AHSG Tag Single Nucleotide Polymorphisms Associate With Type 2 Diabetes and Dyslipidemia

Studies of Metabolic Traits in 7,683 White Danish Subjects

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OBJECTIVE—The gene encoding the α2 Heremans-Schmid glycoprotein (*AHSG*) is a credible biological and positional candidate gene for type 2 diabetes and the metabolic syndrome, and previous attempts to relate *AHSG* variation with type 2 diabetes and obesity in Swedish and French Caucasians have been largely successful. We related seven frequent *AHSG* tag single nucleotide polymorphisms to a range of metabolic traits, including type 2 diabetes, obesity, and dyslipidemia.

RESEARCH DESIGN AND METHODS—The polymorphisms were genotyped in 7,683 white Danish subjects using Taqman allelic discrimination or chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, providing a statistical power of >99% to replicate previous findings. Data were analyzed in case-control and haplotype settings, and quantitative metabolic traits were examined for association. Moreover, epistatic effects between *AHSG* variants and insulin receptor substrate-1 (*IRS1*) and β-2-adrenergic receptor polymorphisms were investigated.

RESULTS—The −469T>G (rs2077119) and IVS6+98C>T (rs2518136) polymorphisms were associated with type 2 diabetes ($P = 0.007$ and $P = 0.006$, respectively, or $P_{corr} = 0.04$ and $P_{corr} = 0.03$, respectively, following correction for multiple hypothesis testing), and in a combined analysis of the present and a previous study −469T>G remained significant (odds ratio 0.90 [95% CI 0.84–0.97]; $P = 0.007$). Furthermore, two *AHSG* haplotypes were associated with dyslipidemia ($P = 0.003$ and $P_{corr} = 0.009$). Thr248Met (rs4917) tended to associate with lower fasting and post–oral glucose tolerance test serum insulin release ($P = 0.02$, $P_{corr} = 0.1$ for fasting and $P = 0.04$, $P_{corr} = 0.2$ for area under the insulin curve) and improved insulin sensitivity estimated by the homeostasis model assessment of insulin resistance (9.0 vs. 8.6 $mmol \cdot l^{-1} \cdot pmol^{-1} \cdot l^{-1}$; $P = 0.01$, $P_{corr} = 0.06$). Indications of epistatic effects of *AHSG* variants with the *IRS1* Gly971Arg polymorphism were observed for fasting serum triglyceride concentrations.

CONCLUSIONS—Based on present and previous findings, common variation in *AHSG* may contribute to the interindividual variation in metabolic traits. ***Diabetes*** **57:1427–1432, 2008**

The α_2 Heremans-Schmid glycoprotein (AHSG) is secreted mainly by the liver and is abundant in plasma. AHSG inhibits insulin-stimulated insulin receptor autophosphorylation, insulin-induced tyrosine phosphorylation of insulin receptor substrate-1 (IRS1), and the association of IRS1 with the p85 subunit of phosphatidylinositol-3 kinase in vitro (1–3). Elevated plasma AHSG was associated with insulin resistance in humans (4), and AHSG levels were higher in subjects with impaired glucose tolerance (5) and women with gestational diabetes (6). *Ahsg* knockout mice demonstrate increased basal and insulin-stimulated insulin receptor phosphorylation, increased glucose clearance, and insulin sensitivity (7). Moreover, even on a high-fat diet and at old age *Ahsg* knockout mice were resistant to weight gain and remained insulin sensitive (7,8), while Ahsg expression was induced in the liver of Osborne-Mendel rats upon a high-fat diet (9). Human *AHSG* (chromosome 3q27) is a positional candidate gene for type 2 diabetes and the metabolic syndrome (10,11). Thus, attempts have been made to associate *AHSG* variation with metabolic phenotypes. Three polymorphisms (−843A>T/rs2248690, −469T>G/rs2077119, and Thr248Met/rs4917) were associated with plasma cholesterol among 291 Swedish women for whom a relationship of −469T>G with insulin-mediated inhibition of lipolysis was also shown (12). Thr248Met and Thr256Ser (rs4918), which are in almost perfect linkage disequilibrium (LD), were examined among 176 Japanese subjects in relation to serum concentrations of AHSG, which proved to be largely dependent on *AHSG* Thr248Met and Thr256Ser genotypes (13). Both of these single nucleotide polymorphisms (SNPs) are in almost perfect LD with a third variant, IVS1-903A>G (rs2593813), which was associated with overweight/obesity among 504 Swedish men, alone and in a haplotype combination with Thr248Met and Thr256Ser (14). In a French study (15) involving 1,655 participants, *AHSG* was resequenced and frequent SNPs (minor allele frequencies [MAFs] >5%) were investigated for association with type 2 diabetes. Thr270Thr (rs1071592) was associated with type 2 diabetes with an odds ratio (OR) of 1.27 (95% CI 1.06–1.52) ($P = 0.008$) (15). Finally, Thr248Met was associated with increased sensitivity to the β_2-adrenergic receptor (ADRB2) in subcutaneous adipose tissue in 93 Swedish men, pointing to a possible role of *AHSG* variation in regulation of lipolysis (16). Here, we tagged the

