Sex-specific association of fatty acid binding protein 2 and microsomal triacylglycerol transfer protein variants with response to dietary lipid changes in the 3-mo Medi-RIVAGE primary intervention study[1–3]

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ABSTRACT

Background: The dietary guidelines targeted at reducing cardiovascular risk lead to largely heterogeneous responses in which genetic determinants are largely involved.

Objectives: We evaluated the effect of fatty acid binding protein 2 (*FABP2*) Ala54Thr and microsomal triacylglycerol transfer protein (*MTTP*) −493G/T allelic variations on plasma lipid markers, at baseline and on the response to the 3-mo Medi-RIVAGE primary prevention study.

Design: Subjects with moderate cardiovascular disease risk (n = 169) were advised to reduce total and saturated dietary fats and to increase intake of monounsaturated and polyunsaturated fats. They were genotyped for *FABP2* Ala54Thr and *MTTP* −493G/T allelic variations, and plasma was processed for cardiovascular risk marker analyses.

Results: At baseline, men and women homozygous for Thr54 presented a significant opposite profile for plasma oleic acid (18:1), triacylglycerol-rich lipoprotein (TRL) cholesterol, and TRL phospholipids. In addition, all Thr/Thr men presented higher 18:1 values than did women. For the *MTTP* −493G/T polymorphism, although all TT subjects presented high apolipoprotein B-48, a genotype × sex interaction was present for palmitic acid, linolenic acid, eicosatrienoic acid, and insulin. The prudent diet clearly improved plasma lipid markers. *FABP2* genotype did not interact much with the amplitude of the response. However, for *MTTP* polymorphism, men homozygous for the T allele displayed a significantly more pronounced response than did men carrying the G allele, which is particularly evident by their larger decrease in the Framingham score.

Conclusions: These 2 polymorphic loci are thus differently associated with the baseline lipid markers as well as with the response to nutritional recommendations, but both presented a marked sex-specific profile, with the response to diet being particularly efficient in men homozygous for the *MTTP* −493T allele. *Am J Clin Nutr* 2007;86:1633–41.

KEY WORDS Serum lipids, dietary fats, fatty acid binding protein 2, *FABP2* polymorphism, microsomal triacylglycerol transfer protein, *MTTP* polymorphism, Mediterranean diet, risk assessment

INTRODUCTION

Cardiovascular diseases (CVDs) represent one of the main causes of death in developed countries and are thus one of the greatest concerns in public health. The various dietary guidelines that are provided by the nutrition research community are aimed at decreasing the incidence of such diseases (1). However, although nutritional recommendations are targeted to the whole population, responses to diet turned out to be largely heterogeneous, and a role for genetic determinants in the interindividual variation is now clearly admitted (2). One of the most widespread recommendations is to replace saturated fat with monounsaturated and polyunsaturated fats, and the variation of the response to such a dietary challenge may be due to the presence of allelic variants in the genes involved in fatty acid (FA) absorption and metabolism.

In the epithelial cells of the small intestine, the absorption of dietary long-chain FAs (LCFAs) represents a multistep process that involves *1*) the uptake of LCFAs at the apical side of the cell, *2*) the intracellular transport of LCFAs and their esterification, and *3*) the formation and secretion of triacylglycerol-rich lipoproteins (TRLs) at the basal side of the cell. Among other processes, the assembly and secretion of TRLs involve the intracellular LCFA trafficking by FA binding proteins (FABPs) and the lipid transfer on the nascent apolipoprotein (apo) B polypeptide chain by the microsomal triacylglycerol transfer protein (MTP).

The human FA binding protein 2 (*FABP2*) gene encodes the intestinal FABP isoform (I-FABP) that is specifically expressed in enterocytes. In 1995, Baier et al (3) reported that a single

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[2] Supported by the French Research Ministry (AQS grant), INSERM (IDS grant), the Provence-Alpes-Côte d'Azur Régional council, the Bouches du Rhône Général council, and the CRITT-PACA.

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Received May 3, 2007.

Accepted for publication July 31, 2007.

nucleotide polymorphism (G to A) within the second exon at codon 54 predicted an amino acid substitution (Ala54Thr) that increases the in vitro binding affinity of the protein to LCFAs. Later, it was reported that, when challenged with an oral fat load, Thr54 homozygotes displayed a significantly greater increase in plasma chylomicron triacylglycerol and VLDL triacylglycerol (4). Another study found an association between Thr54 carriers and higher cholesterol concentration in chylomicron after ingesting olive oil (4, 5). All these modifications could be ascribed to an increased trafficking of FAs toward chylomicron assembly sites.

