Identification of two polymorphisms in the promoter of the microsomal triglyceride transfer protein (MTP) gene: lack of association with lipoprotein profiles

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Abstract **The microsomal triglyceride transfer protein (MTP) catalyzes the transfer of triglyceride, cholesteryl ester, and phosphatidylcholine between phospholipid surfaces. The 97-kD subunit imparts lipid transfer activity and thus plays a role in the assembly of apolipoprotein B (apoB)-containing lipoproteins. We tested whether polymorphisms in the promoter region of the large subunit of the MTP gene might be related to different plasma lipid variables, atherosclerosis, and the risk of myocardial infarction (MI). We screened 838 bp in the promoter region of the MTP gene by PCR-SSCP and identified two polymorphisms at positions −400 (MTP/−400 (A→t)) and −164 (MTP/−164 (T→c)), the latter being situated on a putative sterol responsive element (SRE) consensus sequence. The two polymorphisms, investigated in 622 male patients with MI and in 728 age-matched controls participating in the ECTIM Study, were in nearly complete linkage disequilibrium ($|D'| = +0.98$, less frequent alleles being preferentially associated, $P < 0.001$). There were no significant differences in genotype or allele frequencies between patients with MI and controls. Moreover, no significant associations between the two promoter polymorphisms and several lipid variables measured in the control groups of the ECTIM Study or coronary artery stenosis, angiographically assessed in patients with MI, were detected. We conclude that these MTP polymorphisms are unrelated to lipid variables or coronary heart disease in this study.—**Herrmann, S-M., O. Poirier, V. Nicaud, A. Evans, J-B. Ruidavets, G. Luc, D. Arveiler, C. Bao-Sheng, and F. Cambien. **Identification of two polymorphisms in the promoter of the microsomal triglyceride transfer protein (MTP) gene: lack of association with lipoprotein profiles.** ***J. Lipid Res.*** **1998.** 39: **2432–2435.**

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The endoplasmic reticulum resident microsomal triglyceride transfer protein (MTP) catalyzes the transfer of triglyceride (TG), cholesteryl ester (CE), and phosphatidylcholine between phospholipid surfaces. MTP, a heterodimer composed of a 58-kD protein disulfide-isomerase (PDI) and a 97-kD subunit imparting lipid transfer activity to the complex (1), is primarily expressed in hepatocytes and intestinal enterocytes (2) and has recently been detected in the human heart (3). The subcellular localization and tissue distribution suggested a role of MTP in the assembly of apolipoprotein B (apoB)-containing lipoproteins, such as very low density lipoprotein (VLDL) in the liver, heart, and chylomicrons in the intestine. In fact, several genetic studies have demonstrated that defects of the gene encoding MTP cause the rare autosomal recessive inherited disorder abetalipoproteinemia (ABL) (4–6). This rare disease is characterized by a virtual absence of plasma lipoproteins that contain apoB and by low plasma concentrations of TG and cholesterol (7).

The large subunit of human MTP spans about 55 kb and is situated on chromosome 4q24 (6). We hypothesized that polymorphisms in the 5′ flanking region of the gene coding for the large subunit of MTP could, through altered MTP gene transcription, influence plasma levels of VLDL, TG, or cholesterol and therefore account for atherosclerosis and/or myocardial infarction (MI). The aim of our study was to screen the known 838 bp promoter region of the large subunit of MTP for polymorphisms and to investigate their relation to different lipid profiles and coronary heart disease (CHD) in a multicenter case-control study of MI.

Abbreviations: ASO, allele-specific oligonucleotide; CHD, coronary heart disease; ECTIM, Etude Cas-Témoin de l'Infarctus du Myocarde; HDL, high density lipoprotein; LDL, low density lipoprotein; MI, myocardial infarction; MTP, microsomal triglyceride transfer protein; PCR, polymerase chain reaction; SRE, sterol response element; SSCP, single-strand conformation polymorphism; TG, triglyceride; VLDL, very low density lipoprotein.

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METHODS

Study populations

Details of the populations participating in the ECTIM Study (Etude Cas-Témoin de l'Infarctus du Myocarde) have been provided previously (8). Men aged 25–64 years were recruited between 1988 and 1991 from four WHO MONICA (MONItoring trends and determinants in CArdiovascular disease) centers (9), one in Northern Ireland (Belfast) and three in France (Lille, Strasbourg, Toulouse). Cases were recruited into the study 3–9 months after the event and had to satisfy the WHO criteria for definite acute MI (category I). Controls were randomly recruited from the same areas as the cases and stratification by age was used to match approximately the age distribution of the controls with that of cases. Fasting blood samples were collected and the methodology used to assess plasma levels of lipids, lipoproteins, and apolipoproteins has been described previously (8). Coronary angiograms were available for 93% of French cases but only 18% of Northern-Ireland cases; the results of coronary angiography are thus reported only for French cases. Angiograms were analyzed in each recruitment center and the number of arteries with more than 50% stenosis was used to assess the degree of coronary artery disease (CAD) (10). Informed consent was obtained from the subjects and their family doctors. Among eligible control subjects, 40% in Belfast, 54% in Strasbourg, 49% in Toulouse, and 47% in Lille refused to participate, did not respond, or could not be traced.

