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Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity

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Abstract Adiponectin gene polymorphisms have recently been reported to be associated with obesity, insulin sensitivity, and the risk of type 2 diabetes. We examined a T94G polymorphism of the adiponectin gene in 245 ostensibly normal nondiabetic subjects. The G allele frequency was lower among subjects with higher BMI (≥ 27) than in those with lower BMI. BMI was inversely correlated with the dose of G allele. Multivariate linear regression analyses showed that the adiponectin genotypes were significantly related to BMI after adjusting for age and gender. The dose of the G allele was associated with a reduction of approximately 1.12 kg/m² in BMI. We further found that the relative mRNA levels of G allele were consistently higher than those of T allele in the omental adipose tissue from 21 heterozygous subjects. Finally, we observed that the expression levels of

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adiponectin affected insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. In conclusion, the allele-specific differential expression of this common polymorphism could be responsible for its biological effects observed in this and the other studies.

Keywords Obesity · Insulin resistance · Adiponectin · Polymorphism · mRNA

Abbreviations BMI: Body mass index · SNP: Single nucleotide polymorphism

Introduction

Adipose tissue secretes many adipocytokines to modulate a wide array of biological functions [1]. Along with the well-known examples, such as leptin and resistin [2, 3], a relatively novel adipocytokine, adiponectin has been identified in mouse (adipoQ or ACRP30) [4, 5] as well as in human (apM1 or GBP-28) [6, 7]. Recombinant adiponectin proteins were recently shown to decrease tumor necrosis factor α induced monocyte adhesion, nuclear transcription factor κ B signaling, and the expression of adhesion molecules in endothelial cells in vitro [8, 9, 10]. They also attenuated cholesterol ester accumulation and the expression of class A scavenger receptor in human monocyte-derived macrophages [11]. Lower plasma adiponectin levels are reported in patients with coronary heart disease [8]. Furthermore, immunoreactive adiponectin is detectable in the walls of catheter-injured vessels but not in the intact vascular walls [11, 12]. Taken together these reports indicate that adiponectin could be a protective adipocytokine against atherosclerosis.

A longitudinal observation of rhesus monkeys found a progressive decline in adiponectin paralleled the decrease in insulin sensitivity during the development of diabetes [13]. In mice injection of the recombinant adiponectin decreased plasma glucose and fatty acid levels and improved insulin sensitivity [14, 15, 16]. Long-term injection of adiponectin in mice led to significant weight reduction [14]. Hypoadiponectinemia is also documented in subjects with obesity or diabetes [17, 18]. An inverse correlation between plasma adiponectin levels and body mass index (BMI), fasting insulin, and glucose and triglyceride levels but a positive relationship with high-density lipoprotein cholesterol and measurement of insulin sensitivity in clamp studies has been reported [17, 18, 19]. These findings suggest a role of adiponectin in the pathophysiology of insulin resistance syndrome.

Genetic variations in the human adiponectin gene have recently been reported to be associated with the risk of obesity, insulin resistance, type 2 diabetes, and high levels of low-density lipoprotein cholesterol [20, 21, 22, 23, 24, 25]. In contrast, others have found no association with obesity or type 2 diabetes [25, 26, 27]. Almost all of the studies used a T/G polymorphism in the exon 2 of the human adiponectin gene as the sole genetic marker. One study performed extensive SNP genotyping, ending up with 14 single nucleotide polymorphisms (SNPs) across the whole gene [20]. However, only the T/G polymorphism in exon 2 along with an SNP276 was strongly associated with the risk of type 2 diabetes [21]. Since the T/G polymorphism at exon 2 is a synonymous mutation (GGT→GGG, Gly15Gly) and SNP276 is in intron 2, the exact molecular mechanisms responsible for the biological effects of these variations are not known at present. On the other hand, it was recently reported that SNPs in the adiponectin promoter region are associated with the risk of type 2 diabetes in the French population [24].

