α_2 -Heremans–Schmid glycoprotein gene polymorphisms are associated with adipocyte insulin action

I. Dahlman¹ · P. Eriksson² · M. Kaaman¹ · H. Jiao³ · C. M. Lindgren³ · J. Kere³ · P. Arner¹

¹ Department of Medicine M61, Karolinska University Hospital, Huddinge, Karolinska Institute, Stockholm, Sweden

² Atherosclerosis Research Unit, King Gustaf V Research Institute, Karolinska Institute, Stockholm, Sweden

³ Clinical Research Centre, Department of Bioscience, Karolinska Institute, Stockholm, Sweden

Abstract

Aims/hypothesis. The aim of this study was to investigate the effect of single-nucleotide polymorphisms (SNPs) in the gene encoding the human α_2 -Heremans–Schmid glycoprotein (AHSG) on obesity and insulin action in adipocytes.

Methods. We screened 24 individuals for SNPs in AHSG. Six haplotype-tagging SNPs were genotyped in 188 lean and 176 obese otherwise healthy women for whom common blood chemistry phenotypes were also available. Adipocyte lipolysis and lipogenesis phenotypes were quantified in a subset of 117 lean and 174 obese women.

Results. The $-469T>G$ SNP, which is located in the 5' region of AHSG, was associated with insulin-mediated inhibition of lipolysis and stimulation of lipogenesis, as well as basal and 8-bromocyclic AMP-stimulated lipolysis. Three AHSG SNPs were associated with cir-

culating levels of cholesterol. None of the six genotyped SNPs or inferred haplotypes were associated with BMI, calculated percent body fat, waist circumference, circulating levels of glucose or insulin, or homeostasis model assessment of insulin resistance, which was used as an estimate of in vivo insulin sensitivity.

Conclusions/interpretation. Our results are in agreement with a threshold model of susceptibility for insulin resistance and type 2 diabetes, in which specific genetic loci regulate intermediate molecular phenotypes. When an individual's set of susceptibility alleles at such loci exceeds a threshold, clinical disease occurs. Lipolysis in adipocytes appears to be a phenotype that is particularly sensitive to variation in AHSG.

Keywords Fetuin · Insulin resistance · Lipogenesis · Lipolysis · Type 2 diabetes.

Received: 6 May 2004 / Accepted: 19 July 2004

Published online: 1 December 2004

© Springer-Verlag 2004

I. Dahlman (✉)

Department of Medicine M61,
Karolinska University Hospital, Huddinge,
Karolinska Institute, 141 86 Stockholm, Sweden

E-mail: Ingrid.dahlman@medhs.ki.se

Tel.: +46-8-58580000, Fax: +46-8-58582407

Abbreviations: 8bcAMP, 8-bromocyclic AMP · AHSG, α_2 -Heremans–Schmid glycoprotein · DASH, dynamic allele-specific hybridisation · HOMA_{IR}, homeostasis model assessment for insulin resistance · IR, insulin receptor · SNP, single-nucleotide polymorphism · TK, tyrosine kinase · UTR, untranslated region

Introduction

Western lifestyle has led to a substantial increase in the frequency of obesity and its associated metabolic complications, including insulin resistance. Obesity and type 2 diabetes are complex disorders that are regulated by behavioural, environmental and genetic factors. The identification of genes that predispose individuals to, or protect against, these disorders may highlight important regulatory pathways and suitable markers or targets for intervention. Although numerous susceptibility loci for obesity and related disorders have been identified, the underlying susceptibility genes remain largely unknown [1].

Insulin resistance is central to the pathophysiology of type 2 diabetes. Improving insulin sensitivity offers

a promising approach to the intervention and treatment of this disorder. This implies a role for genes that are physiological regulators of insulin receptor function and insulin sensitivity, such as those involved in insulin signalling through the insulin receptor (IR). An early step in insulin signal transduction is the stimulation of tyrosine kinase (TK) activity [2]. Patients with insulin resistance display impaired IR-TK function [3, 4, 5]. Thus, dysregulation of IR-TK function might be an important component of insulin resistance.

