Variants of the microsomal triglyceride transfer protein gene are associated with plasma cholesterol levels and body mass index

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Abstract The microsomal triglyceride transfer protein (MTP) is required for the assembly and secretion of apolipoprotein B (apoB)-containing lipoproteins from liver and intestine. We set out to study the phenotypic modulation of all common genetic variants in the MTP gene. In addition, we aimed at characterizing the association between the various polymorphisms. A total of 564 healthy men were genotyped for the MTP −493 G/T, −400 A/T, and −164 T/C promoter polymorphisms, as well as the Q/H 95, I/T 128, Q/E 244, and H/Q 297 missense polymorphisms. The −493 G/T, −164 T/C, and I/T 128 polymorphisms showed to be in almost complete linkage disequilibrium. Subjects homozygous for the less common −493 T, −164 C, and T 128 alleles showed significantly lower plasma total and LDL cholesterol levels and plasma LDL apoB levels, and also significantly higher body mass index (BMI) and plasma insulin levels compared with carriers of the common alleles. The associations between plasma total cholesterol and MTP −493 genotype was verified in a cohort consisting of 1,117 disease-free control subjects of the West of Scotland Coronary Prevention Study (WOSCOPS). None of the other polymorphisms showed any significant change in either lipid and lipoprotein levels or anthropometric variables. In summary, two promoter polymorphisms and one missense polymorphism in the MTP gene alter plasma total and LDL cholesterol levels, plasma LDL apoB levels, BMI, and insulin levels. This may, in turn, have implications for genetic regulation of cardiovascular risk factors.—Ledmyr, H., F. Karpe, B. Lundahl, M. McKinnon, C. Skoglund-Andersson, and E. Ehrenborg. **Variants of the microsomal triglyceride transfer protein gene are associated with plasma cholesterol levels and body mass index. *J. Lipid Res.* 2002.** 43: **51–58.**

Supplementary key words polymorphism • cardiovascular disease • LDL cholesterol • microsomal triglyceride transfer protein

The microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein that consists of a large unique 97 kDa subunit and protein disulfide isomerase (PDI) (1). MTP is present in high concentration on the luminal side of the endoplasmatic reticulum (ER) in the liver, intestine, and heart (2–4). The function of MTP is to lipidate the growing apolipoprotein B (apoB) polypeptide chain during translation, allowing apoB to fold correctly and assemble a lipoprotein with a neutral lipid core before secretion (5, 6). The unique subunit confers the lipid transfer activity of the complex, whereas the PDI possesses the ER retention signal that is crucial for its localization (5). Functional MTP is an absolute requirement for the assembly and cellular secretion of apoB-containing lipoproteins, and mutations causing dysfunction of the 97 kDa subunit results in abetalipoproteinemia (7–9). Recent studies indicate that the actual concentration of MTP in the ER is the critical determinant of lipoprotein secretion (10). As the intracellular concentration of MTP is tightly controlled, any constitutive or induced alterations in MTP expression is likely to have an effect on secretion pattern of lipoproteins, and genetic variability may modulate MTP concentration and/or activity. Increased MTP expression has also been associated with obesity and visceral fat accumulation in the rat (11). A common promoter polymorphism in the MTP gene, −493 G/T, has been shown to influence the transcriptional activity and to be associated with low plasma levels of LDL cholesterol in healthy middle-aged men (12). The phenotype is switched to low plasma triglyceride concentrations on a familial hypercholesterolemia background suggestive of a gene-gene interaction with the LDL receptor (13). However, a study of the Framingham Offspring Study cohort was unable to detect a corresponding phenotype (14). In contrast, a recent study of the MTP −493 polymorphism in healthy young black men showed association with high LDL cholesterol and high

Abbreviations: apoB, apolipoprotein B; BMI, body mass index; ER, endoplasmatic reticulum; MTP, microsomal triglyceride transfer protein; PDI, protein disulfide isomerase.

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plasma triglycerides (15). Presently, three promoter polymorphisms and four common missense polymorphisms in the MTP gene have been described. The promoter polymorphisms are located at positions −493 (G/T), −400 (A/T), and −164 (T/C) upstream of transcription start. The polymorphisms in the coding region all lead to exchange of amino acid and are thus putatively functional. These missense polymorphisms, previously reported in Caucasians, are Q/H 95, I/T 128, Q/E 244, and H/Q 297 (16). Against this background, we hypothesized that the three promoter polymorphisms as well as the four amino acid changes in the coding region of the MTP gene may influence plasma lipid and lipoprotein levels. We set out to study this in a large cohort of healthy 50-year-old men in which there was detailed information on plasma lipids, metabolic, hormonal, and anthropometric variables.