The role played by the MTP protein in the chylomicron or VLDL assembly was highlighted by studies showing that MTP activity was absent in abetalipoproteinemic subjects (6) and that a liver-specific deletion of the gene coding for MTP (*MTTP*) produced a dramatic reduction in plasma VLDL triacylglycerol and apo B-100 secretion. The most studied promoter polymorphism at the *MTTP* locus is located 493 bp (base pair) upstream from the transcriptional start site (−493G/T), but the association between this polymorphism and biological markers for risk of CVD is still controversial (7–12) and has never been addressed by intervention studies. The present study was undertaken to examine whether the *FABP2* and *MTTP* gene variants were associated with different responses to a 3-mo dietary intervention targeted to reduce total fat intake concomitantly with a replacement of saturated FAs by monounsaturated and polyunsaturated FAs.

SUBJECTS AND METHODS

Subjects

The design and methods of the Medi-RIVAGE study have been previously reported (13), and a marked reduction of cardiovascular risk was shown (14). Briefly, 169 volunteers with moderate cardiovascular risks were provided with nutritional recommendations. They consumed a low-fat diet for 3 mo: either a Mediterranean-type diet adapted from the traditional model or an adaptation of the commonly prescribed low-fat American Heart Association–type diet. The compliance with dietary recommendations was followed by dietitians. Three-day food records (at entry and after 3 mo) and 24-h unscheduled dietary recalls (once a month) were realized. No differences were observed in the dietary compliance according to sex. The GENI program nutritional database (version 6.1; Micro6, Nancy, France) was used and is based on the French REGAL food database (14). At entry and at the end of the 3-mo period, biochemical analyses were performed as previously described (14). Informed consent was obtained for each subject, and the study was approved by the institution's ethics committee (ethics committee no. 98/25).

Polymorphism detection

Genomic DNA was prepared from white blood cells by a standard proteinase K-phenol method. The *FABP2* Ala54Thr polymorphism and the *MTTP* −493G/T polymorphism were genotyped by a polymerase chain reaction restriction fragment length polymorphism assay (3, 7). For *FABP2* genotyping the forward primer was 5'-CAGGTGTTAATATAGTGAAAAGG, and the reverse primer was 5'-TTACCCTGAGTTCAGTTCCG; the restriction cleavage was performed by *Hha*1 enzyme. For *MTTP* genotyping the forward primer was 5'-AGTTTCACACATAAGGACAATCATCTA and the reverse primer was 5'-GGATTTAAATTTAAACTGTTAATTCATATCAC. The restriction cleavage was performed by *Hph*1 enzyme.

Statistical analyses

Statistical analyses were performed with the SAS statistical software (SAS Institute, Cary, NC). A chi-square test was used to determine whether genotypes at each gene locus were in Hardy-Weinberg equilibrium. Logarithmic transformation was performed on individual values of apo B-48, linolenic acid (18:3), stearidonic acid (18:4), eicosapentaenoic acid (20:5), triacylglycerols, insulin, TRL cholesterol, TRL triacylglycerols, TRL phospholipids, and Framingham score to improve the normality of their distribution. Because the patients were assigned for 3 mo to 2 different diets, it was first checked whether those 2 populations could be merged for the present analysis. A repeated-measures (baseline and 3 mo) general linear model showed that the type of diet had no effect on the variables studied. In addition, it was checked by chi-square test that the distribution of the subjects as a function of their genotypes was not affected by the type of diet.

Before testing the effect of genotypes on the dependent variables, interfering covariables (adjustment factors) were identified. First, each dependent variable measured at baseline was tested in univariate general linear models with independent qualitative variables. Age was always included in the adjustment. Second, linear correlations between the dependent variables and the quantitative covariables were performed, and correlations significant at the 0.05 level were retained. Identified covariables were included as adjustment factors for testing the effect of genotypes at baseline and 3 mo. Professional activity (activity, retirement, inactivity), body mass index (in kg/m^2), alcohol consumption, antihypertensive treatment (yes or no), smoking status (smoker, former smoker, never smoker), and menopausal status in women (yes, no or treated) were entered as covariables into the models. The effects of the genotypes at baseline were tested with general linear models. The effect of the genotypes on the response to the diet was tested in repeated-measures general linear models. Interactions of genotype by time and by sex were tested. Results were given for men and women separately when the interaction was significant.

RESULTS

The distribution of *FABP2* genotypes was 0.51 for Ala54/Ala54, 0.40 for Ala54/Thr54, and 0.09 for Thr54/Thr54. The frequency of *MTTP* genotypes were 0.41 for −493GG, 0.49 for −493GT, and 0.10 for −493TT. The *FABP2* Thr54-encoding allele had a frequency of 0.29, and the *MTTP* −493T variant had a frequency of 0.35. At both *FABP2* and *MTTP* loci, the distribution of genotypes was not significantly different from that expected under the Hardy-Weinberg equilibrium ($P = 0.920$ and $P = 0.593$, respectively).

