

RAPID COMMUNICATION

The 103I Variant of the Melanocortin 4 Receptor Is Associated with Low Serum Triglyceride Levels

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Context: The melanocortin 4 receptor (MC4R) is an essential regulator of energy intake and body weight. Recently, the V103I polymorphism of MC4R has been shown to be negatively associated with body mass index. This suggests that serum lipids and blood pressure in individuals carrying the 103I allele might be influenced as well.

Objective: The objective of this study was to determine whether the most common polymorphism of the MC4R, V103I, affects serum lipid levels and/or blood pressure.

Design, Setting, and Participants: The study participants were 1173 consecutive patients undergoing cardiac catheterization; they were genotyped for the rs2229616 G→A substitution at codon 103 (V103I polymorphism) of the *MC4R* gene. Patients had strictly fasted for at least 12 h before blood samples were drawn. The average age

of the patients was 60.9 yr; 72% were males.

Main Outcome Measures: The main outcome measures were body mass index, serum lipids, aortic and systolic blood pressure, and MC4R polymorphism V103I.

Results: Heterozygous carriers of the 103I allele had significantly lower triglyceride levels than individuals homozygous for the wild-type allele (127 vs. 168 mg/dl mean total triglyceride; $P = 0.001$ or 0.009 after Bonferroni adjustment for seven tests). No homozygous carriers of the 103I allele were present in the study population.

Conclusions: Our study suggests an influence of MC4R activity on triglyceride levels in cardiovascular patients. (*J Clin Endocrinol Metab* 91: 535–538, 2006)

HYPERLIPIDEMIA AND HYPERTENSION are major risk factors for coronary artery disease (CAD), which is the leading cause of death in western countries. Both hyperlipidemia and hypertension can be caused by environmental factors and/or a genetic predisposition. Some monogenic forms of lipid disorders for instance, caused by mutations in the low-density lipoprotein (LDL) receptor gene (*LDLR*) or the apolipoprotein B-100 (apoB-100) gene (*APOB*) have been identified, but these are rare in patients with hyperlipidemia (1). Similarly, monogenic forms of hypertension have been described, e.g. mutations in the epithelial sodium channel (Liddle syndrome) (2, 3) or steroid 11 β -hydroxylase aberrations (glucocorticoid-remediable aldosteronism) (4). All these monogenic disorders are rare; most genetically based, phenotypical variation of these traits is assumed to be due to polygenic factors. Few such factors (e.g. upstream stimulatory factor 1) have been identified to

date (5, 6). Therefore, the search for genetic variations that predispose to elevated blood lipids is ongoing. Because there is a close link among obesity, hyperlipidemia, blood pressure, and CAD (7, 8), we were interested in genes known to be relevant for obesity, such as the melanocortin 4 receptor (*MCR4*) gene (*MC4R*), and their effects on human lipoprotein levels, hypertension, and atherosclerosis. The most common *MC4R* missense variation, V103I, has previously been shown to be associated with lower risk to develop obesity (9), lower body mass index (BMI) (10), lower waist to hip ratio (11), and higher cortisol levels (11). In particular, a trend toward reduced triglyceride levels has been reported in Swedish men carrying the 103I polymorphism (A allele) (11). However, this trend was not detected in a group of mostly female Finish elderly (12). Thus, in this study we investigated whether the V103I polymorphism affects parameters relevant for the pathogenesis of atherosclerosis, such as lipid metabolism and blood pressure, in our study group of 1173 cardiovascular patients.

Subjects and Methods

Study population and procedures

The study population consisted of 1173 Caucasians who had undergone diagnostic coronary angiography at University Hospital Marburg between August 1998 and February 2002. Patients with renal disease (creatinine, >2 mg/dl), malignancies, hypo- or hyperthyroidism, or

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Abbreviations: apo, Apolipoprotein; BMI, body mass index; CAD, coronary artery disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MC4R, melanocortin 4 receptor; SCD 1, stearyl-coenzyme A desaturase 1; VLDL, very low-density lipoprotein.