MATERIALS AND METHODS

Human subjects

A total of 564 healthy 50-year-old men living in the northern region of the Stockholm area were recruited after a random population-based screening. Exclusion criteria were chronic disease of any kind, familial hypercholesterolemia, hyperthyroidism, liver disease, history of coronary heart disease or arterial thromboembolic disease, continuous treatment with antihypertensive or lipid-lowering agents, alcohol abuse or any psychiatric disorders that would interfere with compliance, and participation in other ongoing studies. Specific exclusion criteria were plasma LDL cholesterol levels above 6.5 mM, fasting blood glucose levels above 6.1 mM, serum thyroid-stimulating hormone (S-TSH) above 5 mU/l, and S-Creatinine above 120 μM. Only men of North European descent were included. The procedures described in this study have been approved by the Ethics Committee at the Karolinska Hospital. All subjects gave informed consent to participation. A second cohort consisted of 1,117 disease-free control subjects of the West of Scotland Coronary Prevention Study (WOSCOPS): men 45 to 64 years of age. The sample, taken at random, was used for the study of genotype-phenotype relationship. The subset of 1,117 control subjects was selected after completion of the study to ensure that they had remained disease-free. The WOSCOPS has previously been described in detail (17, 18). The WOSCOPS was approved by the ethics committees of the University of Glasgow, the Karolinska Hospital, Stockholm, Sweden, and all participating health boards.

Blood sampling

Venous blood samples were drawn into precooled sterile tubes (Vacutainer, Becton Dickinson) containing Na_2-EDTA (final concentration 4 mM), and plasma was immediately recovered by low-speed centrifugation (1.750 *g*, 20 min at 1°C). PMSF (10 mM, dissolved in isopropanol) and aprotinin (1.4 mg/ml; Trasylol, Bayer) were immediately added to the isolated plasma to concentrations of 10 μM and 28 μg/ml, respectively.

Determination of major plasma lipids, lipoproteins, and insulin

Fasting plasma concentrations of cholesterol and triglycerides in VLDL, LDL, and HDL were determined by a combination of preparative ultracentrifugation, precipitation of apoB-containing lipoproteins, and lipid determination (19). Blood was collected into vacutainer tubes containing heparin (143 USP units) for determination of insulin. Insulin was measured by ELISA based on a monoclonal antibody according to the manufacturer's advice (DAKO Insulin, DAKO Diagnostics Ltd.).

In the WOSCOPS subjects, plasma total cholesterol was measured twice during the course of the study, and the mean was used as the base-line level (17).

Subfractionation of apoB-containing lipoproteins and determination of apoB content

LDL was isolated by density gradient ultracentrifugation (20). After a 16 h spin at 40,000 rpm and 15°C (Beckman SW40), the top 0.5 ml layer was aspirated (VLDL). The tube was then sliced 57 mm from the top to harvest the fraction (d = 1.006–1.061 kg/l) containing both IDL and LDL. The protein concentration of the isolated fraction was determined according to the method of Lowry et al. (21) after addition of SDS to the reagent mixture to clear turbidity.

