.7	113 ± 15
Glucose (mmol/l)	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.3 ± 0.2	9.4 ± 0.2	10.9 ± 1.3†

Data are *n* or means ± SEM. *$M2^{+-}$ and $M2^{--}$ groups were combined because of a small number of subjects with the $M2^{--}$ genotype (*n* = 2); †$P < 0.01$ and ‡$P < 0.05$ ($M2^{++}$ vs. $M2^{+-/--}$) within the CHD^+ group; §after adjustment for BMI, age, sex, use of lipid-lowering drugs, and smoking (current and former smokers combined); ‖individual values for 2 subjects. Tg, triglycerides.

Table 4—*Comparison of clinical characteristics, HDL cholesterol, apo(a1), serum triglyceride, fasting plasma glucose, and insulin levels among subjects with different* Msp*I1 (−75 bp) genotypes of the apo(a1) gene in nondiabetic subjects without CHD (CHD$^-$), in nondiabetic subjects with CHD (CHD$^+$), and in patients with type 2 diabetes with CHD*

	No diabetes						Type 2 diabetes		
	CHD$^-$			CHD$^+$			CHD$^+$		
	M1^{++}	M1^{+-}	M1^{--}	M1^{++}	M1^{+-}	M1^{--}	M1^{++}	M1^{+-}	M1^{--}
n	49	28	5	201	92	15	153	86	12
Sex (M/F)	49/0	28/0	5/0	145/56	65/27	11/4	101/52	58/28	7/5
Age (years)	54 ± 1	55 ± 1	55 ± 2	58 ± 1	58 ± 1	58 ± 2	65 ± 1	63 ± 1	61 ± 3
BMI (kg/m^2)	25.9 ± 0.4	26.4 ± 0.6	29.5 ± 1.9	28.0 ± 0.3	27.5 ± 0.4	29.1 ± 1.1	29.8 ± 0.4	29.7 ± 0.5	30.1 ± 1.0
HDL cholesterol (mmol/l)	1.29 ± 0.04	1.25 ± 0.04	1.29 ± 0.08	1.21 ± 0.02	1.20 ± 0.03	1.24 ± 0.08	1.08 ± 0.02	1.08 ± 0.03	1.13 ± 0.05
Apo(a1) (g/l)	1.37 ± 0.03	1.38 ± 0.04	1.33 ± 0.03	1.33 ± 0.01	1.36 ± 0.02	1.40 ± 0.06	1.25 ± 0.02	1.30 ± 0.03	1.27 ± 0.04
Tg (mmol/l)	1.49 ± 0.20	1.52 ± 0.09	1.58 ± 0.20	1.81 ± 0.06	1.92 ± 0.10	1.88 ± 0.23	2.43 ± 0.13	2.74 ± 0.31	2.13 ± 0.45
Insulin (pmol/l)	52.6 ± 5.3	53.6 ± 3.9	101 ± 28.5	81.4 ± 3.3	74.3 ± 3.9	85.6 ± 9.9	104 ± 4.6	112 ± 11	116 ± 16
Glucose (mmol/l)	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.5 ± 0.1	5.7 ± 0.2	9.5 ± 0.3	9.4 ± 0.3	9.5 ± 0.9

Data are *n* or means ± SEM. Tg, triglycerides.

with CHD after adjustment for BMI, age, sex, lipid-lowering drugs, and smoking (Table 3). When smokers (current and former smokers) were excluded, the association of the M2$^-$ allele of the apo(a1) gene with elevated apo(a1) and HDL cholesterol levels became even stronger in nondiabetic subjects with CHD. Furthermore, the M2$^-$ allele of the apo(a1) gene was associated with lower total serum triglyceride levels in nondiabetic subjects with CHD (Table 3). Nonsmoking type 2 diabetic subjects with the M2^{+-}/M2^{--} genotype had lower levels of apo(a1) (P = 0.042) (trend for HDL cholesterol, P = 0.082) and higher levels of fasting glucose (P = 0.006) compared with those in subjects with the M2^{++} genotype. The M1$^-$ allele of the apo(a1) gene was not associated with HDL cholesterol, triglyceride, or apo(a1) levels in any of the study groups (Table 4).