At entry, subjects homozygous for the Thr54-encoding allele had a significantly higher percentage of plasma stearic acid (16:0) (**Table 1**). Interestingly, a significant interaction between genotype and sex was observed for the percentage of plasma oleic acid (18:1). This interaction points out that men and women exhibited a significant opposite profile with highest values for Thr/Thr men and lowest values for Thr/Thr women. In addition,

TABLE 1
Plasma fatty acid composition according to the Ala54Thr polymorphism of the fatty acid binding 2 gene

	Thr/Thr	Ala/Thr	Ala/Ala	P^1
	% of total fatty acids	*% of total fatty acids*	*% of total fatty acids*	
16:0 (palmitic acid)[2,3]				
Baseline	26.71 ± 3.92[4]	24.35 ± 3.72	23.78 ± 2.88	0.012
3 mo	25.12 ± 3.27	23.54 ± 2.70	22.94 ± 2.71	0.637
16:1 (palmitoleic acid)[2,5]				
Baseline	2.75 ± 0.87	2.55 ± 1.18	2.45 ± 0.87	0.455
3 mo	2.21 ± 0.84	2.28 ± 1.05	2.32 ± 0.98	0.171
18:0 (stearic acid)[2]				
Baseline	6.69 ± 1.88	6.42 ± 1.67	6.88 ± 1.50	0.178
3 mo	6.52 ± 1.62	6.60 ± 1.40	6.63 ± 1.40	0.055
18:1 (oleic acid)[2,6]				
Baseline	21.83 ± 5.53	20.63 ± 2.84	20.13 ± 2.82	0.378
3 mo	21.90 ± 3.65	21.09 ± 3.31	20.69 ± 3.39	0.764
18:2 (linoleic acid)[2,7]				
Baseline	25.79 ± 5.26	28.58 ± 4.09	28.24 ± 3.99	0.130
3 mo	26.12 ± 3.79	27.91 ± 4.33	27.63 ± 4.41	0.740
18:3 (linolenic acid)[2]				
Baseline	0.35 ± 0.20	0.31 ± 0.24	0.37 ± 0.24	0.110
3 mo	0.31 ± 0.20	0.29 ± 0.20	0.40 ± 0.28	0.254
18:4 (stearidonic acid)[2]				
Baseline	0.04 ± 0.09	0.07 ± 0.12	0.07 ± 0.14	0.463
3 mo	0.04 ± 0.05	0.06 ± 0.13	0.06 ± 0.13	0.944
20:3 (eicosatrienoic acid)[2,6]				
Baseline	1.43 ± 0.52	1.44 ± 0.72	1.70 ± 0.69	0.086
3 mo	1.51 ± 0.58	1.42 ± 0.66	1.59 ± 0.60	0.369
20:4 (arachidonic acid)[2]				
Baseline	6.61 ± 2.05	6.91 ± 1.77	7.44 ± 1.68	0.126
3 mo	6.62 ± 1.55	6.88 ± 1.92	7.33 ± 1.71	0.867
20:5 (eicosapentaenoic acid)[2,8]				
Baseline	0.67 ± 0.44	0.91 ± 0.69	0.94 ± 1.06	0.885
3 mo	1.21 ± 0.96	1.13 ± 1.03	1.20 ± 0.72	0.312
22:6 (docosahexaenoic acid)[2,8]				
Baseline	2.18 ± 0.69	2.54 ± 1.11	2.64 ± 1.07	0.289
3 mo	3.12 ± 0.92	2.91 ± 1.18	3.14 ± 1.08	0.258
18:1[6]				
Men[9,10]				
Baseline	25.26 ± 5.31	21.06 ± 3.01	20.40 ± 2.42	0.002
3 mo	24.17 ± 3.50	22.09 ± 3.24	22.33 ± 3.41	0.076
Women[10,11]				
Baseline	17.91 ± 2.18	20.32 ± 2.72	19.96 ± 3.04	0.105
3 mo	19.31 ± 1.48	20.36 ± 3.20	19.65 ± 2.96	0.419

[1] Comparison between polymorphisms at baseline (tested with general linear models) and in their response to diet (tested with repeated-measures general linear models).
[2] n = 15 Thr/Thr, 67 Ala/Thr, 87 Ala/Ala.
[3] Adjusted for professional activity.
[4] $\bar{x}$ ± SD (all such values).
[5] Adjusted for BMI, menopausal status in women, and smoking status.
[6] Adjusted for alcohol consumption.
[7] Adjusted for menopausal status in women and smoking status.
[8] Adjusted for BMI.
[9] n = 8 Thr/Thr, 28 Ala/Thr, 33 Ala/Ala.
[10] Significant interaction of genotype × sex at baseline (P = 0.0001) and significant interaction of genotype × sex × time after 3 mo (P = 0.028).
[11] n = 7 Thr/Thr, 39 Ala/Thr, 54 Ala/Ala.

men, but not women, homozygous for the Thr54-encoding allele had a significantly higher plasma percentage of oleic acid (18:1) than did men carrying the Ala-encoding allele. No significant differences could be found in any of the biochemical markers (**Table 2**), but, again, according to genotypes, men and women showed significantly opposite profiles for TRL cholesterol and TRL phospholipids. The lowest values for TRL cholesterol and TRL phospholipids were found in women homozygous for the Thr54-encoding allele, whereas highest values were found in men homozygous for the Thr54-encoding allele.