DNA analysis

The −493 G/T and −400 A/T polymorphisms were genotyped as described previously (12). All amplifications were performed in a 25 μl reaction mix containing 100 ng of genomic DNA, 0.8 μM of each primer, 2 mM of each dNTP (Boehringer Mannheim, Germany), 1 unit Taq-polymerase (SDS Promega, Madison, WI), 50 mM KCl, 10 mM Tris-HCl, and 0.1% Triton X-100. For genotyping of the MTP −164 T/C polymorphism, the $MgCl_2$ concentration was 2 mM. Primers used were MTP164-1 (5′-GGTTTGGTTTAGCTCTCAAAAGTG) and MTP164-2 (5′-AGTGAGGGAGTGACCCTCTTC). The amplification cycle started with denaturation at 94°C for 3 min, followed by 40 cycles at 94°C for 45 s, 60°C for 30 s, and 72°C for 1 min. A final elongation at 72°C for 5 min ended the reaction. The analysis of the MTP Q/H 95 polymorphism was performed using primers MTP95-1 (5′-ATGAAGGATGTAAATGTTGAAAATGTGAATCTG CA) and MTP95-2 (5′-AGTTGGAGAAAAAGTTGTGGAATC). Annealing temperature of this PCR was 58°C and the $MgCl_2$ concentration was 2 mM. Also, in the analysis of the I/T 128 polymorphism, primer MTP128-1 (5′-TTTTAACAGCTTTCTTTCTG TTACTC) was used together with a mismatch primer MTP128-2 (5′-GTTGTGGAATCTAAACGCCCCTTTATCCTTCCATGG). The PCR was performed at an annealing temperature of 63°C and a $MgCl_2$ concentration of 3 mM. Primer MTP244-1 (5′-GATGATT ACTTGTTATAAAGATGG) and the mismatch primer MTP244-2 (5′-AAAATTTTAGCATTATCTTACTTCG) were used to analyze the MTP Q/E 244 polymorphism. Amplification was performed with a $MgCl_2$ concentration of 3 mM and annealing temperature of 58°C. The analysis of the MTP H/Q 297 polymorphism was performed with primers MTP297-1 (5′-GAATGATTATAATATAG CATTTCC) and MTP297-2 (5′-GTCTGATGTCATGATTATTCC), and amplification was performed at an annealing temperature of 52°C and with a final $MgCl_2$ concentration of 3 mM. The PCR products were digested with 10 units of *Bsr*I for detection of the −164 T/C and H/Q 297, *Pst*I for detection of the Q/H 95 polymorphism, *Bst*II for the I/T 128 detection, and *Xmn*I for detection of the Q/E 244 polymorphisms, respectively. The digested fragments were separated on 2.5–3% agarose gels containing ethidium bromide for visualization under UV light. The apoE genotype was determined as described previously (22, 23). For the analysis of the MTP −493 G/T polymorphism in the WOSCOPS subjects, 50–100 ng of genomic DNA was amplified in a 50 μl reaction mix containing 0.1 μM of each primer, 3 mM MgCl, 15 mM Tris-HCl, pH 8.0, 50 mM KCl, and 1 unit AmpliTaqGold (Applied Biosystems, NJ). Forward primers used were MTPNhe1 (5′-GCTAGCGCTGATTTGCTCCAAC) and MTP493-U (5′-AGTTTCACACATAAGGACAATCATCTA). Reverse biotin-labeled primer was MTP-4bio (5′-CCAGCTAGGAGTCACTGA GA). The amplification started with activation of the Ampli-

TaqGold at 95°C for 7 min followed by denaturation at 94°C for 45 s. The PCR amplification was performed according to touchdown principle as follows: denaturation at 94°C for 45 s, annealing at 64°C (7 cycles), 60°C (7 cycles), 56°C (8 cycles), 54°C (8 cycles), 52°C (8 cycles), and 48°C (13 cycles) for 30 s, respectively, and elongation at 72°C for 1 min. A final elongation at 72°C for 5 min ended the reaction. Determination of genotypes was performed with real-time sequencing using the Pyrosequencing equipment according to manufacturer's advice (Pyrosequencing AB, Uppsala, Sweden). The MTP493PSQ (5′-AAC ATTATTTTGAAGTGATTGG) or MTP493PSQ2 (5′-TATTTTG AAGTGATTGGT) was used as sequencing primer.

Statistical analysis

Allele frequencies were determined by gene counting. A χ^2 test was used to compare the observed numbers of each MTP genotype with those expected for a population in Hardy-Weinberg equilibrium. The normalized linkage disequilibrium coefficient (D′) for all pairs of MTP polymorphisms was calculated according to Ott (24). Distribution of continuous variables in groups were expressed as means ± SD. Coefficients of skewness were calculated to test deviations from normal distribution. Logarithmic transformation was performed on individual values of skewed variables, and a normal distribution of transformed values was confirmed before statistical computations and significance testing. One-way analyses of covariance (with BMI, insulin, or smoking as covariates) and two-way analyses of variance were performed to test whether genetic variation in the MTP gene was associated with differences in plasma lipid protein levels or anthropometric variables. The Scheffé multiple comparison test was used as post hoc test. All statistical analyses were performed using StatView (SAS Institute Inc.) version 5.0.1. for Windows.

The statistical analysis of the WOSCOPS data was performed using the SAS Package version 6.12.