The α_2 -Heremans–Schmid glycoprotein gene (*AHSG*) is located in a susceptibility locus for type 2 diabetes and the metabolic syndrome on chromosome 3q27 and encodes a protein that is present both in tissue and the circulation [6]. The function of *AHSG* is only partially understood. *AHSG* is a natural inhibitor of insulin-stimulated IR-TK [7, 8]. Mice with targeted disruption of *AHSG* are resistant to weight gain and demonstrate increased insulin sensitivity and glucose clearance in liver and muscle, as well as lower fasting plasma triglycerides [9]. In addition, high *AHSG* expression in liver has been reported to be associated with susceptibility to diet-induced obesity in rats [10]. Adipose tissue was not investigated in these studies. It has been established that *AHSG* inhibits mitogenic signals through IR-TK; however, *AHSG* did not inhibit glucose transport in a rat preadipocyte cell line [8]. In humans, serum levels of *AHSG* have been reported to be higher in gestational diabetes than in normal pregnancies, and were correlated with indirect measurements of insulin resistance [11]. Although it has been proposed that alleles of human *AHSG* with high intrinsic activity could be more common among type 2 diabetic patients, to our knowledge, this has not been investigated [8]. Based on the above observations, we propose that *AHSG* is a potential novel target for the treatment and/or prevention of human obesity and insulin resistance.

The aim of this study was to investigate the effects of *AHSG* polymorphisms on obesity and insulin action in human adipocytes.

Subjects and methods

Subjects. In order to study candidate genes for obesity and insulin action in fat cells, we consecutively recruited 364 unrelated, healthy women who were born in Scandinavia and took no regular medication. There was large inter-individual variation in BMI (18–60 kg/m²) among the women, and obesity was defined as a BMI >30 kg/m². A random subgroup of the women ($n=291$) underwent an *in vitro* metabolic investigation on isolated subcutaneous fat cells.

All women came to the laboratory in the morning after an overnight fast. A venous blood sample was obtained for extraction of DNA and determination of plasma glucose, triglycerides, cholesterol, HDL cholesterol and serum insulin. The homeostasis model assessment for insulin resistance (HOMA_{IR})

was calculated as fasting serum insulin (μ U/ml) $\times$ fasting plasma glucose (mmol/l)/22.5 [12]. In methodological studies on a representative subgroup of this cohort we compared HOMA_{IR} with glucose uptake during euglycaemic–hyperinsulinaemic clamp. A strong correlation was observed between these measurements ($r=0.57$, $p<0.001$) (unpublished results). Body fat content was calculated as (age $\times$ 0.13) + (BMI $\times$ 1.5)–11.5 [13]. In methodological studies on another dataset we compared percent fat as determined by the formula with that determined by dual-energy X-ray absorptiometry (DEXA). An excellent agreement between the two methods was obtained ($r=0.92$), with a slope not different from 1.0 and intercept not different from zero. In yet further methodological experiments in 2735 subjects we compared percent body fat as calculated using the formula above with that obtained with bioimpedance using the formula: $3+0.83 \times \% \text{ fat (impedance)}$. The correlation coefficient was 0.92.

The hospital's committee on ethics approved the study, and written informed consent was obtained from each participant.

Lipolysis and lipogenesis measurements. Abdominal subcutaneous fat biopsies were obtained from 291 women, and lipolysis, lipogenesis and adipocyte cellularity investigated as described previously [14]. Briefly, isolated fat cells were prepared following digestion with collagenase and mean fat cell weight was determined. In lipolysis experiments, diluted cell suspensions were incubated in duplicate for 2 h at 37 °C with air as the gas phase in a medium (pH 7.4) containing albumin (20 g/l), glucose (1 g/l) and ascorbic acid (0.1 g/l). Insulin inhibition of lipolysis was investigated by incubation with adenosine deaminase (1 mU/ml) and the phosphodiesterase-sensitive cyclic AMP analogue 8-bromocyclic AMP (8bcAMP; 10^{-3} mmol/l) in the presence of increasing concentrations of crystalline human insulin (10^{-15} to 10^{-7} mol/l). At the end of the incubation period, an aliquot of the medium was removed for analysis of glycerol release, which was used as an index of lipolysis.