For the *MTTP* −493G/T polymorphism, **Table 3** shows that all subjects homozygous for the T allele exhibited significant lower percentages for stearic acid (18:0) and higher percentages for 18:1. In addition, a significant genotype × sex interaction was

TABLE 2
Fasting biochemical markers according to the Ala54Thr polymorphism of the fatty acid binding protein 2 gene[1]

	Thr/Thr	Ala/Thr	Ala/Ala	P^2
Apo A-1 (g/L)[3]				
Baseline	1.43 ± 0.37[4]	1.54 ± 0.27	1.46 ± 0.29	0.141
3 mo	1.45 ± 0.38	1.46 ± 0.26	1.39 ± 0.25	0.337
Apo B (g/L)[3]				
Baseline	1.30 ± 0.27	1.26 ± 0.26	1.21 ± 0.21	0.337
3 mo	1.19 ± 0.28	1.17 ± 0.26	1.20 ± 0.23	0.033
Apo B-48 (g/L)[3]				
Baseline	0.21 ± 0.15	0.28 ± 0.24	0.22 ± 0.18	0.258
3 mo	0.29 ± 0.21	0.26 ± 0.26	0.28 ± 0.26	0.065
Apo E (mg/L)[3,5]				
Baseline	43.62 ± 15.61	42.32 ± 11.96	41.45 ± 12.62	0.757
3 mo	46.13 ± 30.08	40.07 ± 12.33	39.52 ± 11.90	0.722
Total cholesterol (mmol/L)[3,5]				
Baseline	6.54 ± 1.10	6.60 ± 1.03	6.45 ± 0.88	0.599
3 mo	6.18 ± 0.88	6.09 ± 1.03	6.15 ± 0.91	0.246
HDL cholesterol (mmol/L)[3,6]				
Baseline	1.45 ± 0.52	1.57 ± 0.40	1.52 ± 0.47	0.758
3 mo	1.45 ± 0.50	1.58 ± 0.52	1.51 ± 0.48	0.827
LDL cholesterol (mmol/L)[3,7]				
Baseline	4.13 ± 1.12	4.26 ± 1.03	4.21 ± 0.83	0.845
3 mo	3.70 ± 0.82	3.84 ± 0.89	3.92 ± 0.76	0.596
Triacylglycerols (mmol/L)[3,8]				
Baseline	1.74 ± 1.20	1.52 ± 0.71	1.51 ± 0.98	0.821
3 mo	1.72 ± 1.33	1.29 ± 0.54	1.38 ± 0.86	0.672
TRL cholesterol (mmol/L)[3]				
Baseline	2.02 ± 2.70	1.10 ± 0.60	1.11 ± 0.82	0.910
3 mo	1.99 ± 2.46	0.97 ± 0.50	1.06 ± 0.82	0.678
TRL triacylglycerols (mmol/L)[3,6]				
Baseline	1.83 ± 2.31	0.99 ± 0.59	1.02 ± 0.73	0.980
3 mo	1.55 ± 1.58	0.91 ± 0.52	0.92 ± 0.69	0.130
TRL phospholipids (mmol/L)[3,8]				
Baseline	0.52 ± 0.56	0.34 ± 0.19	0.34 ± 0.22	0.958
3 mo	0.49 ± 0.48	0.32 ± 0.18	0.32 ± 0.22	0.248
Glucose (mmol/L)[3,9]				
Baseline	5.46 ± 0.60	5.27 ± 0.62	5.15 ± 0.66	0.158
3 mo	5.27 ± 0.49	5.01 ± 0.58	5.06 ± 0.62	0.226
Insulin (mU/L)[3,6]				
Baseline	11.78 ± 6.35	10.00 ± 7.51	10.80 ± 6.21	0.169
3 mo	8.78 ± 4.17	7.81 ± 4.37	9.20 ± 5.87	0.716
Framingham score[3]				
Baseline	5.93 ± 3.54	5.72 ± 3.19	6.09 ± 3.13	0.195
3 mo	5.53 ± 3.72	4.47 ± 3.49	5.36 ± 2.96	0.326
TRL cholesterol at baseline (mmol/L)[10]				
Men[11]	3.29 ± 3.24	1.28 ± 0.60	1.37 ± 0.94	0.072
Women[12]	0.56 ± 0.39	0.98 ± 0.58	0.98 ± 0.73	0.312
TRL phospholipids at baseline (mmol/L)[8,13]				
Men[11]	0.83 ± 0.62	0.38 ± 0.18	0.41 ± 0.25	0.072
Women[12]	0.17 ± 0.10	0.32 ± 0.20	0.30 ± 0.19	0.122

[1] Apo, apolipoprotein; TRL, triacylglycerol-rich lipoprotein.
[2] Comparison between polymorphisms at baseline (tested with linear models) and in their response to diet (tested with repeated-measures general linear models).
[3] n = 15 Thr/Thr, 67 Ala/Thr, 87 Ala/Ala.
[4] $\bar{x}$ ± SD (all such values).
[5] Adjusted for menopausal status in women.
[6] Adjusted for BMI.
[7] Adjusted for smoking status.
[8] Adjusted for menopausal status in women and BMI.
[9] Adjusted for BMI, professional activity, and alcohol consumption.
[10,13] Significant interaction of genotype × sex: [10] $P = 0.035$, [13] $P = 0.013$.
[11] n = 8 Thr/Thr, 28 Ala/Thr, 33 Ala/Ala.
[12] n = 7 Thr/Thr, 39 Ala/Thr, 54 Ala/Ala.

