Mutations in the Adiponectin Gene in Lean and Obese Subjects From the Swedish Obese Subjects Cohort

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Adiponectin (also called AdipoQ, gelatin-binding protein 28, Acrp30) DNA sequence variants were determined in 96 unrelated female subjects with severe obesity (mean body mass index [BMI], 42.3 kg/m^2) and in 96 non-obese female controls (mean BMI, 23.0 kg/m^2) from the Swedish Obese Subjects (SOS) cohort. A single base substitution (T45G) at codon 15 of exon 2 resulting in no change in amino acid (Gly15Gly) was found in equal frequencies among obese and control subjects. However, this polymorphism was associated with serum cholesterol and waist circumference (P = .023 and .043, respectively) in the obese group. A IVS2 + G62T sequence variation was also identified, but had similar prevalence rates in obese and control subjects. Blood glucose was highest in the obese female subjects who were homozygotes for the G allele (GG) of the IVS2 + G62T polymorphism (N = 56; P = .033) and all the diabetics (n = 6) in this sample were in this group. IVS2 + G62T polymorphism was also associated with BMI (P = .014), diastolic blood pressure (P = .009), and sagittal diameter (P = .032). A missense point mutation at codon 111 (Tyr111His) was not associated with any obesity-related phenotypes. In conclusion, adiponectin DNA sequence variations might play a role in the complications of morbid obesity and should be further investigated.

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ADIPOSE TISSUE IS an endocrine organ secreting a large number of proteins.[1] Among them, gelatin-binding protein 28 (GBP28), a novel adipose-tissue-specific protein, has a tendency, like collagens, to form complexes.[2] GBP28 is encoded by apM1 mRNA[3] and is the adipose tissue most abundant gene transcript.[4] GBP28 is also known as adiponectin in humans[5] or Acrp30 (AdipoQ)[6] in mice. Interestingly, the protease-generated globular domain of Acrp30 was recently shown to cause weight loss in mice by possibly increasing fatty acid oxidation in muscle.[7] Adiponectin has been shown to decrease insulin resistance by increasing lipid oxidation both in pancreas and muscle.[8,9] Subjects with obesity,[5] diabetes mellitus,[10] or coronary heart disease[11] have decreased plasma adiponectin concentrations. In addition, apM1 mRNA levels were reduced in omental and subcutaneous adipose tissue of type 2 diabetic patients.[12] Although their specific functions are as of yet unknown, Acrp30, and the human homolog, adiponectin, have been proposed as signaling molecules from adipose tissue to muscle.[7] In addition, 2 independent groups have reported significant evidence of linkage for obesity and diabetes-related traits with the region of chromosome 3, which contains the adiponectin structural gene.[13,14] Thus, the adiponectin gene is a potential candidate gene for obesity and its metabolic complications in humans. Recently, the gene coding for adiponectin (GBP28) and its overall genomic structure were characterized.[3] In the present study, we sequenced the region of the gene encoding the entire adiponectin product in 96 extremely obese and 96 non-obese control subjects.

SUBJECTS AND METHODS

Subjects

The Swedish Obese Subjects (SOS) cohort has been previously described.[15,16] Briefly, SOS is an intervention trial designed to determine whether mortality and morbidity rates among obese individuals who lose weight with bariatric surgery differ from those associated with conventional treatment. The SOS study consists of 3 cohorts: a registry of obese subjects, an intervention group selected from the registry, and a normal reference population. A total of 192 subjects including 96 obese females (mean ± SD; age, 42.3 ± 3.4 years) from the obese registry and 96 non-obese females (mean age, 49.6 ± 7.0 years) from the normal reference population were included in the present study. The mean body mass index (BMI) of obese subjects was 42.3 ± 3.4 (range, 39.0 to 55.1) kg/m^2, while that in control subjects was 23.0 ± 1.4 (range, 20.1 to 25.5) kg/m^2.