RESULTS

Allele frequencies and linkage disequilibrium

Three promoter (−493 G/T, −400 A/T, and −164 T/C) and four missense polymorphisms (Q/H 95, I/T 128, Q/E 244, and H/Q 297) in the MTP gene were analyzed in 564 healthy 50-year-old men of North European descent. Normalized linkage disequilibrium coefficients (D′) and allele frequencies according to MTP genotype are listed in **Table 1**. The promoter polymorphisms, −493 G/T and −164 T/C, both showed rare allele frequencies of 0.24, whereas the T allele frequency of the −400 A/T polymorphism was 0.29. The Q/H 95 polymorphism showed a rare allele frequency of 0.04, whereas the I/T 128 rare allele frequency was 0.24. The E allele frequency of the Q/E 244 polymorphism was 0.06 and the less common Q allele of the H/Q 297 polymorphism showed a frequency of 0.23. The −493 G/T, −164 T/C, and I/T 128 polymorphisms were in almost complete linkage disequilibrium with D′ values varying between 0.95 and 0.97 ($P < 0.001$). The −400 A/T polymorphism showed a D′ value of 0.96, which suggests an allelic association with the −493 G/T polymorphism, but other unknown associations could not be excluded. Also, the −493 G/T and the H/Q 297 polymorphisms showed a statistically significant D′ value of 0.20 ($P < 0.001$). Neither of the other polymorphisms, Q/H 95 or Q/E 244, showed any statistically significant D′ values when compared with the −493 G/T polymorphism. None of the allelic distributions deviated significantly from that predicted by the Hardy-Weinberg equilibrium.

TABLE 1. Allele frequencies and normalized linkage disequilibrium coefficients (D′) according to MTP genotype

	Rare Allele Frequency	D′	*P*
−493 G/T	0.24		
−400 A/T	0.29	0.96	[a]
−163 T/C	0.24	0.95	<0.001
Q/H 95	0.04	0.81	[b]
I/T 128	0.24	0.97	<0.001
Q/E 244	0.06	0.84	[b]
H/Q 297	0.23	0.20	<0.001

D′ value indicates linkage disequilibrium with the −493 G/T polymorphism.

[a] Unable to discriminate between allelic and other unknown associations.

[b] Unable to compute a reliable *P* value due to insignificant number of rare homozygotes in the cohort.

Association of the MTP polymorphisms with lipid and lipoprotein levels

Mean plasma concentrations of total and LDL cholesterol are shown according to MTP genotype in **Table 2**. Subjects homozygous for the −493 T allele had lower total cholesterol levels compared with carriers of the G allele (4.89 mM vs. 5.35 mM vs. 5.39 mM, respectively, $P = 0.03$). This was reflected by lower plasma LDL cholesterol levels (3.16 mM vs. 3.54 mM vs. 3.59 mM, $P = 0.03$). Because the −164 T/C and I/T 128 polymorphisms are in almost complete allelic association with the −493 G/T polymorphism, these polymorphisms also showed statistically significant associations with plasma total and LDL cholesterol with similar numerical values (data not shown). To validate these findings, 1,117 disease-free control subjects of the WOSCOPS were genotyped for the −493 G/T polymorphism. Mean plasma concentrations of total and LDL cholesterol in control subjects in the WOSCOPS are shown according to MTP genotype in **Table 3**. Individuals homozygous for the −493 T allele showed significantly lower total cholesterol levels compared with carriers of the G allele (6.84 mM vs. 7.05 mM vs. 7.02 mM, respectively, $P = 0.04$). There was also a trend toward decreased LDL cholesterol concentrations in subjects homozygous for the T allele compared with subjects homozygous for the G allele (4.97 mM vs. 4.84 mM, respectively, $P = 0.04$) Table 3.

No significant change in plasma total or LDL cholesterol levels were observed for any of the −400 A/T (data not shown), Q/H 95, Q/E 244, or H/Q 297 genotype groups (Table 2). In addition, no significant change in plasma total or VLDL triglyceride levels or HDL cholesterol levels were observed with any MTP genotype group (Tables 2 and 3).

Association of the MTP genotypes with plasma total and LDL apoB levels

Mean plasma concentrations of total and LDL apoB measured in a subset of the 564 subjects (between 355 and

TABLE 2. Plasma concentrations of lipids and major lipoproteins in healthy subjects grouped according to MTP genotype