TABLE 3
Plasma fatty acid composition according to the −493 G/T polymorphism of the microsomal triglyceride transfer protein

	T/T	G/T	G/G	P^1
	% of total fatty acids	*% of total fatty acids*	*% of total fatty acids*	
16:0 (palmitic acid)[2,3]				
Baseline	24.95 ± 3.79[4]	24.69 ± 3.49	23.61 ± 3.15	0.024
3 mo	23.04 ± 2.17	23.92 ± 2.97	22.80 ± 2.66	0.043
16:1 (palmitoleic acid)[2,5]				
Baseline	2.66 ± 1.22	2.58 ± 1.12	2.41 ± 0.79	0.416
3 mo	2.25 ± 1.14	2.37 ± 1.07	2.21 ± 0.85	0.576
18:0 (stearic acid)[2]				
Baseline	6.23 ± 1.56	6.48 ± 1.64	7.03 ± 1.54	0.026
3 mo	6.31 ± 1.22	6.53 ± 1.54	6.77 ± 1.29	0.236
18:1 (oleic acid)[2,6]				
Baseline	22.05 ± 1.95	20.57 ± 3.03	19.98 ± 3.45	0.029
3 mo	20.29 ± 2.78	21.13 ± 3.65	20.92 ± 3.18	0.033
18:2 (linoleic acid)[2,7]				
Baseline	26.71 ± 3.78	27.89 ± 4.06	28.82 ± 4.39	0.134
3 mo	28.93 ± 5.54	26.83 ± 4.13	28.22 ± 4.12	0.027
18:3 (linolenic acid)[2]				
Baseline	0.35 ± 0.22	0.34 ± 0.24	0.35 ± 0.24	0.941
3 mo	0.30 ± 0.18	0.35 ± 0.25	0.37 ± 0.26	0.964
18:4 (stearidonic acid)[2]				
Baseline	0.10 ± 0.14	0.08 ± 0.15	0.05 ± 0.08	0.604
3 mo	0.06 ± 0.09	0.07 ± 0.16	0.05 ± 0.07	0.396
20:3 (eicosatrienoic acid)[2,6]				
Baseline	1.42 ± 0.77	1.58 ± 0.71	1.60 ± 0.67	0.424
3 mo	1.12 ± 0.70	1.53 ± 0.66	1.59 ± 0.53	0.132
20:4 (arachidonic acid)[2]				
Baseline	6.68 ± 1.62	7.02 ± 1.76	7.44 ± 1.79	0.092
3 mo	7.03 ± 1.69	7.00 ± 1.69	7.21 ± 1.94	0.270
20:5 (eicosapentaenoic acid)[2,8]				
Baseline	0.94 ± 0.71	0.87 ± 0.58	0.94 ± 1.18	0.631
3 mo	1.46 ± 1.59	1.14 ± 0.70	1.13 ± 0.82	0.971
22:6 (docosahexaenoic acid)[2,8]				
Baseline	2.56 ± 1.22	2.57 ± 1.16	2.55 ± 0.90	0.961
3 mo	3.12 ± 0.99	3.04 ± 1.12	3.04 ± 1.13	0.931
Men[9]				
16:0 at baseline[3,10]	29.44 ± 4.55	25.27 ± 3.48	23.80 ± 2.89	0.006
18:0				
Baseline	4.87 ± 0.67	6.36 ± 1.66	6.71 ± 1.60	0.187
3 mo[11]	6.48 ± 1.49	6.29 ± 1.48	6.34 ± 1.21	0.023
18:3 at baseline[12]	0.09 ± 0.07	0.39 ± 0.28	0.33 ± 0.25	0.046
20:3 at baseline[6,10]	0.51 ± 0.68	1.45 ± 0.61	1.44 ± 0.62	0.025
20:5 at baseline[8,13]	0.52 ± 0.80	0.87 ± 0.53	0.78 ± 0.48	0.123
Women[14]				
16:0 at baseline[3,10]	23.57 ± 2.28	24.27 ± 3.46	23.45 ± 3.38	0.482
18:0				
Baseline	6.65 ± 1.53	6.56 ± 1.63	7.29 ± 1.47	0.056
3 mo[11]	6.26 ± 1.19	6.71 ± 1.58	7.14 ± 1.26	0.241
18:3 at baseline[12]	0.43 ± 0.19	0.30 ± 0.21	0.36 ± 0.23	0.268
20:3 at baseline[6,10]	1.70 ± 0.55	1.67 ± 0.77	1.73 ± 0.70	0.931
20:5 at baseline[8,13]	1.08 ± 0.66	0.86 ± 0.62	1.07 ± 1.53	0.051