In this study we surveyed the frequency of the T94G polymorphism in the exon 2 among 245 ostensibly

normal nondiabetic subjects with a wide range of BMI. We also estimated the relative mRNA levels of T/G alleles in the adipose tissue of heterozygous subjects. Moreover, we measured insulin-stimulated glucose uptake in 3T3-L1 adipocytes stably transfected with either sense or antisense gene of adiponectin. Our data suggest that adiponectin may be a genetic contributor to body weight and the risk of obesity. The allele-specific differential expression of adiponectin is at least one of the causes responsible for the biological effects associated with this polymorphism.

Materials and methods

Subjects

We recruited 245 ostensibly normal nondiabetic subjects (WHO criteria) aged 30 years or over and with a BMI less than 45 (Table 1) during a routine health examination at National Taiwan University Hospital (NTUH). Adipose tissue specimens were obtained from 10 severely obese heterozygous subjects (seven women, three men; aged 32.2 ± 9.6 years; BMI 41.76 ± 4.00) during gastric partition surgery [28] and from 11 lean heterozygous women (age 48.4 ± 4.8 years; BMI 21.56 ± 2.22) during uterine myomectomy. BMI of at least 27.0 was defined as obesity according to the guideline for obesity management issued by the Ministry of Health of Taiwan. Informed consent was obtained. The study was approved by the Medical Ethics Committee of National Taiwan University Hospital.

Laboratory and phenotypic characterization of the subjects

A 75 g oral glucose tolerance test was given to all subjects in health examination. Determination of the concentrations of plasma glucose, total cholesterol, triglyceride, and serum insulin levels and the estimation of insulin resistance index and beta cell function by homeostasis model assessment (HOMA) were carried out as previously reported [28].

Extraction of genomic DNA and genotyping

Total genomic DNA was purified from peripheral blood leukocytes using a DNA extraction kit (Puregene, Minneapolis, Minn., USA). Exon 2 of the human adiponectin gene was amplified using primers, 5'-TAGAAGTAGACTCTGCTGAGATG-3' and 5'-CTC-CCTGTGTCTAGGCCTTAG-3', with the annealing temperatures at 68–60°C stepping down 2°C for every five cycles. The amplified DNA was digested with *Bsp*HI enzyme (New England Biolabs, Beverly, Mass., USA) prior to electrophoresis on a 2% agarose gel. The resulting fragment of PCR was 422 bp. Because the T94G substitution abolished a *Bsp*HI restriction site, two fragments of 265 and 157 bp after digestion indicate the presence of T allele.

Table 1 Characteristics of 245 ostensibly normal nondiabetic subjects in this study

Age (years)	47.7±10.9
Body mass index	26.87±6.59
Gender: F/M	124/121
Hypertension	76 (31%)
Hyperlipidemia	47 (19%)
Diabetes mellitus	0 (0%)

RNA extraction and quantitation of relative mRNA levels of G and T alleles

The adipose tissues were extracted for RNA by Trizol reagent and 1 g of the total RNA was reverse transcribed into cDNA following manufacturer's recommendation (Life Technologies, Rockville, Md., USA). PCR (primers: 5'-ATGCTGTTGCTGGGAGCTGT-3' and 5'-CCATCCAACCTGTGCAGGC-3') was performed with an initial denaturation at 95°C for 10 min, followed by denaturing at 95°C for 10 s, annealing at 60°C for 1 min for a total of 31–37 cycles, then by a final extension at 72°C for 10 min. The amplification of 33 cycles was chosen because the amount of PCR product was located within the linear range of amplification (data not shown). The PCR products were digested with *Sma*I followed by electrophoresis on 2% agarose gels stained with ethidium bromide. Because the T→G change creates a *Sma*I site, the uncut 134-bp band was the T allele, whereas the presence of 79- and 55-bp bands was indicative of the G allele. The band intensity was determined by densitometric analyses (1-D Gel Analysis Software, One-Dscan, Scanalytics, Fairfax, Va., USA). Because the T/G alleles were amplified in the same reaction, and the relative levels between them were used in the analysis, a control gene such as β -actin or GAPDH in the ordinary quantitative PCR was not required.