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Received for publication 25 April 2007 and accepted in revised form 7 February 2008.

Published ahead of print at http://diabetes.diabetesjournals.org on 3 March 2008. DOI: 10.2337/db07-0558.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-0558.

G.A. holds stock in Novo Nordisk.

ADRB2, β-2-adrenergic receptor; AHSG, α_2 Heremans-Schmid glycoprotein; IRS1, insulin receptor substrate-1; LD, linkage disequilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

TABLE 1
Genotype distribution and MAFs of the *AHSG* −469T>G and IVS6+98C>T polymorphisms for the participants stratified according to glucose tolerance status

	Normal glucose tolerance	Type 2 diabetes	P_{GD} ($P_{GD}corr$)	P_{MAF} ($P_{MAF}corr$)
−469T>G	n = 4,750	n = 1,360		
TT	1,392 (29)	441 (32)		
TG	2,315 (49)	657 (48)	0.03 (0.2)	
GG	1,043 (22)	262 (19)		
MAF (95% CI)	46.3 (45.3–47.3)	43.4 (41.6–45.3)		0.007 (0.04)
IVS6+98C>T	n = 4,712	n = 1,336		
CC	1,326 (28)	335 (25)		
CT	2,319 (49)	658 (49)	0.02 (0.1)	
TT	1,067 (23)	343 (26)		
MAF (95% CI)	47.3 (46.2–48.3)	50.3 (48.4–52.2)		0.006 (0.03)

Data are n (%) and frequencies of MAFs are percentages (95% CI). All P values were calculated using Fisher's exact test and compare genotype distributions and MAFs either nominally (P_{GD} and P_{MAF}) or corrected for multiple hypothesis testing ($P_{GD}corr$ and $P_{MAF}corr$). Also, logistic regression was applied with adjustment for age, sex, and BMI; however, this did not change the results. All genotype groups obeyed Hardy-Weinberg equilibrium.

AHSG locus and added SNPs chosen from the current literature in order to examine the relationship of *AHSG* variation with different metabolic phenotypes (type 2 diabetes, obesity, dyslipidemia, and related quantitative traits) in white Danish subjects.

RESEARCH DESIGN AND METHODS

AHSG variants were genotyped in 7,683 white Danish subjects comprising *1*) a population-based sample of middle-aged white Danish subjects sampled at the Research Centre for Prevention and Health (17) (n = 6,256), *2*) a group of type 2 diabetic patients sampled through the outpatient clinic at the Steno Diabetes Center (n = 1,069), and *3*) a population-based group of middle-aged glucose-tolerant subjects recruited from the Research Centre for Prevention and Health and Steno Diabetes Center (n = 358). In study group 1 (3,067 men and 3,189 women), the age was 46 ± 8 years (means ± SD) with BMI 26.3 ± 4.6 kg/m^2. We made no observation of population stratification bias in this study sample (18). In the group of type 2 diabetic patients sampled at the Steno Diabetes Center (653 men and 416 women), age was 59 ± 11 years, age of clinical diagnosis 52 ± 11 years, BMI 29.5 ± 5.1 kg/m^2, and A1C 8.1 ± 1.6%. In study group 3 (174 men and 184 women), age was 62 ± 6 years and BMI 26.1 ± 3.7 kg/m^2. Study groups 1 and 3 underwent a standard 75-g oral glucose tolerance test. All participants were stratified into subgroups for case-control studies of type 2 diabetes, obesity, and dyslipidemia, with many participants being part of more than one group, and participants of study group 1 were analyzed for association with quantitative metabolic traits. Informed written consent was obtained from all subjects before participation. The study was approved by the ethical committee of Copenhagen County and was in accordance with the principles of the Helsinki Declaration. Type 2 diabetes was defined according to the World Health Organization (19). Obesity was defined as described (20), and dyslipidemia was defined as fasting serum triglycerides ≥1.7 mmol/l or HDL cholesterol <0.9 mmol/l for men or <1.0 mmol/l for women and/or current or previous treatment with lipid-lowering drugs (21).