Lipogenesis was investigated by determining the uptake of radiolabelled glucose into lipids. Diluted suspensions of fat cells were incubated in medium (pH 7.4) containing albumin (20 g/l), glucose (5 μ mol/l) and 3-³H glucose (5×10^6 cpm/ml) in the absence or presence of increasing concentrations of crystalline human insulin (10^{-15} to 10^{-6} mol/l) for 2 h at 37 °C with air as the gas phase. Incubation was terminated by the addition of sulphuric acid, and the incorporation of radioactivity into lipids determined for each of the samples.

The concentration (log mol/l) of insulin required to produce half maximum effect was determined using logistic conversion of each concentration response curve. This value was converted to its negative form (pD₂), which reflects hormone sensitivity. The maximum effect or responsiveness of the hormones was determined as glycerol release or glucose incorporation into lipids at the maximum effective hormone concentration.

Single-nucleotide polymorphism harvesting. There is no published information on polymorphisms in *AHSG* in the Swedish population. We therefore chose to sequence the seven exons of the *AHSG* gene, the 3' untranslated region (UTR), and 1940 bp upstream of the ATG site in 24 obese healthy women (age 47 ± 12 years, BMI 39 ± 5 kg/m²). Coding sequences were obtained from Sequence view for *AHSG* on contig NT_005962, the 5' region from AB038689, and the 3' region from AC068631.16. Primer sequences are provided on request. The aim of sequencing was two-fold: (i) to detect potential functional single-nucleotide polymorphisms (SNPs); and (ii) to obtain a broad overview of the haplotypes in the gene [15, 16]. Only obese subjects were screened so as to increase the likeli-

hood of detecting SNPs predisposing for, as opposed to protecting against, obesity. A study cohort of 24 individuals provides 91% power to detect a rare allele with a frequency of 5% by sequencing, i.e. $1-(0.95)^{24}$. Sequencing was performed on an ABI 3100 Genetic Analyzer using the BigDye Terminator v.3 cycle sequencing kit (Applied Biosystems, Foster City, Calif., USA) according to manufacturers instructions. Staden Software was used for sequence analysis [17]. All sequences were scored manually for the presence of SNPs.

Genotyping. The *AHSG* -469T>G SNP was genotyped by dynamic allele-specific hybridization (DASH) [18]. Briefly, for DASH genotyping, a fragment containing the SNP was amplified using forward (5'-GGAAAGCTAACTAAGCTGACA-3') and reverse (5'-GGAGGATAGTCAGCAACTTGATA-3') primers. The forward primer was biotinylated at the 5' end. A genotyping platform (Thermo Hybaid, Ashford, Middlesex, UK) was operated and post-PCR procedures performed according to the manufacturer's protocol. Alleles were determined using a probe with the sequence 5'-AATTCTCCCATGTCAGG-3'.

Our laboratory subsequently changed genotyping platform, and the other SNPs were genotyped using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (Sequenom, San Diego, Calif., USA) [19]. PCR assays and associated extension reactions were designed using SpectroDESIGNER software (Sequenom), and primers (the sequences of which are available on request) were obtained from Metabion (Planegg-Martinsried, Germany). All amplification reactions were run under the same conditions in a 5- μ l reaction containing 2.5 ng of genomic DNA, 1 pmol of each amplification primer, 0.2 mmol/l of each dNTP, 2.5 mmol/l $MgCl_2$ and 0.2 U of HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). Reactions were heated at 95 °C for 15 min and subjected to 45 cycles of amplification (20 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C) before a final extension at 72 °C for 7 min. Extension reactions were conducted in a total volume of 9 μ l using

5 pmol of allele-specific extension primer and the Mass EXTEND Reagents Kit before being cleaned with Spectro-CLEANER (Sequenom) using a MULTIMEK 96 automated 96-channel robot (Beckman Coulter, Fullerton, Calif., USA). Clean primer extension products were loaded onto a 384-element chip (SpectroCHIP, Sequenom) with a nanolitre pipetting system (SpectroJet, Sequenom) and analysed by a MassARRAY mass spectrometer (Bruker Daltonik, Bremen, Germany). Peaks were identified on the resulting mass spectra using SpectroTYPER RT 2.0 software (Sequenom). Two independent scorers confirmed all genotypes for each SNP. Phase software was used to determine haplotypes [20].