Cloning of human adiponectin gene and transfection of 3T3-L1

A PCR reaction using human adipose tissue cDNA as template and primers (5'-ATG CTG TTG CTG GGA GCT GT-3' and 5'-TCA GTT GGT GTC ATG GTA GAG-3') was performed to generate the coding sequences of human adiponectin gene. The PCR product was cloned into pGEM-Teasy (Promega, Madison, Wis., USA), subsequently subcloned into the *Eco*RI site of pCDNA3.1+ (Invitrogen, Groningen, The Netherlands) in either sense or antisense orientation driven by a cytomegalovirus promoter. Of each plasmid 2 μ g was transfected into 3T3-L1 cells by lipofectamine method (Life Technologies). Transfected cells were selected 2 days after transfection in a medium containing 500 μ g/ml G418 for 2 weeks, thereafter maintained in 200 μ g/ml G418 as pools. Each pool had at least 200 colonies from each stable transfection. Because the differentiated 3T3-L1 adipocytes are quite heterogeneous in phenotypes, the experimental results from a few selected clones could be misleading. Therefore we decided to use the pools from a large number of colonies to avoid the bias from selection.

Generation of antibody against adiponectin and western blot analysis

A polyclonal antibody against peptide residues 15–29 (GHD-QETTTQGGPVLL) of adiponectin was produced in rabbits. Crude cellular extracts of stably transfected cells were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by western blot analyses with the antiadiponectin antibody. Mouse β -actin monoclonal antibody (Chemicon International,

Calif., USA) was used as a standard control antibody. The protein of interest was detected by enhanced chemiluminescence (Amersham Pharmacia, Piscataway, N.J., USA).

Glucose transport assays

3T3-L1 cells stably transfected with the plasmids containing vector-alone, sense, or antisense of adiponectin gene were induced to differentiation as previously described [29]. Glucose transport assayed by the uptake of 2-deoxy-D-[³H]glucose, was performed in triplicate as described in the differentiated adipocytes after 24-h incubation in serum-free medium [29]. The count of uptake in the presence of 10 μ M cytochalasin-B was used as the background, therefore subtracted from all the raw values for subsequent analysis. A final insulin concentration of 100 nM was used to stimulate glucose uptake. The data on basal and insulin-stimulated glucose uptake for the cells with sense or antisense adiponectin gene were adjusted using the basal glucose uptake of the cells harboring vector-alone as 100%.

Statistical analyses

Data are presented as mean $\pm$ SD. Statistical analyses by analysis of variance, χ^2 tests, Mann-Whitney rank sum test, and univariate and multivariate linear regression analyses were performed by using the PC version of the Statistical Analysis System (SAS, 6.12 edition, SAS Institute, Cary, N.C., USA) where appropriate. Logistic regression analyses were performed with Stata (College Station, Tex., USA). The original data were log transformed before used for statistical analyses. A *P* value less than 0.05 was considered significant statistically.

Results

We sequenced the protein-coding regions in exons 2 and 3 of the human adiponectin gene using the genomic DNA from 20 unrelated randomly selected Taiwanese subjects. Only one T/G polymorphism at nucleotide 94 in exon 2 was identified. Although it does not alter the amino acid (Gly15Gly), it seemed to be quite common in our population and therefore could be useful as a marker for genetic association studies. We genotyped 245 ostensibly normal nondiabetic subjects. The frequencies of T and G alleles were 0.637 and 0.363, respectively. Comparison of selected characteristics of these subjects categorized by genotypes revealed that only the BMI differed between them, with a borderline significance (Table 2). Further, we found that only BMI was inversely