Biochemical assays and anthropometrical measurements. Height, body weight, and waist and hip circumferences were measured as described (20). A1C, plasma glucose, serum insulin, serum triglycerides, and total and HDL cholesterol were analyzed as described (18).

Genotyping. Eight *AHSG* variants (−726G>T, −469T>G [rs2077119], IVS1–903A>G [rs2593813], Thr248Met [rs4917], IVS6 + 98C>T [rs2518136], Thr256Ser [rs4918], Thr270Thr [rs1071592], and 3′UTR+150C>T [rs11540663]) were selected on the basis of previous positive findings and in order to tag the gene. Discordance between 938 random duplicate samples was <0.85%, and the genotyping success rate was >96%. For gene-gene interaction studies, the *IRS1* Gly971Arg (rs1801278) and *ADRB2* Arg16Gly and Gln27Glu (rs1042713 and rs1042714) (22) polymorphisms were also analyzed. Genotyping of the variants was performed using Taqman allelic discrimination or chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. All genotype groups obeyed Hardy-Weinberg equilibrium in the population-based sample of Danes (P > 0.2).

Statistical analysis. Fisher's exact test was applied to examine differences in MAFs and genotype distributions between affected and unaffected subjects. Also, logistic regression with adjustment for age, sex, and BMI (where appropriate) was applied assuming an additive model. Power calculations were performed assuming ORs of 1.27 and 1.74, as observed in previous reports (14,15), a significance level of 95%, and a sample size of 1,005, which was the smallest number of subjects entering any analysis. A general linear model was used to test quantitative variables for differences between genotype groups among glucose-tolerant subjects not receiving any medication (glucose tolerance status is determined according to 19), and the P values describe an additive model. The degree of LD was estimated using the "Genetics " package of RGui. The meta-analysis was performed using a fixed-effects model, and homogeneity was tested using Mantel-Haenszel. Haplotypes were inferred using the Carlson greedy algorithm on HapMap CEU data, capturing all SNPs with a MAF >5% at r^2 >0.8. Haplotype analysis was performed excluding all haplotypes with a frequency <5% assuming an additive model. The two-way epistasis analysis was performed using general linear models: one with nine parameters (one for each genotype), in which each SNP was assigned an additive and a dominance parameter, and one without interaction coefficients that consists of five parameters describing the epistasis (23). The epistatic effect was tested by comparing the two models (4 df) using ANOVA. The global null hypothesis was that all the parameters except the intercept are zero, and the local null hypothesis was that one of the parameters is zero. All analyses were performed using SPSS version 14.0 and RGui version 2.4.1. A P value of <0.05, following correction for multiple testing using SNPSpD (24), was considered to be significant. The Nyholt method of correction was applied, which takes into account the degree of LD among the SNPs and estimates the effective number of SNPs. An online interface is available at http://genepi.qimr.edu.au/general/daleN/SNPSpD/.

RESULTS

We genotyped eight *AHSG* SNPs (−726G>T, −469T>G, IVS1–903A>G, Thr248Met, IVS6+98C>T, Thr256Ser, Thr270Thr, and 3′UTR+150C>T) in a sample of 7,683 white Danish subjects. Estimates of pairwise LD between the SNPs are shown in the online appendix Table A (available at http://dx.doi.org/10.2337/db07-0558). High degrees of LD are due to some SNPs being chosen based on previous reports of association with metabolic traits. Due to the perfect LD between Thr248Met and Thr256Ser (r^2 = 1), we excluded Thr256Ser from further analyses. The seven remaining SNPs tagged *AHSG* (HapMap phase 2 release no. 21; MAF >5%; r^2 >0.8).

