DOI 10.1002/mnfr.200500241 Mol. Nutr. Food Res. 2006, *50*, 270–274

The L513S polymorphism in medium-chain acyl-CoA synthetase 2 (MACS2) is associated with risk factors of the metabolic syndrome in a Caucasian study population

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Enzymes of the medium-chain acyl-CoA synthetase (MACS) family catalyze the ligation of medium chain fatty acids with CoA to produce medium-chain-acyl-CoA. At least four members of the MACS gene family are clustered on human chromosome 16p12. Association studies in the Japanese Suita cohort of MACS polymorphisms and various phenotypes revealed the contribution of the Leu513Ser polymorphism in MACS2 to multiple risk factors of the metabolic syndrome. Here, we investigated the association between this polymorphism and different risk factors in the Caucasian Metabolic Intervention Cohort Kiel. Seven hundred and sixteen male subjects aged 45–65 years were recruited for a standard oral glucose tolerance test and the postprandial assessment of metabolic parameters after an oral metabolic tolerance test (oMTT; 1017 kcal, 51.6% fat, 29.6% carbohydrates, 11.9% protein). The MACS2 Leu513Ser polymorphism was determined by TaqMan-Assay in 705 subjects. Postprandial triglyceride levels following oMTT [area under the curve (AUC)] were significantly higher in subjects carrying the Ser allele compared to subjects homozygous for the Leu allele (1690 ± 100 mg × h/dL *ersus* 1514 ± 39 mg × h/dL, $p = 0.04$). Significant differences between genotype groups were also found for fasting (108 ± 1.9 mg/dL *versus* 104 ± 0.66 mg/dL, $p = 0.04$) and postprandial (AUC 535 ± 11 *versus* 512 ± 4.0, $p = 0.02$) glucose levels as well as for high-density-lipoprotein, body mass index, waist circumference, systolic and diastolic blood pressure. Carriers of the Ser allele also show an increased risk of impaired glucose metabolism (OR: 1.48, 95% confidence interval: 0.98–2.27, $p = 0.07$), adiposity (1.8, 1.16–2.81, $p = 0.01$) and hypertension (1.5, 0.99–2.17, $p = 0.06$). In conclusion, our results suggest an involvement of the MACS2 Leu513Ser polymorphism in the development of the metabolic syndrome in Caucasian population. Additionally, the higher triglyceride and glucose levels after an oMTT support a possible functional impact of the polymorphism *in vivo*.

Keywords: Medium-chain acyl-CoA synthetase / Metabolic syndrome / Oral metabolic tolerance test / Polymorphis / Postprandial

Received: November 14, 2005; revised: December 16, 2005; accepted: December 18, 2005

1 Introduction

The initial step in fatty acid metabolism is the activation of these molecules with CoA to produce acyl-CoA. These reactions are catalyzed by enzymes of the acyl-CoA synthetase family [1]. Over the past years, a number of acyl-CoA synthetases were described [2–5]. These enzymes are capable of activating long-chain (C16–C20) and very long-chain (C22) fatty acids. More recently, a gene family of medium-chain (C4–C14) acyl-CoA synthetases (MACS) was partially characterized [6–12]. These enzymes have a putative mitochondrial targeting signal at the N terminus of

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Abbreviations: AUC, area under the curve; **BMI**, body mass index; **HDL**, high-density-lipoprotein; **MACS**, medium-chain acyl-CoA synthetase; **MICK**, Metabolic Intervention Cohort Kiel; **oMTT**, oral metabolic tolerance test; **WHR**, waist-to-hip ratio

their primary amino acid sequence and are localized in the mitochondrial matrix [13]. Based on the fatty acid specificity and subcellular distribution, MACS initiate the degradation of medium-chain fatty acids generated by the peroxisomal β-oxidation system from long-chain and very long-chain fatty acids [13]. Members of MACS also play a role in the conjugation of a series of xenobiotic benzoic acid derivatives with glycine [8].