Table 2 Means $\pm$ SD of selected characteristics among 245 normal subjects by adiponectin genotypes (HOMA homeostasis model assessment)

	T/T		T/G		G/G		<i>P</i> ^b
	<i>n</i>	Mean $\pm$ SD ^a	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	
Age (years)	91	48.35 $\pm$ 10.60	130	46.65 $\pm$ 10.69	24	51.13 $\pm$ 12.78	NS*
Triglyceride (mmol/l)	91	1.44 $\pm$ 0.86	130	1.42 $\pm$ 0.10	24	1.32 $\pm$ 0.08	NS
Cholesterol (mmol/l)	91	4.66 $\pm$ 0.85	130	4.66 $\pm$ 1.03	24	4.40 $\pm$ 1.04	NS
Body mass index	91	27.94 $\pm$ 7.45	130	26.53 $\pm$ 6.04	24	24.59 $\pm$ 5.42	0.067
Fasting plasma glucose (mmol/l)	91	5.27 $\pm$ 0.57	129	5.18 $\pm$ 0.65	24	5.10 $\pm$ 0.64	NS
Fasting plasma insulin (pmol/l)	91	69.17 $\pm$ 65.44	126	58.26 $\pm$ 52.16	24	48.28 $\pm$ 26.12	NS
Insulin resistance (HOMA)	91	2.34 $\pm$ 2.37	126	1.94 $\pm$ 1.75	24	1.51 $\pm$ 0.92	NS

^a The original mean $\pm$ SD is presented without transformation; ^b Analysis of variance after log transformation

* *P*>0.1

Table 3 Adiponectin genotype and allele frequencies according to BMI of the total among 245 normal subjects and odds ratios (OR) of obesity by adiponectin genotypes and alleles

Genotype	BMI $\geq$ 27.0		BMI<27.0		OR	95% CI
	<i>n</i>	%	<i>n</i>	%		
G/G	5	6.3	19	11.5	0.42	0.13–1.28
G/T	38	47.5	92	55.8	0.54	0.30–0.97 ^a
T/T	37	46.3	54	32.7	1	–
Allele						
G	48	30.0	130	39.4	0.65*	0.42–0.99*
T	112	70.0	200	60.6	1	–

^aOdds ratios after adjusting for age and gender and treating BMI $\geq$ 27.0 as 1 and BMI<27 as 0. *P* for trend=0.028 for genotypes using T/T as reference in multiple logistic regression model

* χ^2 , *P*=0.043

correlated with the dose of G allele (*P*=0.023, γ =−0.15). Multivariate linear regression analyses showed that the adiponectin genotypes were significantly related to BMI (*P*=0.022, β =−0.05 $\pm$ 0.02), while adjusting for age and gender. The dose of the G allele was associated with a reduction of approximately 1.12 in BMI.

To further illustrate the effect of the adiponectin genotypes on BMI, BMI values of 27 or higher (based on the guideline issued by the Ministry of Health of Taiwan) was used to categorize subjects (Table 3). Significantly lower frequency of G allele was found in the higher BMI group (*P*=0.043). The odds ratio of higher BMI for the G allele relative to the T allele was 0.65. In multivariate logistic regression analyses the odds ratio of higher BMI for the subjects carrying G/T genotype relative to the subjects carrying T/T genotype was 0.54, adjusting for age and gender (*P*=0.028 for linear trend, Table 3). Using 30 as the cutoff point instead of 27 produced similar results. The G allele frequency in subjects with BMI of 30 or higher was less than that with BMI less than 30 (28% vs. 39%; *P*=0.044, χ^2). The odds ratio for obesity for G/T genotype relative to T/T genotype was 0.48 (95% CI 0.24–0.95, *P* for linear trend=0.019) with adjustment for age and gender in logistic regression. Therefore the G allele of the adiponectin gene may be associated with lower BMI and the reduced risk to obesity, defined by BMI value of either 27 or more or one of 30 or more.

