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Human Fetuin-A Rs4918 Polymorphism and its Association with Obesity in Healthy Persons and in Patients with Myocardial Infarction in Two Hungarian Cohorts

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BE 1 György Temesszentandrási
EF 2 Krisztián Vörös
EF 2 Bernadett Márkus
B 1 Zoltán Böröcz
B 3 Edit Kaszás
CD 1,4 Zoltán Prohászka
CD 5 András Falus
ADE 3,6 Károly Cseh
ACDEFG 2 László Kalabay

1 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
2 Department of Family Medicine, Semmelweis University, Budapest, Hungary
3 Department of Internal Medicine, Károlyi Sándor Municipal Hospital, Budapest, Hungary
4 Atherosclerosis Research Group of Hungarian Academy of Sciences, Budapest, Hungary
5 Department of Genetics and Immunology, and Cell Sciences, Semmelweis University, Budapest, Hungary
6 Department of Public Health, Semmelweis University, Budapest, Hungary

Corresponding Author: László Kalabay, e-mail: kalabay.laszlo@med.semmelweis-univ.hu
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Background: Human fetuin A (AHSG) has been associated with the development of obesity, insulin resistance, type 2 diabetes mellitus, and atherosclerosis. Observations on the role of AHSG rs4918 single-nucleotide polymorphism are contradictory. We investigated the association between variants of rs4918 and parameters of obesity, lipid status, tumor necrosis factor-α (TNFα), adipokines (adiponectin, resistin, leptin), and insulin resistance in healthy persons and in patients with previous myocardial infarction.

Material/Methods: This was a cross-sectional study comprising cohort 1 (81 healthy individuals) and cohort 2 (157 patients with previous myocardial infarction). We used the allele-specific KASP genotyping assay to detect rs4918 polymorphism.

Results: In cohort 1, G-nucleotide carriers had significantly lower serum TNFα, adiponectin, and higher leptin concentrations than in non-G carriers. These differences, however, were not observed in cohort 2. In cohort 2, G-carriers had lower BMI and waist circumferences than in non-G carriers. The G allele was more frequent among lean than obese patients (RR=1.067, 95%CI=1.053–2.651, p=0.015). An association between BMI and rs4918 polymorphism was observed among patients without diabetes (CC/CG/GG genotypes: p=0.003, G *vs.* non-G allele: p=0.008) but not in diabetics. In addition, a strong linearity between BMI and the CC/CG/GG genotypes (association value: 4.416, p=0.036) and the frequency of the G allele (7.420, p=0.006) could be identified. In cohort 2, non-obese, non-diabetic G-carriers still had lower BMI and waist circumferences than in non-G carriers.

Conclusions: The rs4918 minor variant is associated with lower TNFα and adiponectin, higher leptin levels in healthy persons, and more favorable anthropomorphic parameters of obesity in cohort 2.

MeSH Keywords: alpha-2-HS-Glycoprotein • Diabetes Mellitus • Myocardial Infarction • Obesity • Polymorphism, Single Nucleotide

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Background

Alpha2HS-glycoprotein/human fetuin A (AHSG) is a multifunctional glycoprotein secreted by liver parenchymal cells in adults [1]. The molecule is a negative acute phase reactant [2] and inhibits extraosseal calcification [3]. Its decreased serum levels predict mortality of patients with liver cirrhosis [4] and end-stage renal disease [5].

The molecule is a natural inhibitor of insulin receptor tyrosine kinase, thus AHSG plays a role in the development of insulin resistance [6–9]. Obesity [10], type 2 diabetes mellitus (T2DM) [11–13], metabolic syndrome [14], adipocyte dysfunction [15] and fatty liver [9,16] have been linked to elevated serum AHSG concentration.

At the molecular level, the synthesis of AHSG is enhanced by free fatty acids (FFAs) via the nuclear factor κB (NFκB) [15]. The AHSG molecule binds to FFA [17]. By binding to the Toll-like receptor 4 (TLR4), the FFA-AHSG complex generates inflammatory signals and insulin resistance [17]. Serum AHSG and FFA interact with each other in predicting insulin sensitivity [18].