Until now, five human members of the human MACS gene family have been identified. O-MACS is specifically expressed in the olfactory epithelium and may play a role in processing odorants in a zone-specific manner [11]. The other members were named MACS1, MACS2, MACS3 and SAH. The acronym of the MACS gene SAH is based on its homology to rat SAH (Spontaneously hypertensive rat-clone A-Hypertension associated). Therefore, SAH is a candidate gene for hypertension [14, 15]. A study in the Japanese Suita cohort of 4039 subjects revealed an association between SAH variations and hypertension, obesity, and hypertriglyceridemia [6]. Since SAH and MACS1–3 are clustered on chromosome 16p12 the association between SAH and multiple risk factors might be due to linkage disequilibrium with polymorphisms in other members of this gene family. Association studies in the Japanese Suita cohort revealed an association of the MACS2 Leu513Ser polymorphism with multiple risk factors of the metabolic syndrome [16]. Further analysis of haplotypes and diplotypes defined by SAH and MACS1–3 polymorphisms demonstrates the importance of the MACS2 Leu513Ser polymorphism for these associations. Against this background, we evaluate the impact of this non-synonymous MACS 2 polymorphism in our Caucasian Metabolic-Intervention-Cohort-Kiel (MICK).

2 Materials and methods

2.1 Metabolic-Intervention-Cohort-Kiel (MICK)

Seven hundred and sixteen male subjects aged 45–65 years were recruited from the resident register of the town of Kiel, Germany. The recruitment period lasted from January 2003 to March 2004. Exclusion criteria were: known diabetes type 1 or 2, diseases with impairment of nutrient digestion or metabolism, intake of lipid-lowering drugs or hormones, operation on the intestine in the past 3 months, hypo- or hyperthyreosis, chronic renal disease, hepatitis, cholestasis, alcoholism or cancer. Blood pressure, body weight, height, waist and hip circumference were determined at recruitment by means of standardized procedures. Unknown type 2 diabetes mellitus was diagnosed based on a standardized oral glucose tolerance test [17]. One hundred and seventy-seven men were diagnosed with impaired glucose metabolism: fasting glucose >110 mg/dL determined on two different occasions or postprandial glucose >140 mg/dL; 49 of these had type 2 diabetes mellitus (fasting glucose >126 mg/dL determined on two different occasions or postprandial glucose >200 mg/dL). Five hundred and twenty-eight had normal glucose tolerance and 11 subjects had to be excluded from analysis because DNA genotyping failed. Serum and plasma was separated from whole blood by centrifugation and stored at −70°C for later determinations. Serum cholesterol, serum high-density-lipoprotein (HDL) cholesterol and plasma glucose were determined using enzymatic methods with Kone Lab 20i analyzer (Kone, Finland). All samples were measured in duplicate. All study participants had given informed consent and the genotype assessment was approved by the local ethics committee. The MACS2 Leu513Ser polymorphism was determined by TaqMan-Assay in 705 subjects. DNA genotyping of 11 subjects failed. Insulin sensitivity was estimated with the homeostasis model assessment (HOMA) [insulin (μU/mL)/glucose (mmol/L)/22.5] according to Wallace *et al*. [18].

2.2 Oral metabolic tolerance test in MICK

A minimum of 3 days after the oral glucose tolerance test participants visited the department after a 12-h-overnight fast for an oral metabolic tolerance test (oMTT). An intravenous catheter equipped with disposable obturators was inserted into a forearm vein for blood sampling and a fasting blood sample (0 h) was obtained. Following this, the subjects drank 500 mL of a standardized high-fat mixed meal containing the following ingredients: 30 g of protein (11.9 kcal%), 75 g of carbohydrate (29.6 kcal%; 93% saccharose, 7% lactose), 58 g of fat (51.6 kcal%; 65% saturated, 35% unsaturated fatty acids), 10 g of alcohol (6.9 kcal%), 600 mg cholesterol and 30000 IU retinylpalmitate. The total energy content was 1017 kcal (4255 kJ). The fatty acid pattern (% w/w) of fat was: caprone – 2%, capryl – 1.2%, caprine – 2.7%, laurine – 3%, myristine – 10.9%, palmitine – 28.4%, stearine – 13.1%, palmitoleine – 2.4%, oleic – 27.1%, linolic – 2.4%, linolenic – 1.6% acid and other acids 5.2%. Accordingly, the medium-chain fatty acids content was 8.9%. The test meal was ingested within 15 min after the drawing of the fasting blood sample. Blood withdrawal was repeated at 30 min, 1, 2, 3, 4, 5, 6, 7, 8 and 9 h after ingestion of oMTT. Subjects were allowed to walk or sit, as they wished, but not to eat or exercise during the test. Intake of water *ad libitum* was permitted.