CONCLUSIONS

Elevated levels of HDL cholesterol and apo(a1) are associated with reduced risk of developing CHD, probably because of the ability of HDL particles to promote cholesterol efflux from the cells (22,23). The heritability of HDL cholesterol level is ~40% (24), and environmental factors, including alcohol intake, obesity, exercise, smoking, and lipid-lowering drugs, may alter the level of circulating HDL cholesterol. Variants in the apo(a1) gene have been recently reported that potentially could modify HDL cholesterol and apo(a1) levels.

A G-to-A transition in the promoter region of the apo(a1) gene (−75 bp) is relatively common, occurring in ~20% of normal Caucasians, and the frequency of the rare M2$^-$ allele (+83 bp) has been reported to be 4.1% (6). In the present study, the M1$^-$ allele frequency (21%) was quite similar, whereas the M2$^-$ allele frequency (2%) was half of that reported in previous studies (5,6). Mechanisms explaining the relationship of T and/or A substitutions with increased HDL cholesterol and apo(a1) levels are currently unknown. However, hypomethylation, which occurs in the T and/or A substitution at the 5′ region of the apo(a1) gene, increases apo(a1) gene expression (25). Alternatively, the T and/or A substitutions, which are located in the 5′-end leader region of the apo(a1) mRNA, could be important for the initiation of mRNA translation (26,27). In the present study, the loss of the *Msp*I site by the C-to-T (+83 bp) and/or G-to-A (+84 bp) substitutions was associated with increased HDL cholesterol level in nondiabetic subjects with CHD, and, in agreement with previous studies (6), this association appeared to be more significant than that of the G-to-A transition at −75 bp. Although the loss of the *Msp*I site in the 5′-untranslated region of the apo(a1) gene (+83 bp) is less common than the G-for-A substitution in the promoter region (−75 bp), it could be an important marker for the risk of CHD if it significantly affects HDL cholesterol levels. In previous studies, the beneficial effect of the −75- or +83-bp polymorphisms on apo(a1) and HDL cholesterol levels was seen among nonsmokers, but not in men who smoked, and it was also absent in women (3). In the meta-analysis, which included >3,000 healthy adults, the association of the G-to-A polymorphism in the promoter area of the apo(a1) gene with elevated apo(a1) levels was small, but significant, and weaker among healthy women than among men (28). In haplotype analysis, combining 2 restriction fragment length polymorphisms, the association of −75-bp and +83-bp polymorphisms of the apo(a1) gene was even more informative compared with single restriction fragment length polymorphism analysis among nonsmoking healthy subjects (29). In the present study, haplotype analysis could not be applied because of lower frequency (2%) of the M2$^-$ allele of the apo(a1) gene in the Finnish study population. In accordance with a previous study (6), triglyceride levels in our study tended to be lower in nondiabetic subjects with the M2^{+-} genotype, but this association was seen only among men (n = 7) because of the small number of women with the M2^{+-} genotype (n = 2).

Although polymorphisms at −75 and +83 bp of the apo(a1) gene have been associated with increased levels of HDL cholesterol and apo(a1) in nondiabetic subjects (29,30), no studies are available on patients with type 2 diabetes. In the present study, the number of subjects with the M2$^-$ allele of the apo(a1) gene in the control group was relatively small (n = 3), which could explain why the association of the M2$^-$ allele and elevated HDL cholesterol and apo(a1) levels was statistically significant only among nondiabetic CHD patients. The −75-bp and +83-bp polymorphisms of the apo(a1) gene were not associated with elevated levels of

apo(a1) or HDL cholesterol in patients with type 2 diabetes with CHD. In fact, nonsmoking subjects with type 2 diabetes and with the $M2^{+-}/M2^{--}$ genotype had lower levels of apo(a1) and higher levels of fasting glucose than subjects with the $M2^{++}$ genotype. This may indicate that the disturbances in lipid and lipoprotein metabolism in type 2 diabetes could be so profound that the variants in the apo(a1) gene are unable to upregulate HDL cholesterol and apo(a1) levels among these patients.

In conclusion, beneficial effects of the −75-bp or +83-bp polymorphisms of the apo(a1) gene are not found in subjects with additional cardiovascular risk factors such as smoking and type 2 diabetes. Thus, it is unlikely that the −75-bp or +83-bp polymorphisms of the apo(a1) gene have a major role in determining lipoprotein and apolipoprotein levels or the risk for CHD in Finnish patients with type 2 diabetes.

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