Case-control studies of individual SNPs. For each of the seven SNPs, four separate case-control studies were performed in which the genotype distributions and MAFs for type 2 diabetic patients were compared with those of glucose-tolerant subjects, obese subjects (BMI >30 kg/m^2) were compared with lean subjects (BMI <25 kg/m^2), dyslipidemic patients were compared with normolipidemic subjects, and finally we compared subjects with high waist circumference (>102 cm for men and 88 cm for

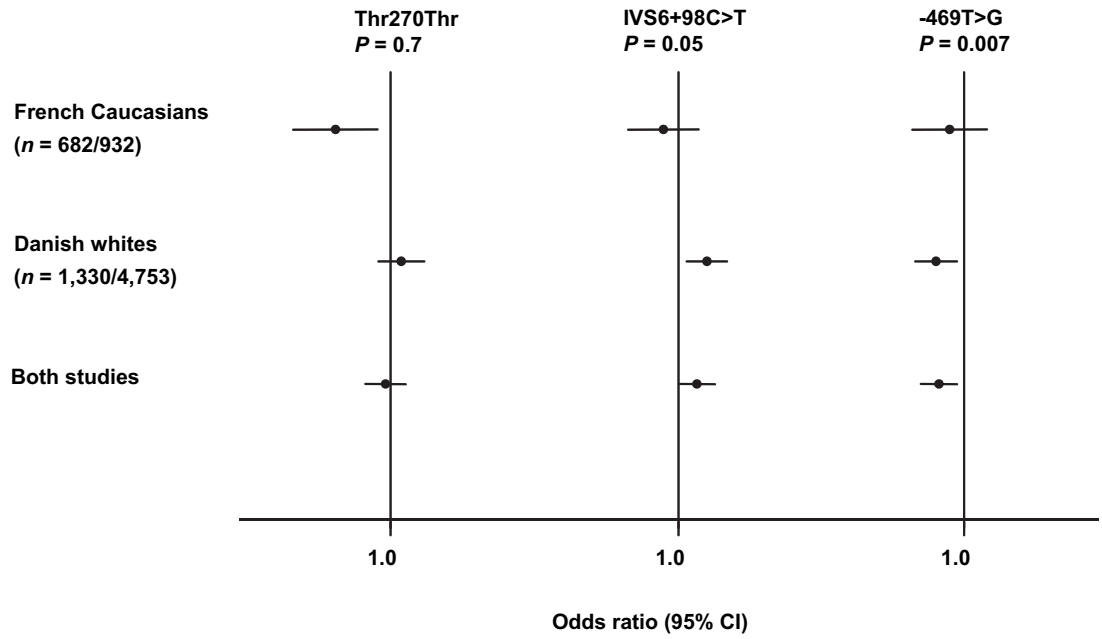

FIG. 1. Combined analyses of data from the present and a previous study (15) of *AHSG* polymorphisms in relation to type 2 diabetes. Data are estimated risks (95% CI) of type 2 diabetes when comparing the numbers of minor alleles of the *AHSG* Thr270Thr, IVS6+98C>T, and −469T>G polymorphisms assuming a general model. Homogeneity between studies was tested (Mantel-Haenszel) but was observed only for −469T>G (P = 0.5) and not for Thr270Thr (P = 0.006) or IVS6+98C>T (P = 0.03).