2.3 Genetic analyses

DNA was isolated from buffy coat (100 μL) using E.Z.N.A.® Blood DNA MiniKits (Peqlab Biotechnologie, Erlangen, Germany) according to the manufacturer's

instructions. Genotyping of MACS2 L513S (C/T) was performed with the TaqMan system (ABI, Foster City, CA, USA), fluorescence was measured with ABI Prism 7900 HT sequence detection system. Sequences of TaqMan assay primers and probes are available on request.

2.4 Statistical analyses

Allele and genotype frequencies were determined by gene counting. Statistics were computed with the Statistics Package for the Social Sciences 11.5 (SPSS, Chicago, IL, USA). Distributions of continuous variables were expressed as means ± SEM. The 0–9-h area under the curve (AUC) was calculated by the trapezoidal method. A p-value of <0.05 was considered statistically significant.

3 Results

In the Metabolic Intervention Cohort Kiel (MICK) of 716 males allele frequencies of the MACS2 Leu513Ser polymorphism were found to be 91% for the Leu-allele and 9% for the Ser-allele (Table 1). Genotype distributions (Leu-Leu: 81.8%, LeuSer: 17.7%, SerSer: 0.4%) in the study population complied with the Hardy-Weinberg equilibrium. Ser-carriers (LeuSer and SerSer) and L513 homozygotes (LeuLeu) were used as analysis groups, due to the low frequency of Ser homozygous subjects.

Table 1. MACS2 L513S polymorphism: genotype and minor allele frequency in MICK ($n = 705$)

SNP	refSNP ID	Base change	Genotype frequencies			Minori allele Frequency
			Leu/Leu	Leu/ Ser	Ser/Ser	
MACS2 L513S	rs16970281	C/T	0.818 ($n = 577$)	0.177 ($n = 125$)	0.004 ($n = 3$)	0.09

Anthropometric and metabolic variables according to MACS2 Leu513Ser polymorphism are shown in Table 2. No significant differences were found for waist-to-hip ratio (WHR) and low-density-lipoprotein levels between MACS2 genotype groups. In comparison to homozygous Leu-carriers, Ser carriers show significantly higher body mass index (BMI) ($p = 0.049$), waist circumference ($p = 0.051$), HDL ($p = 0.022$), systolic blood pressure ($p = 0.013$) and diastolic blood pressure ($p = 0.035$). In compliance with these findings, carriers of the rare allele also showed an increased risk (Table 3) of impaired glucose metabolism (OR: 1.48, 95% confidence interval: 0.98–2.27, $p = 0.07$), adiposity (1.8, 1.16–2.81, $p = 0.01$) and hypertension (1.5, 0.99–2.17, $p = 0.06$).