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receiving systemic glucocorticoids were excluded from the study. The study protocol, according to the Helsinki declaration, was approved by the local ethics committee, and written informed consent was obtained from all patients before the study. These 1173 subjects were a subset of a well-defined cohort of patients that was collected to optimize strategies for the prevention of CAD (13). The mean age of the in-patients, mostly from the Marburg region of Germany, was 60.9 yr; 72% were males. Blood samples for DNA isolation and serum analyses were obtained after a 12-h or longer fasting period. BMI, serum triglycerides, cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, as well as aortic and systolic blood pressures (measured directly during coronary angiography) were analyzed (Table 1). Patients were considered diabetic if fasting glucose was above 7 mmol/liter (according to the guidelines of the American Diabetes Association) or if they were already treated with antidiabetic drugs or insulin injections. The percentage of patients with type 2 diabetes was not significantly different between the groups (wild-type, 16%; carriers of the A allele, 24%; $P = 0.13$). Drug treatments were not discontinued for the purpose of the study. Exact medication data were available for a subset of 81% ($n = 952$) of the patients. Five patients, all wild-type, were treated with fibrates (strong triglyceride-lowering effect). The percentage of statin (strong cholesterol-lowering effect)-treated individuals was virtually identical in carriers of the MC4R wild-type G allele and heterozygous carriers of the A allele (54.2% vs. 54.9% of patients with medication information). The prescription rate of other often-used drugs (*e.g.* β -blockers, inhibitors of angiotensin-converting enzyme, diuretics, and anticoagulants) was not different between the groups. To exclude confounding effects of medication and diabetes mellitus, regression analysis of triglyceride values was repeated in the subset of patients for whom complete information about lipid-lowering drug treatment and diabetes status was available (Table 2).

Determination of lipid parameters and genotyping

Fasting venous blood samples were collected, and plasma was separated from blood cells by centrifugation and immediately used for the

TABLE 1. Logistic regression model for dependent variables

Variable	No.	Estimator regression	SE regression	<i>P</i> regression	Allele	N	Mean	SD
log (TGL)	1173	−0.226	0.070	0.001	V103	1116	TGL (mg/dl)	
					I103	57	167.7	110.9
log (BMI)	1173	0.0096	0.0203	0.638	V103	1116	BMI (kg/m ²)	
					I103	57	126.9	65.1
log (CHOL)	1173	−0.053	0.034	0.117	V103	1116	27.34	4.14
					I103	57	27.59	3.78
log (HDL)	1166	0.054	0.042	0.201	V103	1116	CHOL (mg/dl)	
					I103	57	186.7	46.9
log (LDL)	1104	−0.053	0.049	0.285	V103	1110	HDL (mg/dl)	
					I103	56	175.6	41.0
AODP	1071	−2.853	1.832	0.120	V103	1048	LDL (mg/dl)	
					I103	56	114.8	39.3
AOSP	1074	0.257	3.530	0.942	V103	1017	AODP (mm Hg)	
					I103	54	109.2	36.6
					V103	1020	AOSP (mm Hg)	
					I103	54	71.4	13.3
					V103	1116	68.6	10.8
					I103	57	60.9	12.8
					V103	1116	Age (yr)	
					I103	57	60.7	11.1
					V103	1116	Sex	
					I103	57	315	28.2%
					V103	1116	Females	
					I103	57	13	22.8%

Seven clinical parameters were investigated: triglycerides (TGL), BMI, total cholesterol (CHOL), HDL cholesterol (HDL), LDL cholesterol (LDL), aortic diastolic blood pressure (AODP), and aortic systolic pressure (AOSP). Displayed are the estimates for the clinical parameter in the respective multiple regression model, which adjusts for age, sex and BMI (for BMI, only age and sex). The number of patients varies due to partially incomplete data sets. The reported means and SDs are from the raw data.

TABLE 2. Regression analysis of triglyceride levels

Variable	Estimator regression	SE regression	<i>P</i> regression
Intercept	4.230	0.156	<0.0001
V103I	−0.221	0.085	0.009
Sex	0.013	0.041	0.748
Age	−0.002	0.001	0.001
BMI	0.028	0.004	<0.0001
Diabetes	0.173	0.049	0.001
Medication	0.074	0.038	0.052

Complete logistic regression model for the dependent variable log (triglycerides) with 856 observations.

measurement of lipids. Plasma total cholesterol and triglycerides were determined using the CHOD-PAP (catalog no. 11489437) and GPO-PAP (catalog no. 11488899) kits from Roche (Mannheim, Germany). HDL-cholesterol was determined after precipitation of apoB-containing lipoproteins with phosphotungstate acid/MgCl₂ (catalog no. 543004, Roche). LDL-cholesterol was calculated according to the method described by Friedewald *et al.* (14). Genomic DNA was isolated from EDTA-anticoagulated blood using standard procedures (15). DNA samples were genotyped for the V103I polymorphism (A/G allele at rs2229616) as described previously (16).