	Triglycerides				Cholesterol			
	Plasma Total	VLDL	LDL	HDL	Plasma Total	VLDL	LDL	HDL
	mM				*mM*			
MTP −493 G/T								
GG, n = 316	1.56 ± 0.95	1.15 ± 0.92	0.29 ± 0.10	0.14 ± 0.05[a]	5.39 ± 0.95[a]	0.47 ± 0.38	3.59 ± 0.87[a]	1.24 ± 0.34
GT, n = 218	1.54 ± 0.98	1.12 ± 0.91	0.29 ± 0.10	0.14 ± 0.05[a]	5.35 ± 1.00	0.47 ± 0.41	3.54 ± 0.84	1.24 ± 0.34
TT, n = 30	1.35 ± 0.71	0.96 ± 0.70	0.28 ± 0.07	0.11 ± 0.04	4.89 ± 0.90	0.41 ± 0.31	3.16 ± 0.74	1.15 ± 0.32
P	0.50	0.53	0.55	**0.02**	**0.03**	0.70	**0.03**	0.34
MTP Q/H 95								
QQ, n = 527	1.54 ± 0.95	1.13 ± 0.91	0.29 ± 0.10	0.14 ± 0.05	5.36 ± 0.97	0.46 ± 0.39	3.56 ± 0.85	1.24 ± 0.34
QH + HH, n = 36	1.55 ± 0.94	1.10 ± 0.83	0.32 ± 0.11	0.13 ± 0.06	5.18 ± 0.96	0.49 ± 0.40	3.45 ± 0.89	1.16 ± 0.30
P	0.94	0.81	0.11	0.37	0.30	0.74	0.40	0.21
MTP Q/E 244								
QQ, n = 508	1.53 ± 0.92	1.12 ± 0.87	0.29 ± 0.10	0.14 ± 0.05	5.35 ± 0.97	0.45 ± 0.36	3.56 ± 0.85	1.23 ± 0.33
QE, n = 55	1.66 ± 1.21	1.25 ± 1.16	0.29 ± 0.10	0.14 ± 0.05	5.34 ± 0.99	0.57 ± 0.56	3.46 ± 0.92	1.21 ± 0.37
P	0.54	0.58	0.63	0.56	0.98	0.20	0.38	0.33
MTP H/Q 297								
HH, n = 349	1.53 ± 0.93	1.13 ± 0.89	0.28 ± 0.09	0.14 ± 0.05[b]	5.33 ± 0.95	0.46 ± 0.38	3.52 ± 0.84	1.24 ± 0.34
HQ, n = 188	1.53 ± 0.97	1.11 ± 0.91	0.30 ± 0.11	0.13 ± 0.05	5.36 ± 1.01	0.46 ± 0.37	3.60 ± 0.88	1.21 ± 0.32
QQ, n = 20	1.65 ± 1.20	1.20 ± 1.14	0.28 ± 0.09	0.12 ± 0.04	5.67 ± 1.11	0.60 ± 0.60	3.54 ± 1.02	1.27 ± 0.47
P	0.89	0.60	0.14	**0.001**	0.42	0.56	0.60	0.68

Values are mean ± SD. Differences between genotype groups were assessed by ANOVA. $P \leqslant 0.05$ are shown in bold.
[a] Significantly different from individuals carrying the TT genotype.
[b] Significantly different from individuals carrying the QQ according to Sheffé's post hoc test for continuous variables.

392 individuals, depending on available genotype) are shown according to MTP genotype in **Table 4**. Individuals homozygous for the −493 T allele showed significantly lower plasma LDL apoB levels than individuals carrying one or two copies of the −493 G allele (71.5 mg/l vs. 87.1 mg/l vs. 86.0 mg/l, respectively, $P < 0.01$). Similar values were observed for the −164 T/C and I/T 128 genotype groups (data not shown). This effect was of the same proportional magnitude as that for LDL cholesterol. Also, individuals homozygous for the −400 T allele showed a significantly decreased plasma LDL apoB level compared with carriers of one or two A alleles (77.2 mg/l vs. 87.0 mg/l vs. 84.8 mg/l, respectively, $P = 0.03$). The same effect was seen in individuals hetero- or homozygous for the 95 H allele (79.1 mg/l vs. 86.1 mg/l, respectively, $P = 0.03$). No significant changes in plasma total or LDL apoB levels were observed for either one of the Q/E 244 or H/Q 297 genotype groups (Table 4).

Association of the MTP genotypes with anthropometric variables and insulin

Table 5 shows insulin concentrations and anthropometric variables in the cohort grouped according to MTP genotype. Subjects homozygous for the rare −493 T allele showed a significant increase in BMI (27.5 kg/m^2 vs. 25.8 kg/m^2 vs. 25.7 kg/m^2, respectively, $P = 0.02$) and a significant increase in waist circumference (100.8 cm vs. 95.8 cm vs. 96.4 cm, respectively, $P = 0.02$) compared with subjects carrying one or two copies of the G allele. These associations were also seen in the −164 T/C and I/T 128 genotype groups, and in the −400 A/T genotype group (data not shown). However, when adjusting BMI and waist circumference for insulin, the significance between these variables and MTP genotype groups was lost, which indicates that these variables were interdependent. The significant alterations in plasma total and LDL cholesterol were still statistically significant after adjustment for insulin,

TABLE 3. Plasma concentrations of lipids and major lipoproteins in control subjects grouped according to MTP −493 genotype in the WOSCOPS