women) with subjects having low waist circumference. None of the SNPs were associated with obesity (online appendix Tables B and C), but the genotype distributions tended to differ between subjects with low and high waist circumference for IVS1-903A>G, Thr248Met, and Thr270Thr (online appendix Table C). We observed suggestive evidence for association between dyslipidemia and −469T>G (P = 0.02) (OR 0.91 [95% CI 0.84–0.99]) and IVS6+98C>T (online appendix Table D), albeit not following correction for multiple hypothesis testing. Two SNPs (−469T>G and IVS6+98C>T) were significantly associated with type 2 diabetes (Table 1 and online appendix Table E), in which a difference in the MAFs (P = 0.007, P = 0.006) as well as the genotype distributions (P = 0.03, P = 0.02) were observed, and the differences in MAFs persisted after correction (Table 1). These two SNPs, along with Thr270Thr, which was previously reported to associate with type 2 diabetes (15), were subjected to a combined case-control analysis with previous data (Fig. 1). Only −469T>G remained significant (P = 0.007) using a fixed-effects model, although IVS6+98C>T approached significance (P = 0.05). Attempting to replicate significant associations with obesity among Swedish men (14), we also tested a recessive model for the influence of the rare alleles of SNPs Thr248Met and IVS1-903A>G; however, we failed to replicate the previous finding ($P > 0.05$).

Haplotype analyses. We performed haplotype analyses on type 2 diabetes, BMI-defined obesity, and waist circumference (excluding haplotypes with a frequency of <5%) without any positive findings ($P > 0.05$). One haploblock was present, with two specific haplotypes being significantly associated with dyslipidemia (P = 0.002 and P = 0.008) (Table 2), indicating opposite functions of the minor alleles of −726G>T (haplotype no. 6, frequency 6%) and −469T>G (haplotype no. 1, frequency 40%). The global P value was 0.003 before correction and 0.009 after correction.

Analyses of quantitative metabolic traits. We investigated the relationships of the seven *AHSG* SNPs with quantitative metabolic traits, and results for Thr248Met and IVS6+98C>T are shown in Table 3. Although some indications of a relationship with plasma glucose and serum insulin levels as well as homeostasis model assessment of insulin resistance were observed, these were not statistically significant following correction for multiple testing. Likewise, an observed trend toward the minor −726T allele being related to higher fasting serum cholesterol (P = 0.02) did not remain significant (P = 0.1).

Investigations of epistasis with *IRS1* and *ADRB2* polymorphisms. Due to a report of *Ahsg* knockout mice showing increased insulin receptor phosphorylation in the insulin-stimulated state (7) and the evidence of AHSG inhibiting insulin-induced tyrosine phosphorylation of IRS1 (2), we examined a possible two-way genetic interaction of *IRS1* Gly971Arg with the *AHSG* SNPs. Four *AHSG* SNPs (IVS1–903A>G, Thr248Met, Thr270Thr, and IVS6+98C>T) showed some indications of epistatic effects with the functional *IRS1* Gly971Arg polymorphism on fasting serum triglycerides (uncorrected $P = 1 \times 10^{-5}$, $P = 7 \times 10^{-6}$, P = 0.0004, and P = 0.001, respectively), where a relatively small group consisting of homozygous carriers of both minor alleles had increased triglyceride concentrations. No interaction was observed for fasting or postprandial serum insulin or plasma glucose ($P > 0.05$). Additionally, as Thr248Met was suggested to increase adipose tissue sensitivity to ADRB2 (16), we investigated the interaction of the *AHSG* SNPs with two functional *ADRB2* polymorphisms (Arg16Gly and Gln27Glu). However, no indication of an interaction between SNPs in these two loci was observed for any of the examined traits ($P >$ 0.05).

DISCUSSION

The biological function of AHSG and the *Ahsg* knockout mouse, as well as the chromosomal localization of *AHSG*, suggest a potential involvement of *AHSG* variation in the pathogenesis of metabolic diseases (2,7,10,11), and previous genetic epidemiological studies have shown association of *AHSG* variants with obesity (14), type 2 diabetes (15), and plasma cholesterol levels (12). In the present study we aimed at replicating these findings by genotyping the previously investigated variants, as well as *AHSG* tagSNPs. Given the high MAF of the previously investigated *AHSG* variants, our study has a statistical power of >99% to detect an association with type 2 diabetes with an OR of 1.27 (as observed among white French subjects [15]) or an association with obesity with an OR in the range of 1.74–2.02 (as observed among Swedes [14]). In summary, we found a significant association of *AHSG* variants −469T>G and IVS6+98C>T with type 2 diabetes, a haplotype that is associated with dyslipidemia, and suggestions of epistasis between *AHSG* and *IRS1* variants on fasting serum triglyceride concentrations.