Obesity-Related Phenotypes

Body weight was measured to the nearest 0.1 kg using calibrated balances or electronic scales. Body height was measured to the nearest 1 cm. The BMI was calculated as body weight (kg) divided by squared height (m^2). Systolic and diastolic blood pressure was measured after 15 minutes with patients in a supine position. The patients spent the last 5 of these 15 minutes in complete rest. Sagittal diameter in centimeters was measured by means of a carpenter's spirit level and a ruler. Sagittal diameter was the vertical distance from the examination table up to the horizontal level as measured with a ruler.[16] Serum insulin, blood glucose, and plasma lipids were obtained in the morning after an overnight fast and waist circumference measured as described earlier.[16] Blood glucose was also assayed on samples obtained in a nonfasted state. Diet was not controlled for the days preceding the examination, and the patients were asked not to change their level of physical activity before testing. Albumin in urine was tested by stick. Before receiving a health examination, all subjects completed extensive questionnaires on current and past health status including the level of physical activity.[17] A 12-lead standard electrocardiogram (ECG) was recorded in all subjects from the obese registry.[15] The diagnosis of hypertension and diabetes was based on self-reported data collected in questionnaires.

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Table 1. Summary of Sequence Variations in the Adiponectin Gene

Base Position	Effect on Amino Acid Sequence	Position Within the GBP28 Gene	No. of Subjects		Phenotype Effects in Obese
			Obese	Controls	
T45G	Gly15Gly	Exon2	83 TT	82 TT	Associations with cholesterol and waist circumference
			13 TG	12 TG	
			0 GG	2 GG	
IVS2 + G62T	None	Intron between exon 2 and 3	56 GG	51 GG	All sample diabetics in G62G group
			30 GT	34 GT	
			9 TT	10 TT	
T331C	Tyr111His	Exon 3	91 TT	91 TT	No effect
			4 TC	5 TC	
			0 CC	0 CC	

NOTE. No differences in allele or genotype frequencies between obese and control women.

Sequencing Analysis of the Adiponectin Gene

The human adiponectin gene contains 3 exons. The mature adiponectin product has 244 amino acids and is encoded by part of exons 2 and 3.[3] Two primer pairs were designed to generate products covering exons 2 and 3 encompassing the entire mature adiponectin product. The products were amplified from leukocyte DNA by the polymerase chain reaction (PCR) technique. The primer sequences were as follows: Exon 2: Forward primer, 5′-GAGTCCTTTGTAGGTCCCAAC-3′; Reverse primer, 5′-CTTTCTCCCTGTGTCTAGGC-3′. Exon 3: Forward primer, 5′-CTGTTCTTTGTAGTCACTGAGGTC-3′: Reverse primer, 5′-GAATAATATCTAAAGGCCTCC-3′. The first PCR amplification was performed in a volume of 60 μL containing 200 ng DNA, 0.3 μmol/L of each primer, 0.2 mmol/L of each of the dNTPs< (Amersham Pharmacia Biotech, Piscataway, NJ), 1.25 U Taq polymerase (Qiagen, Valencia, CA). The PCR was started at 95°C for 3 minutes, 60°C (exon 3 - 53°C) for 1 minute, and 72° C for 2 minutes followed by 40 cycles at 95° C for 30 seconds, 60° C (exon 3 - 53° C) for 30 seconds, 72° C for 1 minute and 15 seconds, and 1 cycle at 72° C for 10 minutes using a thermal cycler (Eppendorf Mastercycler Gradient, New York, NY). After purification of the product, a second PCR reaction was performed with the Big Dye Terminator Sequencing Kit as recommended by the manufacturer (Applied Biosystems, Foster City, CA). The amplified product was sequenced using an ABI 3700 as previously described.[18] The PCR products were sequenced in both directions.

Statistical Analysis

All the analyses were performed with the SAS Statistical Software Package (SAS Institute, Cary, NC). Differences in phenotypes between groups were assessed by general linear model procedures. Differences in mutation frequencies between obese and controls were assessed by χ^2 tests.

RESULTS

A summary of the observed sequence variations is given in Table 1 and the positions of the variants are depicted in Fig 1. A polymorphism at codon 15 of exon 2 of the adiponectin gene was identified. This polymorphism was caused by a single base substitution (T45G) that does not cause any amino acid change. The Gly15Gly polymorphism had similar prevalence rates in obese and control subjects. However, obese subjects who were heterozygotes for the T allele (TG) had lower ($P = .023$) serum cholesterol (5.3 $\pm$ 0.3 (SEM) mmol/L) than those who were homozygotes for the T allele (TT) (6.1 $\pm$ 0.1). Waist circumference was also lower ($P = .043$) in TG (112.5 $\pm$ 2.0 cm) than in TT (117.0 $\pm$ 0.8) subjects. A similar tendency was found for the sagittal diameter.