Several SNP-s of AHSG have been studied. Serum AHSG concentration has been linked with the rs4917 [19–22], the AHSG 1–2 [23], and polymorphism of the molecule. The rs4917 SNP has been associated with obesity [22,24,25], T2DM [26,27], atherosclerosis, and mortality [19,27]. In these studies the minor variants seemed to be protective. Others did not find such associations in these clinical settings [26,28–30]. Dyslipidemia has been associated with the –469 T/G (rs2077119) SNP [26,28].

The minor variant of the SNP rs2248690 was associated with lower AHSG concentrations but not with the higher risk of diabetes [27,31]. This SNP in the promoter region of the AHSG gene seems to affect the AHSG gene transcription, possibly by producing different associations with AP-1 [32]. A strong linkage between the rs224869, rs4917, and the risk of myocardial infarction has been observed in the EPIC Potsdam Study [19]. Others found no association between AHSG variants rs4917, rs2248690, and rs2518136, and clinical atherosclerosis [33]. In a magnetic resonance study, AHSG variation was not associated with regional body fat distribution AHSG but it was with the whole body fat composition [20].

Reports on AHSG rs4918 (Thr256Ser) polymorphism in exon 7 are less inconsistent. Lavebratt et al. reported a higher frequency of the G allele among lean Swedish men [22]. Others found the G allele less advantageous. The rs4918 G allele was associated with high mortality in renal transplant patients [34], and conferred higher risk for ischemic stroke than the C allele [35].

In this study we intended to determine the association of the alleles in rs4918 SNP of AHSG with parameters of obesity (BMI), lipid status (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride), proinflammatory cytokines (TNFα), and adipokines (adiponectin, resistin, and leptin), in two unrelated groups of individuals, i.e., among healthy persons (cohort 1) and patients surviving myocardial infarction (cohort 2).

Material and Methods

Cohorts

Cohort 1 consisted of 82 healthy individuals (17 men, 65 women, age: 60.1±6.9 years, mean±SD, range: 42–79) who met the inclusion criteria: healthy status by physical examination, comparable age to group 2, BMI below 30 kg/m^2, and normal results during routine laboratory analysis. These individuals were selected from a larger group of patients as described earlier [25]. The male/female ratios according to the genotypes were: CC: 8/31, CT: 7/25, and TT: 2/8.

Cohort 2 consisted of 157 patients with previous myocardial infarction (103 men, 54 women, age: 59.4±12.2 years, range: 37–85). Only cases with ST-elevation (STEMI) occurring 6–24 months before the start of the study were investigated. The diagnosis of STEMI was established by the alteration of electrocardiography and troponin. Exclusion criteria were: clinical or laboratory signs of acute infection, malignant tumor, hepatic disease, renal failure, immune suppression, and severe medical or surgical conditions such as myocardial infarction within 6 months, stroke (at any time), trauma, or surgical procedures. There were 48 patients with diabetes mellitus (all with type 2 diabetes [T2DM], diagnosed according to WHO criteria) and were treated with diet, metformin, and bedtime insulin. Sixty-five percent of patients in cohort 2 received statins and 70% of them received aspirin. Cohort 2 also included 42 obese (BMI ≥30 kg/m^2) and 115 non-obese patients. The male/female ratios according to the genotypes were: CC: 48/30, CT: 40/18, and TT: 15/6.

The study was approved by the local Ethics Committee of the Károlyi Sándor Municipality Hospital. All persons gave their informed consent prior to their inclusion in the study.