	Triglycerides	Cholesterol			
	Plasma Total	Plasma Total	LDL	HDL	BMI
	mM		*mM*		*kg/m^2*
MTP −493 G/T					
GG, n = 641	1.59 ± 0.63	7.02 ± 0.56	4.97 ± 0.45[a]	1.14 ± 0.25	25.5 ± 3.08
GT, n = 421	1.70 ± 0.75	7.05 ± 0.59	4.94 ± 0.43	1.14 ± 0.25	25.9 ± 3.33
TT, n = 55	1.52 ± 0.61	6.84 ± 0.54	4.84 ± 0.40	1.15 ± 0.26	25.8 ± 3.58
P	**0.04**	**0.04**	0.12	0.92	0.14

Values are mean ± SD. Differences between genotype groups were assessed by ANOVA. WOSCOPS, West of Scotland Coronary Prevention Study. $P \leqslant 0.05$ are shown in bold.
[a] Significantly different from individuals carrying the TT genotype ($P = 0.04$).

TABLE 4. Plasma concentrations of total and LDL apolipoprotein B according to MTP genotype

	Plasma Total ApoB	Plasma LDL ApoB
	mg/dl	*mg/dl*
MTP −493 G/T		
GG	109 ± 2.8 (n = 220)	86.0 ± 18.2[a] (n = 193)
GT	107 ± 21.8 (n = 150)	87.1 ± 19.1[a] (n = 142)
TT	99 ± 27.0 (n = 22)	71.5 ± 15.8 (n = 20)
P	0.06	**0.001**
MTP Q/H 95		
QQ	108 ± 22.8 (n = 368)	86.1 ± 18.4[b] (n = 330)
QH + HH	101 ± 20.3 (n = 24)	79.1 ± 20.9 (n = 25)
P	0.14	**0.03**
MTP Q/E 244		
QQ	108 ± 22.7 (n = 352)	85.2 ± 18.5 (n = 315)
QE	106 ± 23.6 (n = 39)	89.5 ± 19.8 (n = 40)
P	0.71	0.17
MTP H/Q 297		
HH	106 ± 22.9 (n = 218)	83.7 ± 18.1 (n = 203)
HQ	109 ± 21.2 (n = 152)	88.4 ± 18.9 (n = 133)
QQ	112 ± 31.5 (n = 18)	85.9 ± 23.4 (n = 16)
P	0.43	0.08

Values are mean ± SD. Differences between genotype groups were assessed by ANOVA. $P \leq 0.05$ are shown in bold.

[a] Significantly different from individuals carrying the TT genotype.

[b] Significant difference from individuals carrying the QH or HH genotype according to Sheffé's post hoc test for continuous variables.

BMI, and smoking. To analyze the potential gene-environmental interaction between obesity and the MTP polymorphism, the cohort was median split for waist (median 96 cm) and BMI (median 25.6 kg/m^2) **Table 6**. This split enabled the comparison of phenotypic modulation by the MTP polymorphism of strictly nonobese with moderately overweight subjects. The LDL cholesterol modulation by the MTP polymorphism was maintained only in the group with a waist measurement above 96 cm, suggesting that the effect was dependent of obesity. Plasma triglycerides were not affected by the median split. Surprisingly, the BMI gradient was completely lost in the nonobese group, whereas the genotype modulation on BMI was almost completely accounted for by an effect in the moderately obese group. Furthermore, there was a significant aggregation of −493T allele carrier status in the moderately obese group.

The Q/H 95, Q/E 244, or H/Q 297 genotype group could not be significantly associated with any alterations in BMI or waist circumference (Table 5). In concordance with the higher BMI and waist measurements, significantly higher levels of plasma insulin were observed in individuals homozygous for the less common −493T allele (55.5 pM vs. 41.8 pM vs. 42.9 pM, $P = 0.05$). The same effect was seen in individuals hetero- or homozygous for the 95 H allele (50.9 pM vs. 42.6 pM, respectively, $P = 0.04$).

No significant changes in plasma insulin levels were observed for either one of the Q/E 244 or H/Q 297 genotype groups.

DISCUSSION

Secretion of apoB-containing lipoproteins is dependent on expression of the MTP gene and is regulated by lipid availability (5, 6, 25). It is therefore plausible that polymorphisms in the MTP gene would affect the hepatic secretion of apoB-containing lipoproteins.