Statistical analysis. Regression analysis was used to determine the additive effects of alleles on quantitative phenotypes. Similar *p* values were obtained with ANOVA and ANCOVA including BMI and age as covariates. The chi square test was used to determine whether allele frequencies differed between affected and non-affected individuals. Data are shown as means $\pm$ SD. The *p* values shown are nominal, i.e. they are not corrected for multiple comparisons.

Results

Screening *AHSG* for polymorphisms. Sequencing the seven exons of *AHSG*, the 5' region of the gene, and the 3' UTR in 24 obese women identified 20 polymorphisms (Table 1). Twelve of these SNPs were previously published in the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/index.html>). Six SNPs (located upstream of the ATG site, in exons, and in the 3' UTR) were chosen for genotyping. The remaining SNPs were excluded from genotyping for various reasons. Five SNPs had rare allele frequency

Table 1. Single-nucleotide polymorphisms in *AHSG*

Region	Position ^a	dbSNP ID	Rare allele frequency (%)	Sequence
5'	-1880G>A		<5	CCCACTCC(G>A)TTGCCAGG
5'	-1857C>T		8	TGCAGTGG(C>T)GCAATCTTG
5'	-1772G>A		<5	CTACAGGC(G>A)CGCGCCACC
5'	-1690C>A	rs2593814	25	CAATCTCT(C>A)GGCCTCGT
5'	-1650G>A		<5	AAGTGCTG(G>A)AATTACAG
5'	-843A>T	rs2248690	25	GTGTCAAA(A>T)GAGTCAGG
5'	-726G>T		11	GCAGTGAG(G>T)TCTGGAGA
5'	-469T>G	rs2077119	52	CCTGACAT(T>G)GGAGAATT
Exon 1	39C>G	rs4831	12	GCTCAGCT(C>G)TGGGGCTG
Intron 2	3071A>C	rs2282663	6	ATAATAAT(A>C)ATMATCATC
Intron 2	3074C>A	rs2070632	19	ATAATMAT(C>A)ATCATCAT
Exon 4	4126G>A		<5	TGCACGCC(G>A)CGAAAGCT
Exon 6	6670T>C	rs4917	32	GACCTGCA(C>T)GGTGTTC
Intron 6	6907C>T	rs2518136	47	TGCTCTCC(C>T)GTGCTTCT
Intron 6	7205T>C	rs1900618	33	GACATATG(T>C)GTGTAAGT
Intron 6	7388G>A		39	GCCTGTGG(G>A)TTGTCTTG
Exon 7	7452G>C	rs1344306	33	GCCCCGTA(G>C)CTCACAGC
Exon 7	7495C>A	rs1071592	25	GTCCCCAC(C>A)CCCGTGGT
Exon 7	7634C>T		<5	ACGACCTG(C>T)GCCACACC
3' UTR	7939C>T	rs11540663	9	CCCTACTTCC(C>T)GTCATTCTC

^a Numbering is from the A of the ATG. The common allele on the forward strand is written first

Table 2. Phenotypes of study subjects

	Lean subjects (n=188)	Obese subjects (n=176)
Age (year)	38±10	39±10
Waist (cm)	80±9	114±12
BMI (kg/m ²)	23±3	38±5
Body fat (%)	28±4	51±8
Plasma glucose (mmol/l)	4.8±0.5	5.6±1.5
Serum insulin (mU/l)	7±3	14±8
HOMA _{IR}	1.4±0.6	5.2±6.0
Plasma cholesterol (mmol/l)	4.8±1.0	5.2±1.1
Plasma triglycerides (mmol/l)	1.0±0.5	1.7±0.9

	Lean subjects (n=117)	Obese subjects (n=174)
Mean fat cell weight (ng)	430±160	760±140
Basal lipolysis ^a	4.5±3.9	11.6±6.9
8bcAMP lipolysis ^a	19.4±8.4	28.3±13.1
Insulin pD ₂ , lipolysis	14.4±0.6	13.6±1.3
Insulin responsiveness, lipolysis ^a	7.6±3.7	11.6±5.9
Basal lipogenesis ^b	2.7±1.8	1.9±1.5
Insulin pD ₂ , lipogenesis	13.5±1.2	12.3±1.4
Insulin responsiveness, lipogenesis ^b	6.9±4.3	4.4±3.5