The T94G polymorphism in exon 2 of the human adiponectin gene is a synonymous mutation (GGT→GGG, Gly→Gly), which has no biological effects on its own. It is plausible that the polymorphism is in linkage disequilibrium with some other functional genetic alterations, resulting in the difference in mRNA expression levels of the T/G alleles. Therefore we assayed the relative T/G allele-specific expression within the same heterozygous individuals. The relative mRNA levels of T/G alleles should faithfully reflect the effects caused by the genomic changes linked with the T94G polymorphism, since both alleles were in exactly the same “environmental” conditions. Omental adipose tissue from 21 subjects was assayed by semiquantitative PCR (Fig. 1A). The mean level of G allele was significantly higher than that of T allele (*P*<10^{−10}, Fig. 1B). We conclude that the biological

Fig. 1 The allele-specific mRNA expression of adiponectin gene in the omental adipose tissue from 21 subjects with heterozygous T/G genotype assayed by PCR. **A** A representative agarose gel showing the PCR products from five subjects (lanes 1–5) at the 33rd cycle digested with *Sma*I; *M* 100-bp marker; *T* allele 134 bp; *G* allele 79 and 55 bp; The higher intensity of the G allele bands relative to that of the T allele bands indicates higher expression levels. **B** The relative mRNA levels of adiponectin G and T alleles in the omental adipose tissue from 21 heterozygous subjects estimated from the assays shown in **A**. In each individual the total intensity of G/T alleles calculated from densitometric analyses was set to 100% to adjust the relative amount of G/T alleles. Bars means $\pm$ SEM of the data; **P*<1 $\times$ 10^{−10}

cal effects of the T94G polymorphism might be at least in part attributable to the difference in allele-specific expression levels.

We then examined whether the difference in adiponectin gene expression has a direct biological effect on insulin

Fig. 2 **A** The basal and insulin-stimulated glucose uptake and adiponectin protein levels in the differentiated 3T3-L1 adipocyte pools harboring sense, antisense adiponectin gene, or vector alone. The basal glucose uptake of the cells transfected with vector alone (black bar in basal group) was set to 1 to adjust the values from the others. The insulin stimulated glucose uptake for the vector-alone (black bar in insulin+) was enhanced by approximately sixfold. White bars results using sense adiponectin-expression clones; shaded bars results using antisense adiponectin-expression clones (mean and SEM, of seven separate experiments); * $P=0.035$. **B** Western blot gel showing the relative amount of adiponectin (a) and β -actin (b) proteins in the differentiated 3T3-L1 cells harboring the plasmids with vector alone (lane 1), sense (lane 2), or antisense adiponectin gene (lane 3). The adiponectin protein level of the antisense pool was lower adjusted for the level of the control gene β -actin

sensitivity in adipocytes themselves. The plasmids containing sense, antisense, or no adiponectin gene were stably introduced into 3T3-L1 cells. Pools of more than 200 colonies of the stable transfectants were differentiated into adipocytes and assayed for glucose uptake. The basal glucose uptake of the sense-expressing pools was increased by 40%, whereas the basal glucose uptake from the pools expressing antisense was exactly the same as that from the pools with vector-alone. However, they did not differ statistically (Fig. 2A). Insulin-stimulated glucose uptake in the pools with vector alone by an average of sixfold. The insulin-stimulated glucose uptake in the pools expressing sense gene was 135% of that from the insulin-stimulated vector alone. In contrast, the insulin-stimulated glucose uptake in the pools expressing antisense gene was decreased to 75% of that from the insulin-stimulated vector-alone. The difference in mean insulin-stimulated glucose uptake between the sense- and antisense-expressing pools was significant ($P=0.035$, Fig. 2A). The difference in adiponectin gene expression in the differentiated 3T3-L1 adipocytes (Fig. 2B) might be the cause of the difference in insulin-stimulated glucose uptake.