Genotyping

The single-nucleotide polymorphism (SNP) rs 4918 genotyping was carried out using the Kompetitive Allele Specific PCR genotyping system assay (KASPTM) (LGC Genomics, Berlin, Germany) according to the manufacturer's instructions and also described by us earlier [25]. Polymerase chain reaction (PCR) conditions included 20 ng of genomic DNA per sample

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Table 1. Allelic distribution of cohorts 1 and 2.

	rs4918 polymorphism*				Nucleotide count		Nucleotide frequency**	
	CC	CG	GG	Total	C	G	C	G
Cohort 1	39	32	10	81	110	52	0.679	0.321
Cohort 2	81	61	15	157	223	91	0.710	0.290

Chi^2=0.528, p=0.768.

Table 2. Cohort 1: Comparison of the genotypes of rs4918 (mean ±SD).

Parameter	CC homozygotes (n=39)	CG heterozygotes (n=32)	GG homozygotes (n=10)	p*
AHSG (mg/l)	605±84	627±85	613±117	0.185
BMI (kg/m^2)	23.9±1.7	24.1±1.4	24.5±1.6	0.257
Abdominal circumference (cm)	87±11	88±8	87±10	0.872
Total cholesterol (mmol/l)	4.98±1.07	4.96±0.83	5.13±1.35	0.930
LDL-cholesterol (mmol/l)	3.13±0.64	2.65±0.82	1.93±0.40	0.292
HDL-cholesterol (mmol/l)	1.39±0.20	1.54±0.27	1.49±0.41	0.597
Triglyceride (mmol/l)	1.85±1.13	1.71±0.88	1.64±0.75	0.950
TNFα (pg/ml)	**4.11±0.25**	**4.10±0.20**	**3.85±0.22**	**0.013 0.007****
Adiponectin (µg/ml)	12.3±3.37	12.7±2.87	14.3±2.30	0.131
Resistin (ng/ml)	6.64±2.24	5.76±2.67	6.96±3.52	0.264
Leptin (ng/ml)	10.7±9.2	13.8±9.2	14.1±9.8	0.109
C-peptide (ng/ml)	1.07±0.31	1.15±0.42	1.07±0.29	0.247
Glucose (mmol/l)	4.58±0.42	4.39±0.36	4.22±0.34	0.052
Insulin (µU/ml)	5.30±1.16	5.05±0.99	5.62±0.93	0.348

* Kruskal-Wallis test in all parameters; **CC *vs.* GG, when post hoc p<0.05

in a total volume of 8 µl and 37 temperature cycles. PCR reaction was carried out by a 7900HT Fast Real-Time PCR System (Life Technologies). Classical three-cluster pattern for a SNP was considered successful and polymorphic. The genotyping was monitored by using 9 samples (3 parallels for CC, CT, and TT genotypes, each) in every measurement. These genotypes were also determined by KASPTM. The success rate of genotype calls was >99%.

Determination of other laboratory parameters

Serum AHSG concentration by radial immunodiffusion (RID) was as previous described elsewhere [36]. Intra-assay (IACV) and interassay IECV) coefficients of variation were below 5%. Adiponectin levels were measured with radioimmunoassay (IACV: 3.86%, IECV: 8.47%; Linco Research, St Charles, MO, USA). Serum tumor necrosis factor-α (TNFα, Sigma, St Louis, MO, USA; IACV: 4.8%, IECV: 6.7%), resistin (Linco Research Inc., IACV: 4.0%, IECV: 7.0%), and leptin (DRG International, Mountainside, NJ, USA, IACV%: 4.6%, IECV%: 6.6%) were measured by ELISA.

Fasting levels of serum glucose, total cholesterol, low-density (LDL) cholesterol, high-density (HDL) cholesterol, and triglycerides were detected by routine chemical laboratory methods. Insulin concentration was measured by insulin direct human ELISA kit (Invitrogen, Camarillo, CA, USA, 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%).

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Table 3. Cohort 2: Comparison of the genotypes of rs4918 (mean ±SD).

