Association of Human Fetuin-A rs4917 Polymorphism With Obesity in 2 Cohorts

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Background: Previous studies have shown association of the multifunctional hepatic protein α2HS-glycoprotein/human fetuin A with insulin resistance, type 2 diabetes mellitus, metabolic syndrome, obesity, and atherosclerosis. Reports of contribution of α2HS-glycoprotein/human fetuin A rs4917 single-nucleotide polymorphism to the development of these pathologic processes are inconsistent. We aimed to investigate the association between variants of rs4917 and parameters of obesity, lipid status, the proinflammatory cytokine tumor necrosis factor α (TNF-α), adipokines (adiponectin, resistin), and insulin resistance in 2 cohorts.

Methods: Eighty-one healthy persons (cohort 1) and 157 patients with previous myocardial infarction (cohort 2) were included in this cross-sectional study. rs4917 Polymorphism was determined by the allele-specific KASP by design genotyping assays.

Results: In cohort 1, T-nucleotide carriers had lower low-density lipoprotein cholesterol levels compared with non-T carriers. The serum concentration of TNF-α was found to be higher carrying the non-T allele in cohort 1; however, this difference was not observed in cohort 2. In cohort 2, T carriers had lower body mass index and abdominal and waist circumferences than did non-T carriers. The T nucleotide was more frequent in nonobese than in obese patients ($\chi^2 = 5.217$, $P = 0.022$). Nonobese, nondiabetic T carriers still had lower body mass index and waist circumference than did non-T carriers.

Conclusions: Our data suggest that the T nucleotide in rs4917 is associated with more favorable lipid status among healthy persons (i.e., lower low-density lipoprotein cholesterol) and anthropologic parameters of obesity in cohort 2. The protective role of the T allele may also be associated with lower TNF-α levels found in healthy individuals.

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AIMS AND BACKGROUND

α2HS-glycoprotein/human fetuin A (AHSG) is a secretory protein produced by liver parenchymal cells.[1] Today, AHSG is considered a multifunctional protein[2] that acts as a negative acute phase reactant[3] and an inhibitor of calcification.[4] Its decreased serum levels predict mortality of patients with liver cirrhosis[5] and end-stage renal disease.[6]

α2HS-glycoprotein/human fetuin A contributes to the development of insulin resistance.[7–10] Elevated serum AHSG level was observed in obesity,[11] type 2 diabetes mellitus (T2DM)[12–14] and metabolic syndrome,[15] adipocyte dysfunction,[16] and suppressed adiponectin production.[17] Elevated synthesis of AHSG in fatty liver and their link to insulin resistance have also been confirmed.[10,18]

Recent studies have further insight in the molecular action of AHSG. Free fatty acids (FFAs) stimulate AHSG synthesis through the nuclear factor κB.[16] The complex of FFAs and AHSG has been shown to induce inflammatory signals and insulin resistance by binding to Toll-like receptor 4.[19] This molecular mechanism has been further supported by Stefan and Haring,[20] who observed a negative association between serum AHSG and FFA concentration and insulin sensitivity in patients with AHSG and FFA levels above the median. Serum AHSG and FFA interacted with each other in detecting insulin sensitivity. Furthermore, the authors could detect a strongly significant, nearly linear dose-response curve between serum AHSG and FFA levels and insulin sensitivity.

Reports on AHSG rs4917 (Thr248Met) polymorphism are inconsistent. Several authors observed association with serum AHSG levels,[21–25] dyslipidemia,[26,27] obesity,[21,28,29] with T2DM,[27,29] atherosclerosis, and mortality,[23,29] whereas others did not find such associations in these clinical settings.[26,27,30,31]

In our study, we intended to determine the association of the alleles with parameters of obesity (body mass index [BMI]), lipid status (total, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, triglycerides), proinflammatory cytokines (tumor necrosis factor α [TNF-α]), and adipokines (adiponectin, resistin), in 2 unrelated cohorts.

MATERIALS AND METHODS

Cohorts

Individuals of cohort 1 were selected from the 477 subjects screened for allelic distribution of rs4917 in Hungary. Inclusion criteria were as follows: healthy status (by physical examination), leanness (BMI <30 kg/m^2), and normal values during laboratory testing (see Tables 1–3). These individuals (n = 119) were further sorted by applying the age cutoffs (42–79 years) in order to

TABLE 1. Allelic Distribution of Cohorts 1 and 2

	rs4917 Polymorphism				Nucleotide Count		Nucleotide Frequency	
	CC	CT	TT	Total	C	T	C	T
Cohort 1	38	32	11	81	108	54	0.667	0.333
Cohort 2	81	61	15	157	223	91	0.710	0.290

$\chi^2 = 1.033$, $P = 0.597$.

achieve a comparable mean and distribution of age observed in cohort 2. As a result, cohort 1 consisted of 81 control subjects (16 men, 65 women; aged 60.4 [SD, 7.1] years). The male/female ratios according to the genotypes were as follows: CC: 7/31, CT: 7/25, TT: 2/9.