We did not observe any association between Thr270Thr and type 2 diabetes as was found in the French population (15), and in a combined analysis of the French and Danish data only the −469T>G variant remained significant. Despite of our relatively large-scale study, this observation needs validation in future studies, and such validation may be provided by genome-wide association studies. Intriguingly, four imputed *AHSG* SNPs from the publicly available Wellcome Trust Case Control Consortium Study were modestly associated with type 2 diabetes (25) (−469T>G, IVS1-903A>G, Thr248Met, and Thr270Thr; *P* values ranging from 0.002 to 0.08). Even though *AHSG* was not considered to be a first-priority gene in the Wellcome Trust Case Control Consortium genome-wide association study, these results add to the notion of *AHSG* playing a role in type 2 diabetes pathogenesis.

We made no observation of a relationship between *AHSG* variation and obesity, as was suggested by previous reports; however, the present haplotype analyses and case-control studies indicate a role for −469T>G in dyslipidemia. This interpretation is supported by a previous study among Swedes, in which lower plasma cholesterol was observed in subjects homozygous for the minor allele (12). Moreover, there are strong indications that this variant is biologically functional; in a Japanese study, serum AHSG levels were associated with −469T>G, in which the G allele conferred the higher expression (26). Additionally, the results of the haplotype analysis, together with the finding of higher fasting serum cholesterol, suggest that the −726T allele may act in the opposite direction to increase the risk of dyslipidemia, although this was not supported by the case-control study. The relatively low MAF (~6%) and the resulting modest statistical power to detect subtle metabolic changes may account for this discrepancy. Also, the effect of *AHSG* SNPs on fasting serum triglycerides was completely masked in the absence of *IRS1* Gly971Arg, as the increased levels of fasting serum triglyceride were only observed for carriers of both Gly971Arg and one of the *AHSG* SNPs. Ideally, statistical methods should be utilized providing the necessary statistical power to detect what is expected to be small interactive effects in comparison with distinct main effects.

The Thr248Met variant is in perfect LD with Thr256Ser and, as such, represents a double nonsynonymous amino

TABLE 2
Analysis of common *AHSG* haplotypes in relation to dyslipidemia

No.	−726G>T	−469T>G	IVS1-903A>G	Thr24Met	IVS6+98C>T	Thr270Thr	3′UTR+150C>T	Frequency (%)	Score	*P* (P_{corr})
1	G	G	T	C	C	C	C	40	−3.2	0.002 (0.006)
2	G	T	C	T	T	A	C	30	−0.8	0.4
3	G	T	T	C	T	C	T	6	1.1	0.3
4	G	T	C	T	T	C	C	8	1.4	0.2
5	G	T	T	C	T	C	C	6	1.8	0.08
6	T	T	T	C	C	C	C	6	2.6	0.008 (0.02)

Haplotypes with a frequency >5% are shown. The global score *P* value was 0.003 without correction for multiple hypothesis testing and 0.009 with correction.

TABLE 3
Anthropometric and metabolic characteristics of middle-aged glucose-tolerant white Danish subjects stratified according to *AHSG* Thr248Met and IVS6+98C>T genotype