Since postprandial parameters are an important factor for the development of the metabolic syndrome, an oMTT (1017 kcal, 51.6% fat, 29.6% carbohydrates, 11.9% protein) was employed in the whole study population. As shown in Fig. 1a and b, the postprandial triglyceride levels following oMTT, were significantly higher (AUV 1690 ±

Table 2. Anthropometric and metabolic variables according to MACS2 L513S genotypes in MICK

Variable	Leu/Leu[a)]	Leu/Ser+Ser/Ser	p
Subjects (n)	577	128	
Age (years)	59.04 (0.229)	58.56 (0.467)[a)]	0.367[b)]
BMI (kg/m^2)	27.268 (0.1703)	28.062 (0.3684)[a)]	**0.049**[b)]
WHR	0.988 (0.0029)	0.998 (0.0055)[a)]	0.136 [b)]
Waist circumference (cm)	99.69 (0.512)	102.04 (1.058)[a)]	0.051[b)]
Systolic BP (mm HG)	128.57 (0.743)	132.94 (1.561)[a)]	**0.013**[b)]
Diastolic BP (mm HG)	80.1 (0.451)	82.33 (0.934)[a)]	**0.035**[b)]
HDL-C (mg/dl)	53.9351 (0.6178)	50.6308 (1.2394)[a)]	**0.022**[b)]
LDL (mg/dl)	142.81 (1.353)	148.55 (3.079)[a)]	0.076[b)]
HOMA (µU/mL × mmol/L)[e)]	3.12 (0.169)[c)]	3.47 (0.44)[c)]	0.141[d)]
Insulin × Glucose AUC (µU × mg/dL × h)	17 721 (1030)	20 720 (2912)[c)]	**0.045**[d)]

a) Arithmetic mean (SEM).
b) t-Test for equality of means, two-tailed significance.
c) Geometric mean (SEM)
d) Mann-Whitney U-test, two-tailed significance.
e) HOMA, homeostasis model assessment; µU/mL insulin × mmol/L glucose × 22.5^{-1} [18].

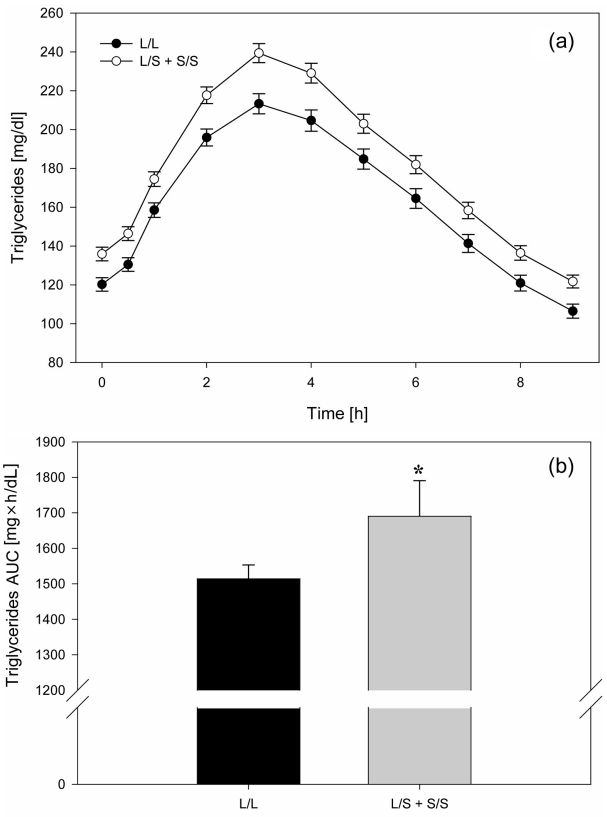

Figure 1. Fasting and postprandial triglyceride levels according to MACS L513S polymorphism in the MICK after an oMTT. Mean (± SEM) triglyceride values for concentrations (a) and AUC (b) in subjects homozygote for the Leu-allele (●) in comparison to Ser-carriers (○; L/S+SS) after an oMTT. *Significantly different from LL genotype, $p > 0.5$.

100, $p = 0.04$) in subjects carrying the Ser allele than in subjects homozygous for the Leu allele. Significant differences between genotype groups were also found for fasting (104 ± 0.66 mg/dL versus 108 ± 1.9 mg/dL, $p = 0.04$) and postprandial (AUC 512 ± 4.0 versus 535 ± 11, $p = 0.02$) glucose levels (Fig. 2a and b). Ser-carriers also show elevated insulin levels, but these differences do not reach a significant level (Fig. 3a and b). Considering the postprandial product of glucose and insulin response (AUC) as a parameter of insulin resistance, these values were also significantly higher in Ser carriers (20 720 ± 2912 versus 17 721 ± 1030, $p = 0.045$, Table 2) after oMTT.