Values are means ± SD ^a Lipolysis is expressed as $\mu\text{mol glycerol} \cdot 2 \text{ h}^{-1} \cdot 10^7 \text{ cells}^{-1}$; ^b lipogenesis is expressed as $\text{nmol glucose} \cdot 2 \text{ h}^{-1} \cdot 10^7 \text{ cells}^{-1}$

(<5%), and the study had low power to detect disease association with alleles with such low frequency. Seven SNPs displayed perfect linkage disequilibrium with at least one genotyped SNP according to SNPHAP and attached linkage disequilibrium software (<http://archimedes.well.ox.ac.uk/cgi-bin/pise/snphap.pl>). Two SNPs were intronic and therefore less likely to be functional.

Relationship between *AHSG* single-nucleotide polymorphisms and phenotypes. In total, 188 non-obese and 176 obese (BMI >30 kg/m²) age-matched women were genotyped for the six chosen *AHSG* SNPs. The phenotypes of these women are displayed in Table 2, and their genotypes are displayed in Table 3. The *AHSG* 39C>G SNP was successfully genotyped in 81% of subjects. The success rate was >98% for all other SNPs. We chose not to repeat 39C>G genotyping since it was a rare SNP, and 39C>G and 7939C>T allelic distribution was in strong linkage disequilibrium in the common haplotypes (Table 4). The 39C>G SNP did not therefore provide much additional information. All SNPs were in Hardy-Weinberg equilibrium (data not shown). Our sample size provides 80% power to detect a susceptibility allele with a rare allele frequency of 35% among controls, approximately what was observed for three of our SNPs (odds ratio 1.5, threshold *p* value 0.05). For an SNP with a rare

Table 3. Genotyping results

SNP	Success rate (%)	Rare allele (%)	Genotype (n)		
			AA	Aa	aa
-843A>T	99	27	189	152	21
-726G>T	99	8	308	48	4
-469T>G	99	46	106	177	77
39C>G	81	5	267	26	2
6670T>C	98	34	152	166	40
7939C>T	99	4	330	31	0

A is the common allele, i.e. the allele written to the left of the > sign in the SNP name; a is the rare allele, i.e. the allele written to the right of the > sign in the SNP name

Table 4. *AHSG* haplotypes

Haplotype number	Alleles present ^a	Chromosomes (n)
1	A G G C C C	329
2	T G T C T C	182
3	A G T C T C	64
4	A T T C C C	56
5	A G T C C C	31
6	A G T G C T	21
7	A G T G C C	19

^a SNPs in order: -843A>T, -726G>T, -469T>G, 39C>G, 6670T>C, 7939C>T

allele frequency of 5%, the power is reduced to 28%. Haplotype analysis identified four common haplotypes (Table 4).

There were no significant differences between obese cases and non-obese control subjects with respect to allele or haplotype frequencies (data not shown). None of the investigated SNPs were associated with BMI, per cent body fat, waist circumference, fat cell weight, HOMA_{IR}, plasma levels of glucose or triglycerides, or serum insulin (data not shown). Bioimpedance measurements of body fat were available for 272 subjects; SNPs were not associated with these values. Three SNPs were associated with levels of plasma cholesterol (Table 5, Fig. 1). The *AHSG* -469T>G genotype was associated with responsiveness to insulin-mediated inhibition of lipolysis (*p*=0.004), responsiveness to 8bcAMP-stimulated lipolysis (*p*=0.006), and pD₂ for insulin stimulation of lipogenesis (*p*=0.05), and was marginally associated with basal lipolysis (*p*=0.06). Similar *p* values were obtained with ANOVA and ANCOVA including BMI and age as covariates. The G allele of -469T>G is specific for *AHSG* haplotype 1. Unfortunately, the number of chromosomes carrying haplotype 5, which is identical to haplotype 1 at all loci except -469T>G, is too small to allow determination of whether the -469T>G SNP or haplotype 1 is primarily associated with adipocyte phenotypes. No additional associations were observed on analysis of the haplotypes.