	Thr248Met (n = 4,455)				IVS6+98C>T (n = 4,358)			
	CC	CT	TT	P (P_{corr})	CC	CT	TT	P (P_{corr})
n (men/women)	1,865 (893/972)	2,033 (913/1,120)	557 (266/291)		1,226 (580/646)	2,145 (980/1,165)	987 (456/531)	
Age (years)	45 ± 8	45 ± 8	46 ± 8		45 ± 8	45 ± 8	46 ± 8	
BMI (kg/m^2)	25.4 ± 3.9	25.6 ± 4.3	25.3 ± 3.9	0.7	25.5 ± 4.0	25.5 ± 4.2	25.5 ± 4.1	0.7
Waist-to-hip ratio	0.84 ± 0.08	0.84 ± 0.08	0.85 ± 0.08	0.8	0.84 ± 0.08	0.84 ± 0.08	0.84 ± 0.08	0.5
Plasma glucose (mmol/l)								
Fasting	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	0.4	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	0.4
30 min	8.2 ± 1.6	8.2 ± 1.5	8.1 ± 1.5	0.2	8.3 ± 1.6	8.2 ± 1.5	8.1 ± 1.5	0.03 (0.2)
120 min	5.5 ± 1.1	5.5 ± 1.1	5.4 ± 1.1	0.5	5.5 ± 1.1	5.5 ± 1.1	5.5 ± 1.1	0.5
Area under the curve	182 ± 103	182 ± 100	175 ± 95	0.05	185 ± 102	181 ± 101	177 ± 98	0.04 (0.2)
Serum insulin (pmol/l)								
Fasting	38 ± 23	38 ± 23	36 ± 25	0.02 (0.1)	38 ± 23	38 ± 23	37 ± 24	0.2
30 min	287 ± 183	292 ± 175	271 ± 157	0.09	286 ± 183	294 ± 183	276 ± 156	0.1
120 min	168 ± 129	172 ± 135	158 ± 122	0.4	167 ± 128	173 ± 139	162 ± 119	0.7
AUC	21,068 ± 13,639	21,398 ± 13,293	19,662 ± 11,814	0.04 (0.2)	20,986 ± 13,602	21,631 ± 13,855	20,078 ± 11,659	0.09
Insulin resistance (mmol · l^{-1} · pmol^{-1} · l^{-1})								
Homeostasis model assessment of insulin resistance	9.0 ± 5.6	9.0 ± 5.7	8.6 ± 5.9	0.01 (0.06)	8.9 ± 5.6	9.0 ± 5.7	8.7 ± 5.7	0.2
Fasting serum lipids (mmol/l)								
Triglycerides	1.2 ± 0.8	1.2 ± 1.1	1.2 ± 0.9	0.8	1.2 ± 0.8	1.2 ± 1.1	1.2 ± 0.8	0.6
Total cholesterol	5.4 ± 1.0	5.4 ± 1.0	5.4 ± 1.1	0.5	5.5 ± 1.0	5.4 ± 1.0	5.5 ± 1.1	0.6
HDL cholesterol	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	0.3	1.5 ± 0.4	1.5 ± 0.4	1.5 ± 0.4	0.2

Data are means ± SD. Values of serum insulin, values derived from insulin variables, and values of serum triglycerides were logarithmically transformed before statistical analysis. All analyses were made using an additive model. Calculated P values were adjusted for age, sex, and BMI; genotype and sex were considered as fixed factors and age and BMI as covariates. Statistically significant P values were corrected for multiple hypothesis testing (*Pcorr*). Homeostasis model assessment of insulin resistance was calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) and divided by 22.5.

acid substitution that has been shown to be functional in the sense that the two minor alleles result in lower in vivo serum levels of AHSG (13). The protein acts in phosphorylation of the insulin receptor tyrosine kinase (1) and has been associated with insulin resistance (4); thus, the observed nominal association in our study of Thr248Met with reduced fasting insulin and insulin resistance may provide a possible explanation of the mechanism by which *AHSG* variation modulates various metabolic phenotypes such as type 2 diabetes. In summary, we find that in the Danish population of middle-aged white subjects, *AHSG* variation is related to several components of the metabolic syndrome. Due to the high degree of LD between some of the SNPs, not all associations should be regarded as independent, and our findings require future validation testing in large-scale settings. Also, future genotyping of as-yet-unidentified *AHSG* variants or other genetic variation in high LD with *AHSG* variants may assist to further explore this relationship.

ACKNOWLEDGMENTS

The study was supported by the Danish Medical Research Council, the Danish Diabetes Association, the Gerda and Aage Haensch Foundation, the A.P. Møller Foundation for the Advancement of Medical Science, and the Velux Foundation. This work is part of the project Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome (HEPADIP) (available at www.hepadip.org), which is supported by the European Commission as an integrated project under the 6th Framework Programme (LSHM-CT-2005-018734). The study received support from The FOOD Study Group/the Danish Ministry of Food, Agriculture, and Fisheries and Ministry of Family and Consumer Affairs (grant no. 2101-05-0044).

The authors thank Annemette Forman, Inge-Lise Wantzin, Lene Aabo, and Marianne Stendal for technical assistance and Grete Lademann for secretarial support.

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