Cohort 2 comprised 157 patients with previous myocardial infarction (103 men, 54 women; aged 59.4 [SD, 12.2] years; range, 37–85 years). Patients had myocardial infarction 6 to 24 months prior to the start of the study. The acute myocardial infarction was diagnosed by the combination of electrocardiographic abnormalities and troponin elevation. Only ST-segment myocardial infarction cases were included. Patients with clinical or laboratory signs of acute infection, malignant tumor, hepatic disease, renal failure, immune suppression, and severe medical or surgical conditions, that is, myocardial infarction within 6 months, stroke (at any time), trauma, or surgical procedure, were excluded. In addition, the post–myocardial infarction patient group comprised 49 patients with diabetes. All of them had T2DM diagnosed according to World Health Organization criteria (fasting plasma glucose ≥7.0 mmol/L [126 mg/dL] or 2-h plasma glucose ≥11.1 mmol/L [200 mg/dL]) and were treated with diet, metformin, and bedtime insulin. Sixty-five percent of patients with previous myocardial infarction received statins and 70% of them aspirin. The post–myocardial infarction patient group included 42 obese (BMI ≥30 kg/m^2) and 115 nonobese patients. The male/female ratios according to the genotypes were as follows: CC: 48/30 CT: 40/18 TT: 15/6.

Genotyping

The single-nucleotide polymorphism rs4917 genotyping was carried out using the Kompetitive Allele Specific polymerase chain reaction genotyping system assay (KASP) (LGC Genomics, Berlin, Germany) according to the manufacturer's instructions. Polymerase chain reaction conditions included 20 ng of genomic DNA per sample in a total volume of 8 μL and 37 temperature cycles. Polymerase chain reaction reaction was carried out by a 7900HT Fast Real-Time PCR System (Life Technologies, Grand Island, NY). Classic 3-cluster pattern for a single-nucleotide polymorphism was considered successful and polymorphic. The genotyping was monitored by using 9 samples (3 parallels each for CC, CT, and TT genotypes) in every measurement. These genotypes were also determined by KASP. The success rate of genotype calls was greater than 99%.

Determination of Other Laboratory Parameters

Serum AHSG concentration by radial immunodiffusion was previous described elsewhere.[32] Intra-assay (IACV) and interassay (IECV) coefficients of variation were less than 5%. Adiponectin levels were measured with radioimmunoassay (IACV: 3.86%, IECV: 8.47%; Linco Research Inc, St Charles, MO). Serum TNF-α (Sigma; St Louis, MO; IACV: 4.8%, IECV: 6.7%) and resistin (Linco Research Inc, IACV: 4.0%, IECV: 7.0%) were measured by enzyme-linked immunosorbent assay.

Fasting serum samples were also used to examine standard clinical laboratory measurements, plasma levels of glucose, insulin, triglycerides, cholesterol, and HDL cholesterol. Insulin concentration was measured by insulin direct human enzyme-linked immunosorbent assay kit (Invitrogen, Camarillo, CA; lowest detectable concentration 0.17 μIU/mL, IACV: 4.8%, IECV: 81%). C-peptide was measured by RIA (Biodata, Rome, Italy; lowest detectable concentration: 0.2 ng/mL, IACV: 5.6%, IECV: 7.3%.

Statistical Analysis

Statistical analysis was carried out using the SPSS version 21 statistical software (SPSS Inc, Chicago, IL). Nonparametric methods, including the Bonferroni (Dunn) post hoc test, were used. $P < 0.05$ was considered as significant.

RESULTS

Allelic Distribution of Cohorts 1 and 2

Allelic distribution of cohorts 1 and 2 is shown in Table 1. They did not differ significantly from each other.