A G62T base change in the intronic region (IVS2 + G62T) between exons 2 and 3 was identified. Thirty heterozygous and 9 homozygous subjects with this sequence variant were found among the obese cases, whereas 34 heterozygotes and 10 homozygotes were identified among control subjects. Obese female subjects who were heterozygotes for the G allele of the adiponectin G62A polymorphism had a higher BMI (Table 2) than those who were homozygotes for the G allele ($P = .014$ for trend). The sagittal diameter was lowest in the subjects with the genotype TT ($P = .032$ for trend). Nonfasting blood glucose was highest among G allele homozygotes ($P = .033$) and

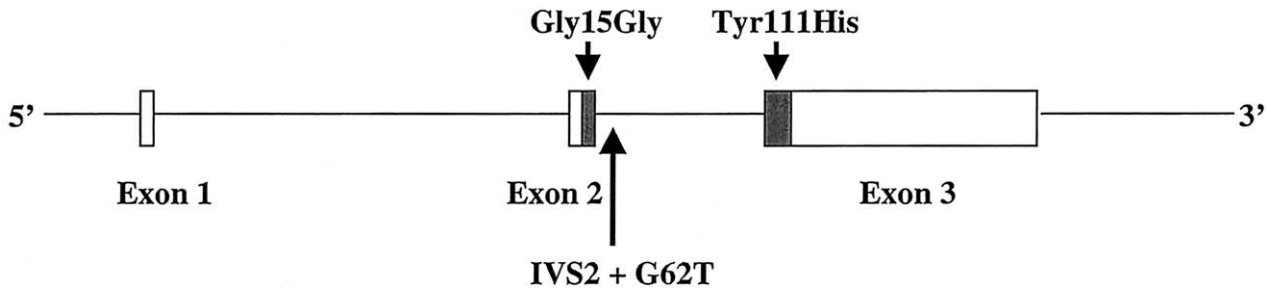

Fig 1. Adiponectin gene and sequence variants. Boxes describe the translated (filled-in) and untranslated regions of the gene.

Table 2. Obesity-Related Phenotypes in SOS Females by Adiponectin IVS2 + G62T Genotype

	GG (n = 56)	GT (n = 30)	TT (n = 9)	*P* for Trend
BMI (kg/m^2)	41.7 (0.4)	43.8 (0.6)*	41.9 (1.1)	.014
Sagittal diameter	28.3 (0.5)	29.7 (0.6)	26.4 (1.2)†	.032
Serum insulin (mU/L)	24.5 (1.6)	21.0 (2.2)	18.9 (3.9)	NS
Blood glucose, fasting (mmol/L)	6.1 (0.2)	5.1 (0.3)	5.5 (0.6)	NS
Blood glucose, nonfasting (mmol/L)	6.1 (0.3)‡	5.0 (0.4)	4.9 (0.7)	.033
Cholesterol, total (mmol/L)	6.0 (0.1)	5.9 (0.2)	6.0 (0.4)	NS
HDL-cholesterol (mmol/L)	1.3 (0.0)	1.3 (0.1)	1.4 (0.1)	NS
Triglyceride, total (mmol/L)	2.2 (0.1)	2.1 (0.2)	1.9 (0.4)	NS
Systolic BP (mm Hg)	142.5 (2.0)	149.9 (2.8)	139.8 (5.1)	NS
Diastolic BP (mm Hg)	88.3 (1.1)	92.7 (1.5)§	83.9 (2.7)	.009

NOTE. Values are means (SE) adjusted for age

*P = .004 between GG and GT, †P = .014 between GT and TT, ‡P = .016 between GG and GT, §P = .019 between GG and GT, P = .006 between GT and TT.

Abbreviation: NS, not significant.

a similar tendency (P = .075) was observed for fasting blood glucose. Diastolic blood pressure was highest among the heterozygotes for the G allele (P = .009).

In Table 3, obesity-related prevalent diseases in SOS female subjects by adiponectin IVS2 + G62T genotype are shown. All the diabetics in this sample were in the G allele homozygote group (6/56). Albuminuria, treated hypertension, and signs of possible or definite ischemia in ECG tended also to be higher in the same group.

A missense point mutation at codon 111 of the adiponectin gene (Tyr111His) was the third sequence variant detected in 4 obese and 5 control subjects (all heterozygotes). This variation was not associated with any obesity-related phenotypes.