[1] Comparison between polymorphisms at baseline (tested with general linear models) and in their response to diet (tested with repeated-measures general linear models).
[2] n = 17 T/T, 83 G/T, 69 G/G.
[3] Adjusted for professional activity.
[4] $\bar{x}$ ± SD (all such values).
[5] Adjusted for BMI, menopausal status in women, and smoking status.
[6] Adjusted for alcohol consumption.
[7] Adjusted for menopausal status in women and smoking status.
[8] Adjusted for BMI.
[9] *n* = 4 T/T, 34 G/T, 31 G/G.
[10, 12, 13] Significant interaction of genotype × sex: [10] $P = 0.049$, [12] $P = 0.015$, [13] $P = 0.008$.
[11] Significant interaction of genotype × sex × time, $P = 0.010$.
[14] *n* = 13 T/T, 48 G/T, 38 G/G.

observed when considering 16:0, 18:3, and eicosatrionioic acid (20:3). Interestingly, only TT men displayed higher 16:0 and lower 18:3 and 20:3 values. For the biochemical markers (**Table 4**), all TT subjects presented significant higher concentrations of apo B-48. Insulin values displayed a genotype × sex interaction with a different pattern in men and women.

As expected, at the end of the 3-mo diet, all subjects reduced their fat intake with a sharp decrease in saturated FAs and a slight increase in monounsaturated and polyunsaturated FA consumption (14). Interestingly, when considering the *FABP2* Ala54Thr polymorphism, the only difference observed in plasma FA composition concerned the 18:1 percentage: men and women exhibited a different profile of response linked to genotype. However, in all subjects homozygous for the Thr allele, the diet induced a significant higher decrease in fasting apo B than did the other 2 genotypes (Table 2). As for the MTP −493G/T polymorphism, this work shows that there were genotype-specific differences in the response to diet. Indeed, TT subjects significantly reduced 16:0 and 18:1 and increased 18:2 (Table 3). In addition, a genotype × sex interaction was also observed: TT men significantly increased 18:0. For the biochemical markers (Table 4), subjects homozygous for MTP −493T also displayed a genotype-specific response in fasting apo B-48 values, total cholesterol, fasting triacylglycerols, and fasting TRL phospholipids. Moreover, this greater genotype-specific susceptibility appears also to be sex specific. Indeed, when we consider the Framingham score, which represents a "global" evaluation of risk, we observed in men a marked decrease of this score that is significantly more important than in women.

DISCUSSION

Our results show the interaction of sex with 2 gene polymorphisms and how the response to diet can be modulated by these polymorphisms. In this study, we did not find any relation between the Ala54Thr polymorphism at the *FAB2* locus and fasting glucose or insulin concentrations, which is consistent with some previous data (15–19). An association between this polymorphism and insulin resistance was previously described in the Pima population, a group known to have a high prevalence of type 2 diabetes mellitus (3). Nevertheless, it was shown later that this association is likely to be linked to promoter variations, which are in complete genotypic concordance with the Ala54Thr substitution in Pima but not in white populations (20). Moreover, in young French-Canadians, among the components of the metabolic syndrome, only plasma triacylglycerol concentrations were shown to display an interaction with the *FABP2* polymorphism (11). In our study, only men homozygous for the Thr allele showed a tendency to higher triacylglycerol concentrations (data not shown).

Previous experiments showed that in vitro, compared with the Ala isoform, the human I-FABP Thr protein presented a 2-fold higher affinity for 18:1 FAs (21). In addition, expression studies showed that Caco-2 cells transfected with the Thr-encoding allele exhibited a 2-fold increase in FA uptake and a 5-fold increase in triacylglycerol secretion than did Caco-2 cells transfected with the Ala-encoding allele (22). Finally, it was shown that human intestinal explants from carriers of the Thr allele displayed a marked increase in triacylglycerol secretion (23). In agreement with those studies, we showed here a significant association between the Thr54 variant and an altered fasting plasma FA profile (increase in 16:0 associated in men with an increase in 18:1). Our study also showed an interaction between the Ala/Thr polymorphism and sex because the Thr/Thr men showed high TRL-cholesterol and TRL-phospholipid concentrations. These latter alterations together with the increases in 16:0 and 18:1 might be associated with an increased risk of CVD. Such an interaction between this polymorphism and sex has already been described. Although not strictly comparable, data reported from the Framingham cohort showed that women but not men homozygous for the Ala54-encoding allele displayed lower total and LDL-cholesterol concentrations (24). Such a sex-specific difference, as observed in our study, can also be linked to recent data obtained from I-FABP knockout mice (25) in which females did not present any altered liver histology, whereas males showed centrolobular vacuolated hepatocytes.