Parameter	CC homozygotes (n=81)	CG heterozygotes (n=61)	GG homozygotes (n=15)	p*
AHSG (mg/l)	682±125	680±94	611±102	0.185
BMI (kg/m^2)	28.6±3.8	27.2±4.5	27.5±5.0	0.048 0.204**
Abdominal circumference (cm)	103±10	101±12	100±11	0.333
Waist circumference (cm)	**106±8**	**102±9**	**102±9**	**0.005** **0.026****
Total cholesterol (mmol/l)	5.38±1.26	5.10±1.13	5.43±1.25	0.673
LDL-cholesterol (mmol/l)	3.23±0.97	3.11±0.96	3.32±0.76	0.618
HDL-cholesterol (mmol/l)	**1.23±0.28**	**1.20±0.27**	**1.10±0.27**	**0.112**
Triglyceride (mmol/l)	1.90±1.17	1.76±0.80	1.66±0.58	0.934
TNFα (pg/ml)	6.07±1.70	6.15±1.88	5.89±1.75	0.848
Adiponectin (µg/ml)	9.29±4.13	8.63±4.30	8.53±3.21	0.486
Resistin (ng/ml)	9.24±5.73	10.19±7.11	8.93±4.03	0.756
Leptin (ng/ml)	32.2±19.3	29.2±18.0	31.0±10.6	0.676
C-peptide (ng/ml)	3.36±2.26	3.05±1.79	3.87±3.35	0.866
Glucose (mmol/l)	5.51±1.68	5.93±1.74	5.43±0.75	0.275
Insulin (µU/ml)	24.2±16.0	22.1±12.9	27.6±22.6	0.850

* Kruskal-Wallis test in all parameters; ** CC *vs.* GG, when post hoc p<0.05

Statistical analysis

Statistical analysis was carried out using the SPSS v.21 statistical software (SPSS Inc., Chicago, IL, USA). Non-parametric methods, including the Bonferroni (Dunn) post-hoc test, were used.

Results

The allele frequencies did not differ between the two cohorts (Table 1).

Cohort 1. Association of the rs4918 alleles with parameters of obesity

Anthropologic and laboratory parameters of cohort 1 are shown in Table 2. During multiple comparisons we observed significant differences in TNFα levels only. Compared to non-G carriers (n=39), the presence of the G nucleotide (n=42) was associated with lower TNFα (3.85±0.22 *vs.* 4.10±0.23 pg/ml, p=0.003), higher leptin (13.9±9.23 *vs.* 10.7±9.19 ng/ml, p=0.036), and lower adiponectin levels (12.4±3.14 *vs.* 14.3±2.30 µg/ml, p=0.047).

Cohort 2. Association of the rs4918 alleles with parameters of obesity

On multiple comparisons we found significant differences in waist circumference (Table 3). There was a trend towards lower values of obesity in patients with the G allele. Indeed, G-carriers (n=76) had significantly lower BMI (27.3±4.6 *vs.* 28.6±3.8 kg/m^2, p=0.017) and waist circumference (102±9 *vs.* 106±8 cm, p=0.002) than those without the G nucleotide (n=81). Other parameters, including abdominal circumference (101±12 *vs.* 104±10 cm, p=0.168), did not differ significantly.

Comparison of diabetic and non-diabetic individuals in cohort 2

There were 48 patients with T2DM in cohort 2. They differed from the non-diabetic individuals (n=109) only in parameters of insulin resistance – glucose (7.08±1.99 *vs.* 5.03±0.68 mmol/l, p<0.001), insulin (28.4±16.4 *vs.* 21.7±14.8 µU/ml, p=0.008), C-peptide (3.93±2.27 ng/ml *vs.* 3.00±2.13 ng/ml, p=0.008), HOMA A (7.62±3.45 *vs.* 4.67±3.04 p<0.001), and HOMA B (194±133 *vs.* 260±147, p=0.004), but not in those of obesity – BMI (28.8±4.51 *vs.* 27.7±3.93 kg/m^2, p=0.187), abdominal

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Table 4. Cohort 2: Comparison of laboratory parameters (mean±SD) of obese and non-obese patients (mean±SD).