Cohort 1: Association of the rs4917 Alleles With Parameters of Obesity

Anthropologic laboratory parameters of cohort 1 are shown in Table 2. During multiple comparisons, we observed significant differences in LDL cholesterol and TNF-α levels only. There was a trend of serum total cholesterol decreasing with the presence of the T allele. On pairwise analysis, members with CC had higher LDL cholesterol and TNF-α levels than TT homozygotes (Mann-Whitney U test, $P = 0.017$ in both parameters). The presence of the T nucleotide was also associated with lower total cholesterol (4.08 ± 0.51 vs 5.41 ± 0.86 mmol/L, n = 43, $P = 0.018$), LDL cholesterol (2.13 ± 0.21 vs 3.03 ± 0.72 mmol/L, $P = 0.020$), and TNF-α levels than non-T nucleotide (3.90 ± 0.021 vs 4.10 ± 0.24 pg/mL, n = 38, $P = 0.010$).

Cohort 2: Association of the rs4917 Alleles With Parameters of Obesity

On multiple comparisons, we found significant differences in waist circumference (Table 3). Body mass index and abdominal circumference also showed a trend toward lower values with the T allele. Indeed, T carriers (n = 76) had significantly lower BMI (27.2 ± 4.6 vs 28.6 ± 3.8 kg/m^2, $P = 0.019$) and abdominal (101 ± 12 vs 103 ± 10 cm, $P = 0.040$) and waist circumferences (102 ± 9 vs 106 ± 8 cm, $P = 0.003$) than did those who had no T nucleotide (n = 81) in rs4917.

Comparison of Diabetic and Nondiabetic Individuals in Cohort 2

There were 49 patients with T2DM in cohort 2. They differed from the nondiabetic individuals (n = 108) only in parameters of insulin resistance, that is, glucose (7.19 ± 2.13 vs

TABLE 2. Cohort 1: Comparison of the Genotypes of rs4917 (Mean ± SD)

Parameter	CC Homozygotes (n = 38)	CT Heterozygotes (n = 32)	TT Homozygotes (n = 11)	*P**	
AHSG, mg/L	609 ± 85	625 ± 88	573 ± 100	0.269	
BMI, kg/m^2	24.0 ± 1.8	24.1 ± 1.5	24.1 ± 1.6	0.831	
Abdominal circumference, cm	87 ± 11	88 ± 9	85 ± 11	0.610	
Total cholesterol, mmol/L	5.41 ± 0.86	4.99 ± 0.83	4.80 ± 0.51	0.492	
LDL cholesterol, mmol/L	3.39 ± 0.44	2.66 ± 0.82	2.28 ± 0.32	0.020	0.027†
HDL cholesterol, mmol/L	1.33 ± 0.14	1.55 ± 0.28	1.57 ± 0.30	0.174	
Triglycerides, mmol/L	1.91 ± 1.14	1.69 ± 0.88	1.54 ± 0.72	0.497	
TNF-α, pg/mL	4.11 ± 0.27	4.09 ± 0.02	3.89 ± 0.21	0.017	0.037†
Adiponectin, μg/mL	12.23 ± 3.40	12.80 ± 2.80	13.8 ± 2.74	0.101	
Resistin, ng/mL	6.76 ± 2.31	5.60 ± 2.57	6.95 ± 3.36	0.113	
Glucose, mmol/L	4.56 ± 0.42	4.41 ± 0.37	4.29 ± 0.40	0.102	
Insulin, μU/mL	5.26 ± 1.15	5.13 ± 1.10	5.54 ± 0.73	0.337	

*Kruskal-Wallis test in all parameters.

†CC vs TT post hoc *P*, when significant.

5.03 ± 0.68 mmol/L, $P < 0.001$), insulin (28.3 ± 16.3 vs 21.7 ± 14.08 μU/mL, $P = 0.008$), C-peptide (3.91 ± 2.25 ng/mL vs 3.00 ± 2.13 ng/mL, $P = 0.008$), Homeostatic Model Assessment A (HOMA-A) (7.69 ± 3.46 vs 4.67 ± 3.04, $P < 0.001$), and HOMA-B (191 ± 133 vs 260 ± 147, $P = 0.004$) but not in those of obesity: BMI (28.8 ± 4.47 vs 27.7 ± 3.93 kg/m^2, $P = 0.176$) and abdominal circumference (104 ± 12 vs 101 ± 11 cm, $P = 0.232$) or waist circumference (105 ± 9 vs 104 ± 8 cm, $P = 0.420$). The small sample size did not allow comparison either of CC (n = 25) and TT (n = 3) homozygotes or C- (n = 46) and non-C carriers (n = 3) within the diabetic group (data not shown). There was no statistical difference when the T (n = 24) was compared with non-T allele (n = 25), either (data not shown).