Our study also highlighted a sex-specific link between allelic variation at the *MTTP* −493 locus and lipid metabolism at baseline. MTP is known to play a critical role in the assembly of triacylglycerol-rich particles in liver and intestine. However, some clues seem to indicate that the expression of the *MTTP* gene could be different for the tissue and could lead to compensatory effects. For example, an intestine-specific MTP deficiency in mice led to an increase in hepatic VLDL secretion (26). MTP inhibitors also may present different effects on intestine or liver (27).

The *MTTP* T allele was shown to be associated with an increase in gene expression (7) and with an increased production of small apo B-48–containing lipoproteins in the postprandial state (28). It is thus not surprising to observe in our study an increase in plasma apo B-48 concentration, the specific chylomicron apolipoprotein, in the subjects homozygous for the T allele. A longitudinal study in adult men has shown that T allele carriers presented a significantly increased risk of coronary heart disease (8). Paradoxically, however, this study also put forward that this increased risk was linked to a decrease in plasma total cholesterol in this subpopulation. Other works however did not show any modification in circulating cholesterol (10–12). In our study we can only observe a tendency to an increase in the T homozygous population. The increased risk observed by Ledmyr et al (8) might be associated to the modification of other lipid compounds that have long been linked to CVD risk. As a matter of fact, we observed in men homozygous for the T allele, an increase in the saturated FA 16:0 together with a decrease in polyunsaturated FAs (18:3 and 20:3). This pattern is known to be associated with CVD risk. Interestingly, this FA pattern appears to be sex specific because it is restricted to the male population.

Because several studies have cast light on the possible regulation of the *MTTP* gene expression by insulin (29, 30), we have searched for a possible link between fasting plasma insulin concentrations and the *MTTP* −493 polymorphism. Although no significant modification of this marker could be found according to genotype in the whole population, a significant genotype × sex interaction was observed.

The effect of the 3-mo diet resulted in a clear improvement of most biological markers (14). We must note that no differences in the dietary intakes of men and women were observed at baseline and at the end of the diet period. However, the response to diet differed according to the 2 loci. Indeed, the response did not seem to be greatly modulated by the Ala/Thr *FABP2* polymorphism, except for the decrease in apo B in the Thr/Thr population and the different pattern of response in Thr/Thr men and women

TABLE 4
Fasting biochemical markers according to the −493 G/T polymorphism of the microsomal triglyceride transfer protein[1]

	T/T	G/T	G/G	P^2
Apo A-1 (g/L)[3]				
Baseline	1.48 ± 0.21[4]	1.47 ± 0.28	1.51 ± 0.32	0.475
3 mo	1.40 ± 0.30	1.41 ± 0.28	1.44 ± 0.26	0.883
Apo B (g/L)[3]				
Baseline	1.29 ± 0.24	1.24 ± 0.25	1.22 ± 0.22	0.349
3 mo	1.17 ± 0.28	1.22 ± 0.26	1.15 ± 0.21	0.179
Apo B-48 (g/L)[3]				
Baseline	0.30 ± 0.18	0.27 ± 0.23	0.20 ± 0.17	0.011
3 mo	0.24 ± 0.27	0.28 ± 0.22	0.27 ± 0.28	0.015
Apo E (mg/L)[3]				
Baseline	45.4 ± 13.5	42.7 ± 12.2	40.3 ± 12.7	0.294
3 mo	36.9 ± 8.5	41.2 ± 12.9	40.2 ± 17.4	0.057
Total cholesterol (mmol/L)[3,5]				
Baseline	6.98 ± 0.89	6.49 ± 1.05	6.43 ± 0.84	0.088
3 mo	6.11 ± 1.09	6.16 ± 1.00	6.09 ± 0.87	0.030
HDL cholesterol (mmol/L)[3,6]				
Baseline	1.52 ± 0.48	1.51 ± 0.42	1.56 ± 0.47	0.337
3 mo	1.56 ± 0.56	1.48 ± 0.40	1.59 ± 0.58	0.293
LDL cholesterol (mmol/L)[3,7]				
Baseline	4.64 ± 0.75	4.20 ± 1.03	4.15 ± 0.83	0.099
3 mo	4.00 ± 0.80	3.93 ± 0.83	3.76 ± 0.80	0.122
Triacylglycerols (mmol/L)[3,8]				
Baseline	1.62 ± 0.87	1.58 ± 0.88	1.46 ± 0.94	0.256
3 mo	1.11 ± 0.46	1.44 ± 0.80	1.36 ± 0.86	0.036
TRL cholesterol (mmol/L)[3]				
Baseline	1.36 ± 0.76	1.18 ± 0.86	1.18 ± 1.39	0.112
3 mo	0.90 ± 0.48	1.16 ± 0.85	1.11 ± 1.30	0.087
TRL triacylglycerols (mmol/L)[3,6]				
Baseline	1.10 ± 0.65	1.12 ± 0.87	1.05 ± 1.15	0.258
3 mo	0.77 ± 0.45	1.02 ± 0.76	0.97 ± 0.86	0.079
TRL phospholipids (mmol/L)[3,8]				
Baseline	0.38 ± 0.18	0.38 ± 0.26	0.33 ± 0.29	0.135
3 mo	0.26 ± 0.14	0.36 ± 0.24	0.32 ± 0.27	0.042
Glucose (mmol/L)[3,9]				
Baseline	5.24 ± 0.65	5.19 ± 0.56	5.28 ± 0.73	0.340
3 mo	5.00 ± 0.71	5.04 ± 0.53	5.09 ± 0.65	0.483
Insulin (mU/L)[3,6]				
Baseline	9.69 ± 5.08	10.13 ± 5.98	11.31 ± 7.90	0.825
3 mo	7.39 ± 4.44	8.38 ± 4.41	9.13 ± 6.10	0.370
Framingham score[3,10]				
Baseline	6.76 ± 3.09	6.09 ± 3.21	5.52 ± 3.14	0.191
3 mo	4.47 ± 3.70	5.33 ± 3.37	4.78 ± 3.03	0.002
Insulin at baseline (mU/L)[6,11]				
Men[12]	12.63 ± 6.47	11.63 ± 5.94	10.96 ± 8.61	0.221
Women[13]	8.79 ± 4.49	9.09 ± 5.85	11.59 ± 7.38	0.136
Framingham score[10,14]				
Men[12]				
Baseline	7.00 ± 4.97	6.42 ± 3.06	5.72 ± 2.67	0.247
3 mo[13]	2.75 ± 5.85	5.91 ± 2.97	4.65 ± 3.27	0.008
Women[13]				
Baseline	6.69 ± 2.56	5.86 ± 3.33	5.37 ± 3.48	0.448
3 mo[13]	5.09 ± 2.70	4.94 ± 3.60	4.89 ± 2.86	0.089