4 Discussion

The initial publication of the MACS2 L513S polymorphism by Iwai *et al*. [16] reported a minor allele frequency of 20% in the Japanese Suita cohort. In contrast, we found a frequency for the minor allele of 9% in our Caucasian cohort.

Table 3. Association of MACS2 Leu513Ser with impaired glucose metabolism, adiposity and hypertension in MICK

	Impaired glucose metabolism				Adiposity (BMI ≥ 30)				Hypertension			
	Cases (%)	Controls (%)	OR[c] (95% CI[a])	p[b]	Cases (%)	Controls (%)	OR (95% CI)	p[b]	Cases (%)	Controls (%)	OR[c] (95% CI)	p[b]
Leu/Leu	135 (77)	442 (83)	1.0		104 (74)	470 (84)	1.0		288 (79)	288 (85)	1.0	
Leu/Ser +Ser/Ser	40 (23)	88 (16)	1.48 (0.98 – 2.27)	**0.07**	36 (26)	90 (16)	1.8 (1.16 – 2.81)	**0.01**	75 (21)	51 (15)	1.5 (0.99 – 2.17)	**0.061**

a) CI, confidence interval.
b) Fisher's exact test, two-tailed significance.
c) OR, odd's ratio.

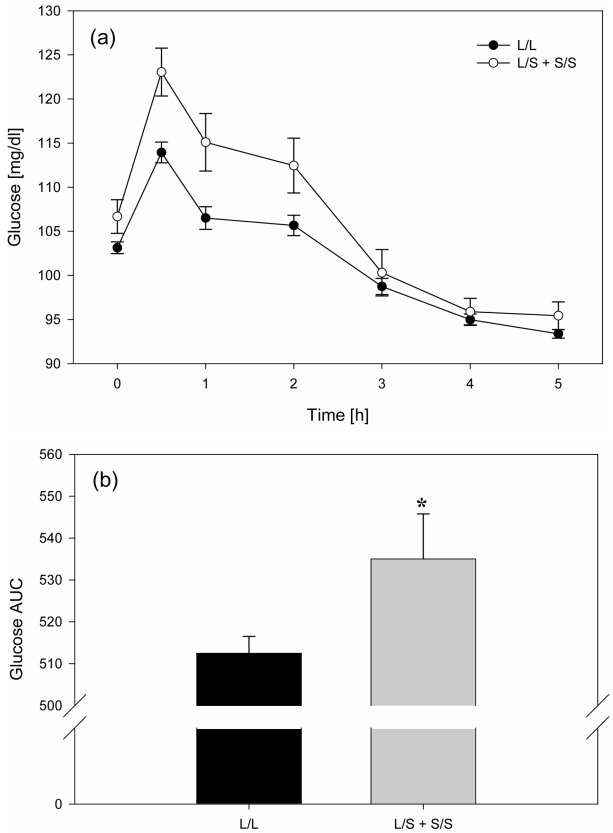

Figure 2. Fasting and postprandial glucose levels according to MACS L513S polymorphism in the MICK after an oMTT. Mean (± SEM) glucose values for concentrations and AUC in subjects homozygote for the Leu-allele (●) in comparison to Ser-carriers (○; L/S+SS) after an oMTT (Fig. 1a). *Significantly different from LL genotype, $p > 0.05$. AUC units are: mg × h/dL.