The present study shows that two promoter polymor-

TABLE 5. MTP genotypes in relation with anthropometric variables and insulin

	BMI	Waist Circumference	Insulin
	kg/m^2	*cm*	*pmol/l*
MTP −493 G/T			
GG	25.7 ± 2.97[a] (n = 315)	96.4 ± 8.7[a] (n =316)	42.9 ± 24.7 (n = 315)
GT	25.8 ± 3.16[a] (n = 218)	95.8 ± 9.1[a] (n = 218)	41.8 ± 21.1 (n = 217)
TT	27.5 ± 4.08 (n = 30)	100.8 ± 11.1 (n = 30)	55.5 ± 34.2 (n = 30)
P	**0.02**	**0.02**	**0.05**
MTP Q/H 95			
QQ	25.8 ± 3.10 (n = 526)	96.4 ± 8.9 (n = 527)	42.6 ± 23.7[b] (n = 525)
QH + HH	26.4 ± 3.69 (n = 36)	96.5 ± 1.1 (n = 36)	50.9 ± 28.8 (n = 36)
P	0.28	0.93	**0.04**
MTP Q/E 244			
QQ	25.8 ± 3.16 (n = 507)	96.4 ± 9.1 (n = 508)	43.2 ± 24.3 (n = 506)
QE	26.0 ± 2.97 (n = 55)	96.6 ± 8.7 (n = 55)	43.1 ± 22.8 (n = 55)
P	0.61	0.84	0.92
MTP H/Q 297			
HH	25.8 ± 3.15 (n = 349)	96.5 ± 9.3 (n =349)	43.3 ± 23.4 (n = 349)
HQ	25.9 ± 3.12 (n = 187)	96.2 ± 8.6 (n = 188)	42.1 ± 25.3 (n = 186)
QQ	26.5 ± 3.41 (n = 20)	96.7 ± 11.3 (n = 20)	49.8 ± 25.5 (n = 20)
P	0.52	0.92	0.22

Values are mean ± SD. Differences between genotype groups were assessed by ANOVA. $P \leq 0.05$ are shown in bold.

[a] Significantly different from individuals carrying the TT genotype.

[b] Significantly different from individuals carrying the QH or HH genotype according to Sheffé's post hoc test for continuous variables.

TABLE 6. MTP 493 G/T genotypes in relation to LDL cholesterol, VLDL triglycerides, insulin, and BMI

	LDL Cholesterol	VLDL TG	Insulin	BMI
	mM	*mM*	*mU/ml*	*kg/m²*
Waist ≤96 cm				
GG (n = 157)	3.55	0.90	33.5	23.8
GT (n = 117)	3.40	0.83	34.5	23.9
TT (n = 8)	3.11	0.55	31.2	23.0
P	0.28	0.21	0.26	0.44
Waist ≥96 cm				
GG (n = 159)	3.62	1.41	52.1	27.7[a]
GT (n = 101)	3.71[a]	1.45	50.3	28.0
TT (n = 22)	3.18	1.10	64.3	29.2
P	**0.04**	0.24	0.18	**0.04**
BMI ≤25.6 kg/m²				
GG (n = 156)	3.49	0.81	34.4	23.4
GT (n = 117)	3.45	0.89	34.8	23.6
TT (n = 9)	3.21	0.64	32.2	22.7
P	0.75	0.55	0.32	0.23
BMI >25.6 kg/m²				
GG (n = 157)	3.69[a]	1.48	51.2	28.0
GT (n = 98)	3.63[a]	1.40	49.5	28.4
TT (n = 19)	3.10	1.10	62.6	29.3
P	<0.01	0.28	0.22	0.07

Values are expressed means. Differences between genotype groups were assessed by ANOVA. The cohort is split in two groups according to median value. TG, triglycerides. $P \leq 0.05$ are shown in bold.

[a] Significantly different from individuals carrying the TT genotype according to Sheffé's post hoc test for continuous variables.

phisms and one missense polymorphism in the MTP gene are in almost complete linkage disequilibrium. These three polymorphisms significantly affect lipid, lipoprotein, and LDL apoB levels in healthy 50-year-old men. Furthermore, disease-free slightly hypercholesterolemic men homozygous for the −493 T allele also showed significantly lower plasma total cholesterol levels, verifying the lipid findings in the middle-aged North European cohort.

The −493 G/T and to some extent the −164 T/C polymorphism, have been investigated in other cohorts with differing results. Genotyping for the −493 G/T polymorphism of the Coronary Artery Risk Development in Young Adults population (young healthy black men) showed an increase in total plasma triglycerides as well as in LDL cholesterol (15). In patients diagnosed with familial hypercholesterolemia, serum triglycerides levels were 40% lower in subjects homozygous for the −493 T allele, but the LDL cholesterol level was not affected (13). A previous study in healthy individuals showed a significant decrease in plasma total and LDL cholesterol in subjects homozygous for the rare −493 T allele (12). Although the Framingham Offspring Study is a prospective study consisting of individuals 35–54 years of age and similar ethnic background, there were no significant associations between the MTP −493 G/T polymorphism and lipid, lipoprotein, or anthropometric variables. A possible explanation for the lack of associations in the Framingham Offspring Study is that the data from subjects with cardiovascular disease have been combined with data from disease-free individuals, and analyzed together. Approximately 12.6% (155/1,226) of subjects had cardiovascular disease in the Framingham Offspring Study, whereas both populations in the present study consist of 1,681 disease-free individuals. Another difference between the Framingham Offspring Study and the cohorts in the present study is the fact that some individuals in the Framingham Offspring Study are closely related individuals, which is not the case in the present study.