Fig. 1. Associations between $-469T>G$ genotype (black bars: TT , $n=91$; hatched bars: GT , $n=134$; white bars: GG , $n=63$) and lipolysis (**a**) and lipogenesis (**b**) phenotypes. See subjects and methods for details of phenotype quantification. The value pD_2 is the concentration (log mol/l) of insulin that produces half

maximum effect, converted to its negative form. Data are shown as means $\pm$ SD. The p values were obtained by regression of quantitative phenotypes on additive allele effects. * $p=0.06$; ** $p=0.05$; *** $p<0.01$

Table 5. Associations between single-nucleotide polymorphisms in *AHSG* and plasma cholesterol

Variable	$-843A>T$ genotype			p value	$-469T>G$ genotype			p value	$6670T>C$ genotype			p value
	AA ($n=174$)	AT ($n=141$)	TT ($n=18$)		TT ($n=96$)	GT ($n=163$)	GG ($n=72$)		TT ($n=38$)	TC ($n=153$)	CC ($n=139$)	
Plasma cholesterol (mmol/l)	4.9 $\pm$ 1.0	5.2 $\pm$ 1.1	5.2 $\pm$ 1.3	0.02	5.2 $\pm$ 1.2	5.0 $\pm$ 1.0	4.9 $\pm$ 0.9	0.03	5.4 $\pm$ 1.5	5.1 $\pm$ 1.0	4.8 $\pm$ 0.9	0.008

Values are means $\pm$ SD. Plasma cholesterol was determined using standard procedures. The p values were obtained using simple regression of phenotypes on additive allele effects

Discussion

In this study we have demonstrated associations between an *AHSG* polymorphism and lipid metabolism in fat cells. The $-469T>G$ SNP was strongly associated with insulin inhibition of lipolysis and 8bcAMP-stimulated lipolysis in adipocytes, and was more weakly associated with insulin stimulation of lipogenesis and basal lipolysis.

Insulin resistance and type 2 diabetes are complex phenotypes, with disturbed glucose and lipid homeostasis in adipose tissue, liver and muscle, and changes in insulin production in the pancreas. The identification of individual underlying susceptibility or protective alleles for these complex phenotypes has proved difficult. Attempts have been made to increase power by analysing several loci simultaneously for phenotypic association, analysing a subgroup that display a more homogenous phenotype, or analysing intermediate phenotypes that may display a stronger association with an individual locus. The latter has proven particularly successful in animal models, probably due to access to abundant tissue and the ability to control for exogenous impact on phenotypes. For practical and economic reasons we used rather simple measure-

ments of body fat (calculation) and insulin sensitivity ($HOMA_{IR}$), since methodological studies had shown that these measures provided valid information.

Our results indicate that *AHSG* variants have a local impact in adipose tissue on insulin regulation of lipogenesis and lipolysis. In particular, there appears to be a strong association between variants and anti-lipolytic pathways. Whether *AHSG* polymorphisms also regulate glucose homeostasis is unclear; we did not observe an association with the indirect measurement of insulin sensitivity on glucose metabolism, $HOMA_{IR}$. This does not exclude an association with direct measurements such as glucose uptake during euglycaemic-hyperinsulinaemic clamp. However, it is difficult to obtain in a large enough dataset on this phenotype to make genetic analysis meaningful. In addition, separate methodological experiments in our laboratory demonstrated that measurements of $HOMA_{IR}$ and glucose uptake during euglycaemic-hyperinsulinaemic clamp were strongly correlated ($r=0.57$). Our results are in agreement with a threshold model of susceptibility for type 2 diabetes. In this model, individual genetic loci regulate intermediate molecular phenotypes, and when the individual's set of susceptibility alleles at such loci exceeds a threshold, clinical disease may oc-

cur. Insulin action on adipocytes may be a phenotype that is sensitive to variation in *AHSG*.

We observed a weak association between *AHSG* allele and plasma cholesterol. However, given that *AHSG* has no obvious primary role in plasma lipid regulation, we interpret this result with caution.