Identification of polymorphisms on the MTP gene promoter and genotyping

Genomic DNA was prepared from white blood cells by phenol extraction. For PCR-SSCP analysis (11) of the promoter region of the large subunit of the MTP gene, 20 individuals with MI were selected from the ECTIM Study. From the published sequences of the MTP gene promoter (12), two overlapping fragments less than 450 bp in length were enzymatically amplified. Each amplification was performed using 250 ng of DNA in a total volume of 50 μl containing 10 mm Tris-HCl (pH 9), 50 mm KCl, 2.5 mm $MgCl_2$, 0.1% Triton-X100, 0.2 mg/ml bovine serum albumin, 200 μm dNTPs, 25 pmol of each primer, and 0.2 U Taq polymerase; 95°C for 5 min to denature, followed by 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s for 30 cycles and 72°C for 10 min. DNA from patients presenting a different SSCP pattern of migration was reamplified by PCR with unlabeled primers. PCR products were purified by precipitation with Bio-spin 6 columns (Bio-Rad). Sequencing was performed by the method of Sanger, Nicklen, and Coulson (13) in 25 cycles of PCR with [γ^{32}P]dATP end-labeled primer using a direct sequencing kit (AmpliCycle™, Perkin-Elmer, Roche Molecular Systems, Inc., Nutley, NJ). Genotyping of all subjects was done by using allele specific oligonucleotides (ASO) (14) and performed as previously described (15). The amplimers for the 785 bp fragment which contained both polymorphic sites were: upper primer: 5′ CTC TTC CTA GAA ATG AGA TT 3′; lower primer: 5′ CAG CTA GGA GTC ACT GAG AA 3′. The ASOs for the −400 (A→t) and −164 (T→c) polymorphisms were: 5′ ACA AGA AAA ATT AAA AT 3′ to detect the adenine, 5′ ACA AGA AAT ATT AAA AT 3′ to detect the thymine and 5′ TTT CCT CAT TGG GTG AA 3′ to detect the thymine, 5′ TTT CCT CAC TGG GTG AA 3′ to detect the cytosine, respectively.

Statistical analysis

To simplify the presentation of the data, the three French centers were considered together after having checked that the results were not statistically heterogenous across centers. Data were analyzed, using the SAS statistical software (SAS Institute Inc., Cary, NC). Pairwise linkage disequilibrium was estimated in the control sample; coefficients are reported as the ratio of the unstandardized coefficients to their minimal/maximal value (|D′|) (16). Hardy-Weinberg equilibrium was tested by a χ^2 test with 1 degree of freedom. Haplotype frequencies were estimated with the MYRIAD program (17).

For the case-control comparisons of genotype and allele frequencies, controls with CHD were excluded. Genotype and allele frequencies were compared between cases and control subjects and between Northern Ireland and France by a χ^2 test. In controls, levels of lipids were compared between carriers and noncarriers of the less frequent allele by analysis of variance (SAS-PROC GLM) taking age and country into account. To remove positive skewness, TG and VLDL-cholesterol were log-transformed, but untransformed means are presented in the tables.

RESULTS

We screened the 838 bp region in 5′ of the gene coding for the large subunit of the MTP and found two polymorphisms at positions −400 (A/t) and −164 (T/c) from the transcription start site according to the sequence reported by Sharp et al. (12). The −164 polymorphism lies in a putative consensus sequence (−174 to −163) which is homologous to the human LDL receptor promoter sterol response element (SRE) (18, 19). These biallelic polymorphisms were then studied in 622 male patients with a history of MI and in 728 age-matched controls participating

TABLE 1. Genotype and allele frequencies of the −164 and −400 polymorphisms by country and case-control status

Country, Status	Genotype TT n	TT %	Tc n	Tc %	cc n	cc %	Less Frequent Allele Frequency
−164							
Belfast							
Cases	103	53.9	72	37.7	16	8.4	0.272
Controls	102	57.9	61	34.7	13	7.4	0.247
France							
Cases	226	52.6	175	40.7	29	6.7	0.271
Controls	251	49.3	215	42.2	43	8.5	0.296
	AA		At		tt		
−400							
Belfast							
Cases	78	40.8	84	44.0	29	15.2	0.372
Controls	80	45.7	67	38.3	28	16.0	0.351
France							
Cases	192	44.6	192	44.6	47	10.8	0.332
Controls	203	39.7	234	45.8	74	14.5	0.374

MTP haplotypes	−164 / −400	T / A	T / t	c / t
Belfast				
Controls		0.65	0.11	0.24
France				
Controls		0.62	0.08	0.29
All				
Cases		0.65	0.08	0.27
Controls		0.63	0.09	0.28

No significant difference in genotype or allele frequencies between countries or cases and controls were found. The MTP haplotypes were similarly distributed across countries and in cases and controls. For these comparisons, control subjects with a history of CHD were excluded.