Previously, only one study involving the −164 T/C polymorphism has been published. The subjects investigated were patients diagnosed with myocardial infarction and age-matched controls [the Etude Cas-Témoins sur l'Infarctus du Myocarde (ECTIM) study]; this study was not able to detect any significant association between alterations of plasma triglycerides or LDL cholesterol (26). This is in agreement with unpublished observations from our group showing that the MTP −493 G/T polymorphism has no effect on plasma lipid levels in a group of 200 survivors of a first myocardial infarction before the age of 45 and, therefore, argues that disease states influencing lipid metabolism may override the phenotypic modulation by the polymorphism.

The present study shows a significant 9% decrease in plasma total cholesterol levels, a 12% decrease in LDL cholesterol levels, and a 17% decrease in plasma LDL apoB levels in subjects homozygous for the rare MTP −493T/−164C/T128 alleles. Differences in inclusion criteria as well as disease state are likely to contribute in explaining the contradictory results. The association between the −493 G/T polymorphism and plasma total cholesterol levels was verified in a large cohort consisting of 1,117 disease-free moderately hypercholesterolemic men participating in the WOSCOPS. Furthermore, a significant decrease of LDL cholesterol concentration was noted for subjects homozygous for the T allele compared with subjects homozygous for the G allele. The WOSCOPS population was recruited on the basis of moderate hypercholesterolemia and the selection was made within a rather narrow limit (4.5–6.0 mM LDL cholesterol) (17). This may have restricted the expression of the MTP phenotype, which was smaller in magnitude compared with our selected Swedish population.

The polymorphism located at −493 upstream from transcription start is the only one in this study that has been investigated in vitro, and the study showed an increased expression of the MTP gene with the rare T allele (12). Because homozygous carriers of the T allele have been shown to have fewer but more lipid-rich particles, we hypothesized that an increased expression of the MTP gene would lead to a more efficient lipidation of immature VLDL particles (12). As large VLDLs are not direct precursors of LDL, the input from VLDL to the LDL fraction will decrease and this, in turn, would explain the lower LDL cholesterol level seen in subjects homozygous for the MTP −493 T allele.

No gene dose effect is obvious for the −493 G/T polymorphism, which is in agreement with findings regarding the previously reported MTP mutations causing abetalipoproteinemia (7, 9). No major change in lipid or lipoprotein concentration has been thus noted for heterozygous carriers of MTP mutations.

MTP polymorphisms have not previously been associ-

ated with the degree of obesity such as BMI or waist circumference but, in this study, we show an increase in both BMI and waist circumference in individuals homozygous for the rare −493T/−164C/T128 alleles. The basis of the unexpected but apparent relationship is difficult to understand. Although it may be speculated that the triglycerides of large VLDL may be preferentially deposited into adipose tissue, this does not explain the full effect, as it must also involve an imbalance between energy intake and expenditure. Therefore, it cannot be assumed that these variations in BMI and waist circumference are caused by a specific MTP genotype. One cannot exclude the possibility of linkage disequilibrium between the rare −493/−164/128 alleles and functional polymorphism in an adiposity-regulating gene located in the same chromosomal region. Recently, a genome-wide scan for genes influencing the propensity to store fat in the abdominal area showed evidence of linkage to the long arm of chromosome 4 (27). The MTP genotypic effect shown in the present study seemed to be independent of obesity: Numerically, the same group differences were obtained in the obese and nonobese groups, but the lower LDL cholesterol level in the homozygous carriers of the less common variant was not statistically significant in the nonobese group, as it contained only nine subjects.

In summary, we found that two polymorphisms in the promoter region, and one missense polymorphism in exon 3 of the MTP gene are in almost complete linkage disequilibrium, and individuals homozygous for the three rare alleles show significantly decreased levels of plasma total and LDL cholesterol and plasma apoB. The same individuals also show significantly higher BMI and waist circumference measurements and plasma insulin levels. Based on these findings, we hereby suggest that genetic variation in the MTP gene may have important implications for the development of cardiovascular disease in humans.

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