Parameter	Obese (n=48)	Non-obese (n=109)	p*
AHSG (mg/l)	699±126	661±105	0.053
BMI (kg/m²)	**23.1±2.9**	**26.0±2.4**	**<0.001**
Abdominal circumference (cm)	**112±8**	**98±10**	**<0.001**
Waist circumference (cm)	**111±8**	**102±7**	**<0.001**
Waist/hip ratio	**1.00±0.07**	**0.96±0.08**	**<0.001**
Total cholesterol (mmol/l)	5.20±1.31	5.29±1.14	0.520
LDL-cholesterol (mmol/l)	3.14±0.99	3.20±0.90	0.653
HDL-cholesterol (mmol/l)	1.14±0.23	1.22±0.29	0.107
Triglyceride (mmol/l)	1.99±1.04	1.80±0.95	0.066
TNFα (pg/ml)	**6.69±1.77**	**5.91±1.72**	**0.009**
Adiponectin (µg/ml)	8.53±4.78	9.24±4.12	0.198
Resistin (ng/ml)	11.6±8.13	8.96±5.58	0.057
Leptin (ng/ml)	38.1±20.5	29.9±17.4	0.122
C-peptide (ng/ml)	3.76±2.41	3.10±2.10	0.097
Glucose (mmol/l)	5.69±1.47	5.70±1.71	0.553
Insulin (µU/ml)	27.0±16.9	22.4±14.8	0.106

* Mann-Whitney test.

circumference (104±12 *vs.* 101±10 cm, p=0.200), or waist circumference (105±10 *vs.* 104±8 cm, p=0.510). Due to the small size of the minor allele G among diabetics, statistical analysis could not be done among CC (n=24), CG (n=21), and GG (n=3) variants. Comparison of C- (n=45) with non-C (n=3) or G- (n=24) with non-G-carriers (n=24) showed no significant differences in the diabetic group (data not shown).

In patients without diabetes, we found no significant differences between CC and GG homozygotes. On the other hand, G-carriers (n=52) had lower serum AHSG (602±108 *vs.* 676±110 mg/l, p=0.043), BMI (26.4±4.0 *vs.* 28.7±3.8 kg/m², p=0.001), and waist circumference (100±7 *vs.* 106±8 cm, p<0.001) and mildly lower abdominal circumference values (99±11 *vs.* 103±10 cm, p=0.068) than non-G carriers (n=57).

Comparison of obese and non-obese individuals in cohort 2

Forty-eight patients were clinically overweight or obese as defined by BMI ≥30 kg/m². They differed from non-obese individuals only in BMI, abdominal and waist circumferences, waist/hip ratio, and TNFα, but not in other parameters (Table 4). Serum AHSG and resistin concentrations were also marginally higher in obese than in non-obese patients.

Nineteen percent (12/64) of the lean individuals carried the GG genotype in contrast to 9% (3/32) of obese patients. Accordingly, the minor allele G was found to be more frequent in lean (62/114) than in obese patients (14/43, chi-square: 5.957, RR=1.067, 95% CI=1.053–2.651, p=0.015).

Since AHSG has been implicated both with obesity and diabetes, further analysis of the genotype and allelic distribution among obese and non-obese was performed with respect to diabetes (Table 5). Association between BMI and rs4918 polymorphism (CC, CG, and GG genotypes, and G allele) could be observed among patients without diabetes but not in diabetics. In addition, a strong linearity between the frequency of the G allele and lower BMI could be identified. Subgroup analysis of the anthropometric and metabolic parameters in obese patients according to different genotypes and alleles did not result in significant statistical differences (data not shown).

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Table 5. Cohort 2: Association between BMI and rs4918 polymorphism. A: genotypes (CC, CG, GG), B: distribution of the G allele.