TABLE 2. Mean (SEM) lipid and lipoprotein levels (g/l) according to −164 and −400 genotypes in controls, adjusted for age and country

	−164			−400		
	TT n = 379	Tc n = 291	cc n = 57	AA n = 306	At n = 316	tt n = 106
Total cholesterol	2.34 (0.02)	2.33 (0.03)	2.31 (0.06)	2.33 (0.03)	2.35 (0.03)	2.31 (0.04)
ApoB	1.33 (0.02)	1.31 (0.02)	1.31 (0.05)	1.32 (0.02)	1.32 (0.02)	1.33 (0.04)
LDL-C	1.56 (0.02)	1.55 (0.02)	1.58 (0.05)	1.54 (0.02)	1.57 (0.02)	1.54 (0.04)
VLDL-C	0.276 (0.009)	0.265 (0.011)	0.245 (0.023)	0.275 (0.010)	0.262 (0.010)	0.274 (0.017)
HDL-C	0.51 (0.01)	0.52 (0.01)	0.48 (0.02)	0.51 (0.01)	0.51 (0.01)	0.50 (0.01)
Triglycerides	1.54 (0.04)	1.49 (0.05)	1.53 (0.10)	1.52 (0.05)	1.50 (0.05)	1.56 (0.07)

There was no significant association between the polymorphisms and different plasma lipid variables.

in the ECTIM Study. In control subjects, the distribution of genotypes of both polymorphisms was not significantly different from that expected under Hardy-Weinberg equilibrium. The linkage disequilibrium between both polymorphisms was nearly complete ($|D'| = +0.98$, $P < 0.001$), the less frequent alleles being preferentially associated) and not significantly different between cases and controls in Northern Ireland and France. There was no significant difference in genotype or allele frequencies between countries or between cases and controls (**Table 1**). The frequencies of haplotypes combining the polymorphisms were also similar in the different subgroups. In **Table 2**, we report the mean values of several lipid variables according to MTP genotypes in the control group of the ECTIM Study. No association between lipid variables and genotypes could be detected. This was true both in the Northern Ireland and French groups and irrespective of whether patients receiving lipid lowering drugs were excluded or not (Table 2). Ninety-three percent of French patients underwent coronary angiography after their MI; there was no association between the degree of CAD and the MTP genotypes (not shown).

DISCUSSION

We have identified two polymorphisms in the 5′ flanking region of the MTP gene, designated −400 (A/t) and −164 (T/c). None of the variants showed any association with angiographically assessed coronary stenosis or plasma lipid profiles measured in the ECTIM Study. There was no significant difference in genotype or allele frequencies for both polymorphisms between countries or between cases and controls. A number of arguments suggested that the MTP gene might be a candidate for CHD. MTP plays an important role in the assembly of VLDL and chylomicrons (5). MTP transports newly synthesized lipids, such as TG, CE, and phospholipid from the endoplasmic reticulum (ER) membrane to apoB-containing particles in the lumen of ER (20). Its important function is supported by the fact that mutations in the coding region of the MTP gene cause abetalipoproteinemia, a rare genetic disorder characterized by a virtual absence of plasma lipoproteins that contain apoB and by low plasma concentrations of TG and cholesterol (4–6).

Conceivably, polymorphisms in the promoter region of the MTP gene might influence transcription of the gene and the assembly and transport processes to which MTP contributes. Furthermore, the MTP/−164 polymorphism lies in a consensus sequence (−174 to −163) that shows homology to the human LDL receptor promoter SRE and other physiologically related genes (19). Hagan et al. (18) recently compared the transcription of human and hamster MTP genes and found highly conserved regions within the promoter of the two species. They could demonstrate by transient transfection analysis in HepG2 cells that the deletion of sequences 5′ to −239 bp had no effect on promoter activities, whereas further deletion from −239 to −121 bp increased the promoter activity to approximately 250%.

Whether the polymorphism in the putative SRE affects the transcription of the human MTP gene cannot be inferred from our study; this would have to be tested in vitro. It must also be recalled that the sensitivity of the SSCP technique to detect molecular variants is about 90% (personal data), so that we might have missed a potentially functional polymorphism. However, the two polymorphisms have clearly no influence on the plasma lipoprotein profile of the subjects investigated. It might be worthwhile to clone and investigate more distal parts of the human MTP gene promoter in order to detect possible functional polymorphisms. In conclusion, our results do not provide any evidence for an association between two polymorphisms in the promoter region of the MTP gene and plasma lipid variables, CAD or MI.

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