[1] Apo, apolipoprotein; TRL, triacylglycerol-rich lipoprotein.
[2] Comparison between polymorphisms at baseline (tested with general linear models) and in their response to diet (tested with repeated-measures general linear models).
[3] n = 17 T/T, 83 G/T, 69 G/G.
[4] $\bar{x}$ ± SD (all such values).
[5] Adjusted for menopausal status in women.
[6] Adjusted for BMI.
[7] Adjusted for smoking status.
[8] Adjusted for menopausal status in women and BMI.
[9] Adjusted for menopausal status in women, BMI, professional activity, alcohol consumption, and antihypertensive treatment.
[10] Adjusted for menopausal status in women, BMI, smoking status, and professional activity.
[11] Significant interaction of genotype × sex, P = 0.045.
[12] n = 4 T/T, 34 G/T, 31 G/G.
[13] n = 13 T/T, 48 G/T, 38 G/G.
[14] Significant interaction of genotype × sex × time, P = 0.036.

for 18:1. This lack of reactivity is not surprising because, to date, few studies reported the influence of the FABP2 Ala54Thr polymorphism on the efficiency of a diet in terms of modulation of the magnitude of the response. One study reported on a greatest high-fiber diet-induced response in plasma total and LDL cholesterol among dyslipidemic subjects homozygous for the Thr-encoding allele (31). A second study was performed in normolipidemic subjects who were submitted to a change from saturated fat to monounsaturated FAs. That study showed that carriers of the Thr-encoding allele presented an enhanced decrease in insulin sensitivity compared with Ala/Ala homozygous subjects. Although in that latter study and in ours, the type of diets was comparable, we did not detect any diet-induced difference in insulin sensitivity: all subjects had decreased fasting plasma insulin concentrations regardless of genotypes.

Finally, our study showed a clear interaction between the *MTTP* −493G/T variation and the magnitude in the response to the diet. Indeed, although biological markers for the risk of CVD clearly decreased after the 3-mo intervention, this improvement is particularly marked in men homozygous for the T allele than in the other subjects. The TT subjects, as a whole, showed a significantly higher response in reducing fasting cholesterol, apo B-48, TRL phospholipids, 16:0, and 18:1 and in increasing 18:2 values, but only TT men showed a major improvement of the Framingham score. This sex-related sensitivity to diet can be linked to the recent finding that, in men with familial hypercholesterolemia but not in women, this polymorphism modulated after-treatment plasma triacylglycerol values (32).

In conclusion, important new findings in this study show that *FABP2* Ala54Thr and *MTTP* −493G/T polymorphisms are differently involved in the contribution to cardiovascular risk in men and women. These 2 polymorphisms interact with sex *1*) at baseline on fasting biological markers showing an increased risk of CVD for *FABP2* Thr/Thr and *MTTP* TT men and *2*) after the 3-mo diet leading to a better response in *MTTP* TT men.

We thank Chantal Bideau, Danielle Iniesta, and Nicole Peyrol for their technical help in SNP determination.

The author's responsibilities were as follows—MG: was involved in the interpretation of the data and wrote the manuscript; SD and MD: performed statistical analysis of data; HP and RP: were involved in blood analyses, CD: was involved in the collection of data; DL: coordinated the study, RP: designed the genetic study and contributed to the writing of the manuscript. None of the authors had a personal or financial conflict of interest.

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