DISCUSSION

In an earlier study, a proteolytic cleavage product of Acrp30, the mouse homolog of adiponectin, induced weight loss in mice and decreased blood glucose.[7] The latter effect was hypothesized to be caused by relieving the fatty acid–mediated inhibition of glucose utilization by muscle cells. In addition, decreased plasma adiponectin levels have been suggested to play a causative role in the development of insulin resistance[10] and type 2 diabetes. Adiponectin has been shown to decrease insulin resistance by decreasing triglyceride content in muscle and liver in obese mice.[8] It is interesting to note that, in the present study, homozygotes for the G allele of the IVS2 + G62T polymorphism had higher plasma glucose levels and more diabetic cases than T allele homozygotes or heterozygotes. Our results are in accordance with an earlier observation in the Japanese population in which the IVS2 + G62T variant was associated with type 2 diabetes and insulin resistance.[19] A haplotype that included the IVS2 + G62T and another polymorphism, Gly15Gly, was also associated with obesity and other features of the metabolic syndrome in another study.[20] Although the sample sizes were small, the GG group in the present study tended to exhibit more diabetic complications. Sagittal diameter, a predictor of visceral adipose tissue level,[21] was lowest among T allele homozygotes. It is likely that variation in sagittal diameter among IVS2 + G62T genotypes in obese female subjects only increases the predisposition to a morbid condition. Therefore, some additional mechanisms are undoubtedly involved. For instance, one could speculate that the GG subjects had decreased adiponectin levels due to a genetic alteration in the adiponectin gene. The latter may have caused decreased muscle oxidation and clearance of free fatty acids from the bloodstream setting the stage for insulin resistance and vascular disease. However, the IVS2 + G62T polymorphism is not located in a coding region of the adiponectin gene and the functional implications of the variant need to be further investigated.

Table 3. Prevalence of Obesity-Related Diseases in SOS Females by Adiponectin IVS2 + G62T Genotype

	GG	GT	TT
Diabetes	10.7% (6/56)	0% (0/30)	0% (0/9)
Albumin in urine test (>0.1 g/L)	13.5% (7/52)	11.1% (3/27)	0% (0/9)
Treated hypertension	33.9% (19/56)	36.7% (11/30)	22.2% (2/9)
Signs of ischemia* in ECG	7.1% (4/56)	3.3% (1/30)	0% (0/9)

*Definite plus possible cases.

In a population of 219 Japanese subjects, Takahashi et al[22] reported 1 case with a missense mutation in codon 112 of exon 3 in the adiponectin gene. The subject had markedly low plasma adiponectin concentrations. In the present study, we did not find any subject with this mutation or another missense mutation (I164T) that has been associated with low plasma adiponectin concentrations and type 2 diabetes in an earlier study.[23] However, a mutation causing a tyrosine to histidine change in codon 111, in the globular domain of the adiponectin protein, was observed. Four obese and 5 non-obese heterozygote carriers of the mutation were found, supporting the concept that the mutation was not associated with obesity.

One conservative variant in codon 15 within exon 2 of the adiponectin gene has been associated with obesity and insulin sensitivity in a German population.[24] However, the effect of this variant on the risk of obesity was observed only in individuals without a family predisposition to type 2 diabetes.[24] In

the present study, the Gly15Gly polymorphism was associated with cholesterol among obese subjects in agreement with the findings of an earlier study.[25] How could adiponectin gene sequence variations have effects on plasma lipid values? It is possible that the mechanisms hypothesized to influence fat and glucose metabolism may also have affected plasma lipid concentrations. Adiponectin has been shown to increase lipid oxidation both in muscle and other tissues.[8,9] The Gly15Gly polymorphism is in the nonhelical region of the adiponectin gene without homology to known proteins. One cannot rule out the possibility that its putative effects could be mediated by linkage disequilibrium with another functional mutation elsewhere in the same gene or in another gene in linkage disequilibrium. More than 20 polymorphisms have been identified in the promoter region of the adiponectin gene. A complete screening of the adiponectin gene showed that variants in the promoter and exon 3 region of the gene contributed to the variation in blood adiponectin levels and risk for type 2 diabetes in a French Caucasian population.[26]

In conclusion, a IVS2 + G62T polymorphism in the adiponectin gene was associated with BMI, sagittal diameter, blood glucose, and the prevalence of diabetes mellitus in morbidly obese SOS subjects. A Gly15Gly silent polymorphism was associated with serum cholesterol and waist circumference variability. A Tyr111His variant was not associated with obesity or its comorbidities. These data suggest that adiponectin sequence variations may play a role in the development of the metabolic complications of morbid obesity.

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