In patients without diabetes and with the CC genotype (n = 50), these parameters did not differ from the TT genotype (n = 8), nor did C (n = 56) and non-C (n = 52), respectively (data not shown). Body mass index and waist circumference of T- and non-T carriers, however, still differed from each other markedly (Table 4).

Comparison of Obese and Nonobese Individuals in Cohort 2

Forty-two patients were clinically overweight or obese as defined by BMI of 30 kg/m^2 or greater. Apart from BMI (33.2 ± 3.01, vs 26.0 ± 2.43 kg/m^2, $P < 0.001$) and abdominal (112 ± 8 vs 98 ± 9 cm, $P < 0.001$) and waist circumferences (112 ± 9 vs 102 ± 7 cm, $P < 0.001$), waist-hip ratio (1.01 ± 0.07 vs 0.97 ± 0.08 cm, $P < 0.001$) and TNF-α (6.69 ± 1.17 vs 5.91 ± 1.72 pg/mL, $P = 0.009$) in obese and nonobese patients (n = 115) did not differ from each other significantly.

We observed a trend of relatively lower frequency of the CC and higher proportion of the TT genotype among lean compared with obese patients (Table 5A). When rs4917 nucleotide distributions were compared, the association of leanness with the T allele became more prevalent (Table 5B). Subgroup analysis of obese patients did not result in significant statistical differences of the anthropometric and metabolic parameters between different genotypes and alleles (data not shown).

TABLE 3. Cohort 2: Comparison of the Genotypes of rs4917 (Mean ± SD)

Parameter	CC Homozygotes (n = 81)	CT Heterozygotes (n = 61)	TT Homozygotes (n = 15)	*P**	
AHSG, mg/L	668 ± 112	689 ± 112	641 ± 103	0.483	
BMI, kg/m^2	28.6 ± 3.8	27.2 ± 4.5	27.5 ± 5.0	0.065	
Abdominal circumference, cm	103 ± 10	101 ± 13	100 ± 11	0.418	
Waist circumference, cm	106 ± 8	102 ± 9	102 ± 9	0.009	0.034†
Total cholesterol, mmol/L	5.37 ± 1.25	5.12 ± 1.13	5.53 ± 1.24	0.623	
LDL cholesterol, mmol/L	3.39 ± 0.44	2.66 ± 0.82	2.28 ± 0.32	0.567	
HDL cholesterol, mmol/L	1.23 ± 0.28	1.20 ± 0.28	1.13 ± 0.29	0.275	
Triglycerides, mmol/L	1.90 ± 1.17	1.75 ± 0.80	1.67 ± 0.58	0.963	
TNF-α, pg/mL	6.09 ± 1.72	6.15 ± 1.88	5.89 ± 1.75	0.841	
Adiponectin, μg/mL	9.45 ± 4.35	8.63 ± 4.30	8.53 ± 3.21	0.419	
Resistin, ng/mL	9.25 ± 5.72	10.19 ± 7.11	8.93 ± 4.03	0.784	
Glucose, mmol/L	5.52 ± 1.67	5.93 ± 1.74	5.43 ± 0.75	0.317	
Insulin, μU/mL	24.09 ± 16.02	22.14 ± 12.93	27.62 ± 22.62	0.883	

*Kruskal-Wallis test in all parameters.

†CC vs TT post hoc *P*, when significant.

TABLE 4. Cohort 2: Comparison of Laboratory Parameters (Mean ± SD) of Nondiabetic T- and Non-T Carriers of rs4917 (Mean ± SD)

Parameter	T (n = 52)	Non-T (n = 56)	P*
AHSG, mg/L	669 ± 122	671 ± 108	0.941
BMI, kg/m^2	26.4 ± 4.0	28.6 ± 3.8	0.002
Abdominal circumference, cm	98 ± 11	104 ± 9	0.034
Waist circumference, cm	101 ± 7	106 ± 8	<0.001
Waist-hip ratio	0.98 ± 0.08	0.97 ± 0.06	0.400
Total cholesterol, mmol/L	5.31 ± 1.22	5.28 ± 1.25	0.540
LDL cholesterol, mmol/L	3.34 ± 0.91	3.20 ± 0.91	0.566
HDL cholesterol, mmol/L	1.21 ± 0.28	1.25 ± 0.31	0.485
Triglycerides, mmol/L	1.72 ± 0.80	1.82 ± 1.09	0.820
TNF-α, pg/mL	6.05 ± 1.61	6.07 ± 1.71	0.944
Adiponectin, μg/mL	8.99 ± 4.23	9.05 ± 3.79	0.606
Resistin, ng/mL	9.19 ± 5.35	8.91 ± 5.09	0.731
Glucose, mmol/L	5.08 ± 0.65	4.97 ± 0.66	0.432
Insulin, μU/mL	22.0 ± 16.6	21.4 ± 13.2	0.708

*Mann-Whitney *U* test.