A	Genotype	BMI (kg/m²)				Total
		0–25	26–29	30–39	40 or more	
Patients without diabetes	CC	13	22	22	0	57
	CG	16	20	4	0	40
	GG	5	4	2	1	12
	Total	34	46	28	1	109
	Chi square: 19.671, p=0.003 **Linear-by-linear association value: 4.416, p=0.036**					
Patients with diabetes	CC	7	8	9	0	24
	CG	6	5	9	1	21
	GG	1	2	0	0	3
	Total	14	15	18	1	48
	Chi square: 4.201, p=0.649 **Linear-by-linear association value: 0.011, p=0.918**					
All patients in cohort 2	CC	20	30	31	0	81
	CG	22	25	13	1	61
	GG	6	6	2	1	15
	Total	48	61	46	2	157
	Chi square: 11.479, p=0.075 **Linear-by-linear association value: 3.314, p=0.075**					
B	**Allele**	**BMI (kg/m²)**				**Total**
		0–25	**26–29**	**30–39**	**40 or more**	
Patients without diabetes	G	21	24	26	1	52
	Non-G	13	22	22	0	57
	Total	34	46	28	1	109
	Chi square: 11.908, p=0.008 **Linear-by-linear association value: 7.420, p=0.006**					
Patients with diabetes	G	7	7	9	1	24
	Non-G	7	8	9	0	24
	Total	14	15	18	1	48
	Chi square: 1.067, p=0.785 **Linear-by-linear association value: 0.111, p=0.739**					
All patients in cohort 2	G	28	31	15	2	76
	Non-G	20	30	31	0	81
	Total	48	61	46	2	157
	Chi square: 11.479, p=0.075 **Linear-by-linear association value: 3.314 p=0.075**					

Mantel-Haenszel statistics

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Analysis of non-obese, non-diabetic patients in cohort 2

In order to eliminate the confounding effects of diabetes and obesity, we analyzed non-obese, non-diabetic patients separately in cohort 2 (n=83). On comparison of patients with the CC (n=37), CG (n=37) and GG genotypes (n=9), no significant differences were observed. Patient with G allele (n=37), however, still had significantly lower waist circumference (103±6.8 *vs.* 99±7.3 cm, p=0.026) and mildly lower BMI (25.3±2.6 *vs.* 26.5±2.3 kg/m^2, p=0.048) than the non-G carriers (n=46). Other parameters showed no differences (data not shown).

Discussion

Our results suggest that in rs4918 SNP of AHSG, the presence of the G nucleotide is associated with lower serum TNFα and adiponectin and higher leptin concentrations in cohort 1 compared to that of the C nucleotide. In cohort 2, waist circumference and BMI values were lower in G-carriers than in C-carriers. In cohort 2, the association between the rs4918 polymorphism and obesity was relevant only in patients without diabetes. The small sample size could not allow analysis in the diabetic subgroup. The link between obesity parameters and the rs4918 polymorphism, however, could still be observed among non-obese and non-diabetic patients. Leanness, again, was associated with higher prevalence of the G allele.

There were obvious differences between the anthropometric, metabolic, adipokine, and other cytokine levels of the two cohorts; therefore, we did not intend to compare them. Cohort 1 consisted of healthy individuals with normal BMI (below 25), whereas members in cohort 2 were chosen based on a hard cardiovascular endpoint (myocardial infarction) and had multiple cardiovascular risk factors (vascular disease, obesity, diabetes, and elevated proinflammatory cytokine levels).

Reports on AHSG rs4918 (Thr256Ser) polymorphism are contradictory. The rs4917 TT and rs4918 GG haplotypes, along with rs2593813: G, conferred an increased likelihood of leanness in Swedish men [22]. We also found the minor T allele of rs4917 was more frequent among lean patients [25]. On the other hand, others found the G allele less advantageous. In a follow-up study on dialysis patients, the CG and GG genotypes were associated with lower AHSG levels and slightly increased mortality; however, no causative effect on lethal outcome was observed [37]. The rs4918 G allele was associated with lower serum AHSG levels [23], the latter being a determinant of aortic calcification and high mortality in renal transplant patients [34]. Similarly, Ma and others found the G allele confers a higher risk for ischemic stroke than the C allele [35]. In addition, they found a higher frequency of the GG genotype and the G allele in patients with ischemic stroke or atherosclerotic cerebral infarction than in healthy controls in a Northern Han Chinese population [35].