It would be appropriate to confirm our results using an independent dataset. However, to our knowledge, there is no other large human dataset on adipocyte phenotypes. Instead, additional molecular functional studies on the role of the investigated *AHSG* polymorphism on insulin signalling in adipocyte are needed. In mice, *AHSG* has been shown to inhibit insulin signalling in muscle; however, its effect on adipose tissue has not been investigated. The association between *AHSG* and 8bcAMP-stimulated lipolysis indicates that the gene may also control insulin-independent signalling pathways in lipolysis regulation.

In summary, *AHSG* is not a strong candidate for obesity and type 2 diabetes, but may control insulin signalling in adipose tissue. *AHSG* is an attractive candidate gene for disturbed adipocyte lipolytic function in obesity and insulin resistance.

Acknowledgements. We thank E. Sjölin, K. Hertel, B.-M. Leijonhufvud, M. Johansson, K. Wåhlén and B. Burt for excellent technical assistance. This study was supported by the Tore Nilsson Foundation, the Swedish Medical Society, the Swedish Society for Medical Research and the Swedish Research Council. None of the authors of this manuscript have a relationship with a company or organisation that could benefit financially from the publication of the data in this manuscript.

References

1. Snyder EE, Walts B, Perusse L et al. (2004) The human obesity gene map: the 2003 update. *Obes Res* 12:369–439
2. Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414:799–806
3. Arner P, Pollare T, Lithell H, Livingston JN (1987) Defective insulin receptor tyrosine kinase in human skeletal muscle in obesity and type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 30:437–440
4. Comi RJ, Grunberger G, Gorden P (1987) Relationship of insulin binding and insulin-stimulated tyrosine kinase activity is altered in type II diabetes. *J Clin Invest* 79:453–462
5. Grunberger G, Geiger D, Carpentier JL, Robert A, Gorden P (1989) Receptor-mediated endocytosis of insulin: inhibition of [¹²⁵I]iodoinsulin internalization in insulin resistant diabetic states of man. *Acta Endocrinol (Copenh)* 121: 581–586
6. Kissebah AH, Sonnenberg GE, Myklebust J et al. (2000) Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci USA* 97:14478–14483
7. Auberger P, Falquerho L, Contreres JO et al. (1989) Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification, and anti-mitogenic activity. *Cell* 58:631–640
8. Srinivas PR, Wagner AS, Reddy LV et al. (1993) Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. *Mol Endocrinol* 7: 1445–1455
9. Mathews ST, Singh GP, Ranalletta M et al. (2002) Improved insulin sensitivity and resistance to weight gain in mice null for the *Ahsg* gene. *Diabetes* 51:2450–2458
10. Lin X, Braymer HD, Bray GA, York DA (1998) Differential expression of insulin receptor tyrosine kinase inhibitor (fetuin) gene in a model of diet-induced obesity. *Life Sci* 63:145–153
11. Kalabay L, Cseh K, Pajor A et al. (2002) Correlation of maternal serum fetuin/alpha2-HS-glycoprotein concentration with maternal insulin resistance and anthropometric parameters of neonates in normal pregnancy and gestational diabetes. *Eur J Endocrinol* 147:243–248
12. Bonora E, Targher G, Alberiche M et al. (2000) Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 23:57–63
13. Gallagher D, Visser M, Sepulveda D, Pierson RN, Harris T, Heymsfield SB (1996) How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol* 143:228–239
14. Reynisdottir S, Eriksson M, Angelin B, Arner P (1995) Impaired activation of adipocyte lipolysis in familial combined hyperlipidemia. *J Clin Invest* 95:2161–2169
15. Botstein D, Risch N (2003) Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nat Genet* 33 [Suppl]:228–237
16. Johnson GC, Esposito L, Barratt BJ et al. (2001) Haplotype tagging for the identification of common disease genes. *Nat Genet* 29:233–237
17. Dear S, Staden R (1991) A sequence assembly and editing program for efficient management of large projects. *Nucleic Acids Res* 19:3907–3911
18. Howell WM, Jobs M, Gyllenstein U, Brookes AJ (1999) Dynamic allele-specific hybridization. A new method for scoring single nucleotide polymorphisms. *Nat Biotechnol* 17:87–88
19. Jurinke C, van den Boom D, Cantor CR, Koster H (2002) Automated genotyping using the DNA MassArray technology. *Methods Mol Biol* 187:179–192
20. Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978–989