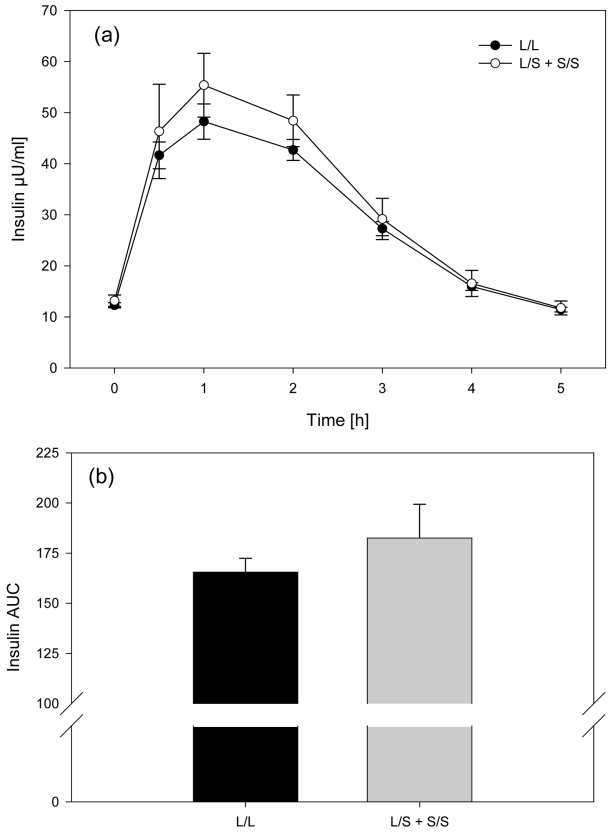

Figure 3. Fasting and postprandial insulin levels according to MACS L513S polymorphism in the MICK after an oMTT. Mean (± SEM) triglyceride insulin values for concentrations and AUC in subjects homozygotes for the Leu-allele (●) in comparison to Ser-carriers (○, L/S+SS) after an oMTT (Fig. 1a). $p > 0.05$. AUC units are: Insulin, µU × h/mL.

This kind of racial differences is common but its importance is frequently debated in clinical, epidemiological and molecular research [19]. Furthermore, Iwai *et al*. found associations between the MACS2 L513S variant and various phenotypes including triglycerides, HDL, WHR, BMI and HOMA. Likewise, in our Caucasian study population, we found also associations between the non-synonymous MACS2 polymorphisms and the traits of the metabolic syndrome. This includes postprandial triglyceride and glucose levels, adiposity, waist circumference, systolic and diastolic blood pressure and HDL. Therefore, the MACS2 L513S variant seems to contribute to multiple risk factors independent of the race of study population.

Medium-chain fatty acids are present in a normal diet in coconut oil, milk and milk products. In our test meal, the fat fraction contained 8.9% w/w medium-chain fatty acids. Therefore, the standardized test meal mimics in some extents a high-fat meal that contains medium-chain fatty acids. It has been proposed in a number of studies that medium-chain triglycerides increase energy expenditure and help to prevent obesity [20]. Because MACS2 seems to have acyl-CoA synthetase activity towards medium-chain fatty acids, one may assume that an altered MACS2 enzyme plays a role in triglcyceride metabolism, fat metabolism, energy expenditure and therefore insulin resistance. Furthermore, the higher postprandial triglcyceride and glucose levels observed in MACS2–513Ser carriers after an oMTT support the functional impact of the polymorphism. However, direct evidence for the consequence of the L513S amino acid substitution on MACS2 enzyme activity is lacking *in vitro*. Since branched-chain amino acids at position 513 are conserved in all members of the MACS family [6, 13, 16] it is likely that the L513S polymorphism perturbates the MACS2 enzyme activity of. This assumption should be tested by comparing wild-type and mutant proteins in future studies.

In conclusion, our results suggest a possible connection between the MACS2 Leu513Ser polymorphism and development of the metabolic syndrome in a Caucasian study population. The observed postprandial triglyceride and glucose levels support an influence of the polymorphism on the functionality of the enzyme *in vivo*.

We thank Y. Dignal, D. Stengel and S. Kaschner for excellent technical assistance. This work was financially supported by the Federal Ministry of Education and Research (Project: "Fat and Metabolism – gene variation, gene regulation and gene function"; AZ 0312823A/B).

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