There are increasing number of observations suggesting that ethnic differences may explain the contradictory effects of different AHSG genotypes and their putative function. It is known that the minor variant AHSG-2 is linked with higher estradiol levels and higher bone density in Caucasian women [38,39]. Jiang and others found evidence of linkage between the AHSG-SacI polymorphism (rs4918) and bone geometry in Caucasians, but not in Chinese [40]. Other studies also demonstrated significant differences between genes determining bone density in the Caucasian and Chinese populations [41,42].

In theory, change in its serum concentrations or altered molecular structure or function of AHSG (or both) may explain the different activities of the rs4918 variants.

Elevated AHSG levels play a role in the development of obesity and insulin resistance [6–10,14]. Since the first report on AHSG SNP-s, including rs4918 [23], several other studies have confirmed that minor variants of AHSG (AHSG2) are linked with lower serum AHSG levels [19,20,22]. In spite of the small sample size, we also noticed a similar trend in cohort 2. The mean of the AHSG levels was lower in the GG than in the CC in cohort 2, although the difference was not statistically significant, probably because of the small sample size.

Elevated serum AHSG is also associated with increased risk of diabetes [11,12,43] but this association is not as clear as that with non-diabetic healthy or obese individuals [27,44]. Jensen et al. found that serum AHSG concentration was inversely correlated with cardiovascular mortality in old, non-obese, non-diabetic individuals [27]. This trend was reversed in 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 non-obese persons with normal HOMA-IR below the median value [27].

In the EPIC Potsdam Study, Fisher et al. observed a strong association between the C allele of rs4917 and occurrence of myocardial infarction [19]. They suggested the pathogenic role of AHSG in the development of cardiovascular diseases. We found no statistically significant differences of rs4918 genotypes and alleles between the two small cohorts we studied. Nevertheless, it is remarkable that in cohort 1 (healthy persons with normal BMI), TNFα levels significantly differed among rs4918 homozygotes and the G was associated with lower TNFα and adiponectin and higher leptin levels than the C allele. These differences could not be detected in cohort 2, probably because of the existing subclinical inflammation and prevalence of obesity among patients. Signal transduction changes in the peri-infarct area may also affect infarct size and post-infarction cardial remodelling [45].

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In theory, the rs4918 polymorphism, which affects the intron 7 of the molecule (D3 domain region), may also result in altered function of the molecule. It has been demonstrated that the TT variant of rs4917 has 35 times higher lipolytic sensitivity than its CC variant, but no functional differences for rs4918 have been reported [46]. Since AHSG connects metabolic and inflammatory processes by linking FFAs and TLR4-s, it seems relevant to investigate the FFA and TLR4 binding activities of the various rs4918 AHSG polymorphisms [15–17].

One limitation of our study is that the sample size did not allow for subgroup analysis, especially the minor (G) nucleotide frequency in cohort 2. Secondly, the cross-sectional design did not allow drawing causal relationships between rs4918 polymorphism and the parameters we investigated. The obligatory antilipemic (mainly with statins), platelet aggregation inhibiting (salicylates, clopidogrel), and antidiabetic treatment (bedtime insulin) may mask the differences in serum AHSG concentrations and their correlation with rs4918 variants in cohort 2.

Conclusions

Our observations (made in a Caucasian population) are in line with those suggesting that the minor variant G of rs4918 is linked with more favorable parameters than is the C allele. In addition, AHSG seems to be correlated much more with obesity than with diabetes mellitus. Large-scale prospective studies could determine the causative relationship and functional impact of AHSG variants in different populations.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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