Statistical analysis

The relationship between seven clinical parameters and the V103I polymorphism was studied by generalized linear models. Data were log-transformed if the parameter showed a skewed distribution. The (transformed) clinical parameters were used as the dependent variable in the models; the binary 103I carrier status, sex, age, and BMI were used as independent variables. Thus, the resulting models show the dependency between the clinical parameters and the V103I polymorphism

adjusted for sex, age, and BMI. The estimates for 103I carrier status and its SE from the multiple regression models were used to perform *t* tests, and the results are displayed in the tables. Bonferroni adjustment for the seven clinical parameters relevant for the questions treated in this study resulted in a required $P < 0.007$ ($0.05/7$) to meet the overall significance level of 5%. Six additional clinical parameters were investigated (body surface, creatinine, apoA1, apoB, ejection fraction, and cardiac index), for which we did not assume influence of the V103I carrier status; adjustment for all 13 parameters would not change the main results.

Results

Of the 1173 individuals studied, 4.9% were heterozygous for the V103I polymorphism. Homozygous carriers of the 103I allele were not present in the study group. Heterozygous carriers of the 103I allele of the MC4R V103I polymorphism exhibited significantly lower fasting triglyceride levels (mean total triglyceride, 127 *vs.* 168 mg/dl; unadjusted $P = 0.001$; after Bonferroni adjustment for all seven tested parameters, $P = 0.009$) compared with homozygous carriers of the V103 allele (Table 1). The values in homozygous V103 carriers ranged from 31–1220 mg/dl (median, 140 mg/dl) and in the heterozygous carriers of the 103I allele from 44–450 mg/dl (median, 115 mg/dl). For LDL cholesterol and HDL cholesterol, a nonsignificant trend toward lower LDL cholesterol and higher HDL cholesterol was observed in carriers of the 103I allele. There was no significant difference between wild-type and variation carriers in age, BMI, or aortic and systolic blood pressures. Additionally, a regression model was investigated for the subset of 856 individuals for whom information about lipid-lowering medication and diabetes mellitus status was available (Table 2). The model revealed that the V103I genotype is an independent predictor of triglyceride levels. Not unexpectedly, both BMI and type 2 diabetes proved to be additional predictors. Analysis of two-way interactions between the V103I polymorphism and each of the variables (diabetes mellitus, lipid-lowering medication, and BMI) did not reveal significant interactions (data not shown).

Discussion

The prime finding of this study is the substantially lower triglyceride levels in carriers of the A allele at rs2229616 in the MC4R gene, affirming a previous trend reported by Rosmond *et al.* (11), who described lower triglyceride levels in 13 carriers of the MC4R 103I allele (122.5 *vs.* 157.5 mg/dl; $P = 0.163$) in a study group of 284 Swedish men (mean age, 51 yr). In contrast, Rutanen *et al.* (12) did not confirm this finding in a group of 1031 elderly Finnish probands (mean age, 70 yr; 35% men) from the Kuopio region. Because the sex distribution varies strongly between these study groups, and our own group consists of 72% men, we explored the possibility that a sex effect explains the observed differences in triglyceride levels. However, no sex differences in triglycerides were detectable in our cohort (data not shown). Other potential reasons underlying these differences could be the selection of the study group, for example, with respect to age, ethnicity, or compliance with the required fasting period in epidemiological studies. In contrast to the other two studies, all of our patients presented for cardiac catheterization. Confounding effects of lipid-lowering medication and diabetes mellitus could be excluded in our study group. Finally, as in

all association studies, false positive or false negative findings cannot be excluded.