Analysis of Nonobese, Nondiabetic Patients in Cohort 2

We checked whether the differences we observed in cohort 1 could still be observed in those individuals of cohort 2, who lack the confounding effects of obesity and diabetes. In this subgroup (n = 83), there were no differences between either CC (n = 32) and TT (n = 5) homozygotes or the C (n = 74) and non-C (n = 9) groups. T carriers, however, still had significantly lower BMI (25.3 ± 2.6, n = 46, vs 26.5 ± 2.3 kg/m^2, n = 37; $P = 0.048$) and waist circumferences (99 ± 7 vs 103 ± 6 cm, $P = 0.026$) than did those with the non-T variant. Although the mean of the abdominal circumferences was also lower in the T-allele group (97.7 ± 10 vs 99.2 ± 8 cm), the difference was not significant. Other parameters showed no differences (data not shown).

DISCUSSION

Despite relatively small size, our data suggest that the presence of the T nucleotide in rs4917 was associated with favorable lipid status and TNF-α levels in cohort 1.

The association of the T nucleotide with more favorable anthropologic parameters (BMI and abdominal and waist circumferences) could be observed in cohort 2, essentially in the subgroup without diabetes. Detailed analysis according to different alleles could not be done because of the small sample size of patients with diabetes, but these differences still remained significant when the nondiabetic, nonobese subgroup was analyzed. Leanness, again, was associated with higher prevalence of the T allele.

We observed a difference regarding measures of obesity and metabolism between cohorts 1 and 2. The explanation for this difference is that cohort 1 consisted of healthy individuals with normal BMI (<25 kg/m^2), whereas members in cohort 2 were chosen based on a hard cardiovascular end point (myocardial infarction) and had multiple cardiovascular risk factors (vascular disease, obesity, diabetes, and elevated proinflammatory cytokine levels).

Several studies have addressed the significance of rs4917 polymorphism in hyperlipidemia, obesity, and diabetes. Many of them indicate that AHSG plays role in the development of obesity and insulin resistance.[7–11,15]

There are observations indicating the association between rs4917 and obesity. The rs4917 TT and rs4918 GG haplotypes, along with rs2593813:G, conferred an increased risk for leanness in Swedish men.[21] The rs4917 has been associated with whole-body fat content, too.[24] In a lifestyle intervention study, rs4917 CC homozygotes increased muscle mass and basal metabolic rate and decreased total fat content better than did those with the T allele, but the authors found no relationship between rs4917 variants and the decrease in BMI.[28] Others found no relationship between rs4917 variants and obesity at all.[26,27]

In 1 study, the rs4917 polymorphism was not associated with diabetes.[30] In another study, the CC allele of rs4917 was

TABLE 5A. Cohort 2: Distribution of rs4917 Genotypes Among Obese and Nonobese Patients

	Genotype			
	CC	CT	TT	Total
Obese	28	11	3	42
Nonobese	53	50	12	115
Total	81	61	15	157

$\chi^2 = 5.241$, $P = 0.073$.

TABLE 5B. Cohort 2: Distribution of rs4917 Alleles Among Obese and Nonobese Patients

	Allele		
	T	Non-T	Total
Obese	14	28	42
Nonobese	62	53	115
Total	76	81	157

$\chi^2 = 5.217$, $P = 0.022$.

Relative risk, 0.618 (95% confidence interval, 0.390–0.979); odds ratio, 0.427 (95% confidence interval, 0.204–0.895).

associated with worse HOMA, increased fasting and OGTT-stimulated plasma insulin levels than in the TT variant. The CC allele was associated with dyslipidemia, as well. Nevertheless, no association with obesity and diabetes was found.[27] In the recent study of Jensen et al.,[22] the rs4917 variants were associated with AHSG concentrations. Higher blood glucose came with higher serum AHSG. Nevertheless, they could not find relationship between the genetically determined AHSG level and the risk of T2DM or fasting glucose level. These findings argue against the casual relationship between AHSG concentration and T2DM.[22]

In 1 study, the CC variant was associated with lower total cholesterol than the TT variant.[26] A possible increase in total cholesterol in AHSG rs4917 T allele carriers was demonstrated in Czech Post-Monica Study.[33] In this study, however, the authors did not confirm any other previous observations regarding its influence on LDL cholesterol, central obesity, or blood glucose. Like us, Andersen et al.[27] found no differences between total, HDL cholesterol, and triglyceride levels between the CC and TT variants.

In theory, both different serum concentrations of AHSG or different molecular structure may explain the different activities of the rs4917 variants. Since its first report,[25] several other studies have confirmed that minor variants of AHSG (AHSG2) are linked with lower serum AHSG levels.[21,23,24]

We did not observe any significant associations between serum AHSG levels and rs4917 polymorphism, although the mean of the AHSG levels was lower in the TT than in the CC groups in both cohorts. One explanation for this finding is the obviously lower statistical power of our study in this analysis.

Elevated serum AHSG is clearly associated with increased risk of diabetes, as well.[12,13,34] Studies on AHSG levels in diabetes, however, do not show trends that have been found among healthy or obese individuals.[29,35] Other confounding factors such as hyperlipidemia,[26,27] antidiabetic,[36] and antilipemic[37] treatment should also be considered in the evaluation of AHSG in diabetes. In their large-scale prospective study, Jensen et al.[29] found that serum AHSG inversely correlated with cardiovascular mortality in old, nonobese, nondiabetic individuals. This trend was inverted in patients with diabetes. Obesity and insulin resistance had similar modifying effects in individuals without diabetes. Thus, the association with elevated AHSG and lower mortality was present only in nonobese persons with normal HOMA of Insulin Resistance below the median value.[29]

In the EPIC Potsdam Study, Fisher et al.[23] observed a strong association between rs4917 and occurrence of myocardial infarction. The higher serum AHSG concentration in the CC variant was independently determined by this polymorphism by more than 20%. Moreover, the presence of the C allele was associated with the development of myocardial infarction. They suggested the pathogenic role of AHSG in the development of cardiovascular diseases.[23] Probably, because of small sample size, we did not find such difference, not even between CC and TT but rs4917CC:rs4918CC and rs4917TT:rs4918GG patients either. It is interesting, however, that TNF-α concentration can differ significantly even in sera of healthy persons with normal BMI, that is, the C allele is associated with higher TNF-α levels than the T allele. This difference could not be detected in cohort 2 probably because of the existing subclinical inflammation and prevalence of obesity among patients. This finding warrants further studies.

In theory, the rs4917 polymorphism, which affects exon 6 of the molecule (D3 domain region), may also result in altered function. Apart from the observation that the TT variant of rs4917 showed 35-times higher lipolytic sensitivity than the CC variant, we have no evidence for different functional activity.[38] In light of the novel findings of the molecular mechanism suggesting that AHSG is the "missing link" between metabolic diseases and inflammation,[16,18,19] it would be interesting to directly compare FFA- and Toll-like receptor 4–binding activities of the various rs4917 AHSG polymorphisms.

Our study has its limitations. The cross-sectional design does not allow for drawing casual relationships between rs4917 polymorphism and parameters of metabolism we have investigated. The relatively small sample size does not allow for subgroup analysis especially the minor (T) nucleotide frequency among post–myocardial infarction patients. As in several studies as well, treatment of hypercholesterolemia (mainly with statins) and diabetes (bedtime insulin) masks putative differences of serum lipid and AHSG concentrations and their correlation between rs4917 variants.

During subgroup analysis of obese and nonobese patients of cohort 2, we found no significant genotype-associated differences. There are several explanations for not detecting these differences. First, the sample size may be too small. Second, the differences observed in cohort 1 can easily be masked by the pathologic processes associated with cardiovascular disease (subclinical inflammation, diabetes, obesity) in cohort 2. Third, and equally importantly, most of these patients received antihypertensive, antilipemic, and antidiabetic and treatment and acetylsalicylates. These medications may also blunt the putative differences stemming from different genotypes. The rs4917 polymorphism, however, was found to be associated with obesity: carriers of the T allele were definitely leaner compared with carriers of C allele.

In summary, our results are in accord with the observations that the minor variant T of rs4917 is linked with more favorable parameters than the C allele. Our observations are in accord with those that find AHSG correlate much more with obesity than with diabetes mellitus. Large-scale prospective studies are needed to evaluate causative relationship between variants and functional differences of AHSG.

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