Because obesity is closely associated with elevated serum triglycerides (7), altered triglyceride levels might be explained by an influence of MC4R on body weight. The A allele (I103) has recently been shown to be negatively associated with BMI (change in BMI, 0.52 kg/m² in two surveys combining data from 7937 people) (10). This finding suggests a tighter control of body weight in subjects carrying the 103I variation, which, in turn, would lead to lower triglyceride levels. However, in the study group of cardiovascular patients analyzed, we were not able to find a significant difference in BMI of heterozygous 103I carriers *vs.* noncarriers. Furthermore, we adjusted triglyceride levels for BMI in the statistical analysis to control for even weak confounding BMI effects. There is still the possibility that in our particular study group of patients undergoing heart catheterization, only body composition (*i.e.* percent body fat), not BMI, is altered by the V103I polymorphism; due to the lack of body composition data, we cannot exclude this possibility. However, even if this would be the case, it remains questionable whether the presumably small effect on body fat can cause such a relatively substantial difference in triglyceride levels. Therefore, it seems reasonable to consider a direct effect of MC4R activity on triglyceride levels. There are two main sources for serum triglycerides: 1) food intake and secretion into the lymph as chylomicrons, and 2) liver synthesis and secretion as very low-density lipoprotein (VLDL) particles. All patients studied had been strictly fasting for 12 h or more before blood drawing. Therefore, triglycerides were unaffected by chylomicrons, leaving liver synthesis as the only triglyceride source. How, then, should MC4R affect liver metabolism? The first clues stem from animal models. In rats, intracerebroventricular infusion of the MC3/4R synthetic agonist MTII reduces the mRNA expression of stearoyl-coenzyme A desaturase 1 (SCD 1) in liver, whereas the MC3/4R antagonist SHU9119 has the opposite effect (17). This suggests that modulation of MC3/4R activity in the brain can affect liver metabolism. SCD 1 is a key enzyme required for synthesis of triglycerides and *Scd 1*^{-/-} mice show severely reduced VLDL secretion by the liver (18). Therefore, we hypothesize that a more active form of the MC4R linked with the 103I allele (9) entails a reduced SCD 1 activity, resulting in reduced triglyceride synthesis and VLDL secretion. Interestingly, sympathetic denervation of rat liver leads to greatly decreased incorporation of ¹⁴C-labeled oleate into the triglyceride fraction of VLDL (19). Our hypothesis is also supported by physiological studies involving intracerebroventricular injection of antagonist SHU9119 into prostate tumor-bearing rats. Central blockade of the melanocortin receptors increased serum triglyceride levels more than 2-fold those in untreated tumor rats or healthy control rats (20).

As of today no functional differences have been found upon comparison between the wild-type MC4R and the 103I variant (21). It has been argued that the mean BMI reduction of 0.5 kg/m² in polymorphism carriers is due to a more active form of the receptor (9, 10) either as a consequence of the valin to isoleucine exchange directly or of other single nucleotide polymorphisms in linkage disequilibrium with V103I. If the assumption is correct that the 103I variation is associated with

elevated MC4R function, one would expect that MC4R loss of function mutations lead to increased serum triglyceride levels. Unfortunately, the relative rareness of functionally relevant MC4R mutations combined with high interpersonal variability of triglyceride levels hamper statistically significant conclusions. Vaisse *et al.* (22) reported increased triglyceride levels in heterozygous MC4R mutation carriers, but these levels were similar to those found in obese, weight-matched controls. In a similar study, Potoczna *et al.* (23) compared a group of heterozygous MC4R variation carriers with a group of BMI-, sex-, and age-matched controls. In contrast to the findings by Vaisse *et al.* (22), triglyceride levels were significantly higher in the variation carriers after a 12-h fasting period ($P < 0.001$). However, both studies comprise only the small number of 20 and 19 mutation carriers, respectively, and the functional consequences of the diverse mutations are still being debated. Therefore, caution should be exercised when interpreting these data. Other studies describing cases of severely increased triglyceride levels (24, 25) or levels within the normal range (26, 27) were reported. Again, no clear-cut conclusions can be drawn from these reports.

Clearly, more data need to be collected to assess the phenotypic consequences of the V103I polymorphism of the MC4R in human populations. Because all our patients presented for cardiac catheterization, the findings of our study may only be relevant for subjects with CAD or a high risk of CAD. However, in this group, which represents a substantial subset of the population due to the high prevalence of CAD, carriers of the 103I variation exhibit significantly reduced triglyceride levels. Although confirmation and additional research are required, we believe that our finding may provide new insights into the complex regulation of serum triglyceride levels in man.

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