Discussion

This study provides the genetic evidence that adiponectin influences BMI, which is consistent with the findings in several very recent genetic reports [20, 21, 22, 23].

Most importantly, we found the differential expression of the alleles associated with the polymorphism. We also demonstrate that the difference in expression levels of adiponectin can lead to different glucose uptake in differentiated 3T3-L1 adipocytes.

Since the T94G polymorphism is a synonymous mutation without a biological effect on its own, all these genetic association studies in a sense led to the same conclusions that the adiponectin gene is a genetic contributor to insulin sensitivity, BMI, and risk of type 2 diabetes [20, 21, 22, 23, 24]. Exactly what genetic alterations are in linkage disequilibrium with this polymorphism is not clear at present. A difference in the allele-specific expression of an SNP of the gene has been suggested recently to correlate functionally with disease phenotype [30]. Our study showed for the first time that the steady-state mRNA levels transcribed by the G allele are higher than those by the T allele in the adipose tissue of heterozygous subjects, suggesting that the promoter, the introns, and/or the other potential regulatory elements such as upstream enhancers or locus control regions of the adiponectin gene should be carefully examined. Recently the SNPs in the adiponectin promoter region were shown to be associated with type 2 diabetes [24]. Whether these SNPs are functional and are responsible for the allele-specific expression observed in our study has not been explored. Alternatively, this synonymous mutation may affect steady-state mRNA levels by altering RNA splicing or stability. The T/G or linked polymorphism nearby may affect mRNA splicing or stability [31]. The allele-specific differential expression of adiponectin in the adipose tissue is also consistent with the observations that higher plasma adiponectin was associated with reduced risk of obesity, diabetes, insulin resistance and coronary heart disease in humans [8, 17, 18]. Our data provide an explanation for the indirect genetic association studies at the molecular levels. However, the exact genetic mechanisms responsible for the allele-specific expression await further exploration.

Our transfection experiments further demonstrated that the expression level of adiponectin affects insulin-stimulated glucose uptake in adipocytes. Previous studies have shown the insulin-sensitizing effects of adip-

onectin in liver and skeletal muscle [15, 16]. This is the first to show that adiponectin also affects insulin sensitivity on adipocytes themselves. Therefore adiponectin may act in the adipose tissue via an autocrine or paracrine manner. However, our experiments were too preliminary to address the exact mechanism of actions. In conclusion, our study suggests that the human adiponectin gene is an important genetic factor linking insulin resistance syndrome and related disorders such as obesity. The allele-specific differential expression of adiponectin at least partially explains the biological effects observed in many genetic association studies.

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References

- Funahashi T, Nakamura T, Shimomura I, Maeda K, Kuriyama H, Takahashi M, Arita Y, Kihara S, Matsuzawa Y (1999) Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. *Intern Med* 3:202–206
- Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. *Nature* 395:763–770
- Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Laza, MA (2001) The hormone resistin links diabetes to obesity. *Nature* 409:307–312
- Hu E, Liang P, Spiegelman BM (1996) AdipoQ is a novel adipocyte-specific gene dysregulated in obesity. *J Biol Chem* 271:10697–10703
- Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746–26749
- Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1. *Biochem Biophys Res Commun* 221:286–289
- Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M (1996) Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem (Tokyo)* 120:803–812
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 100:2473–2476
- Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y (2000) Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 96:1723–1732
- Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y (2000) Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 12:1296–1301
- Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y (2001) Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* 103:1057–1063
- Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, Igura T, Inui Y, Kihara S, Nakamura T, Yamashita S, Miyagawa J, Funahashi T, Matsuzawa Y (2000) An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res* 32:47–50
- Hotta K, Funahashi T, Bodkin NL, Ortmeier HK, Arita Y, Hansen BC, Matsuzawa Y (2001) Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 50:1126–1133
- Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010
- Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946
- Berg AH, Combs TP, Du X, Brownlee M, Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7:947–953
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoaka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83
- Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599
- Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935
- Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otobe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Kimura S, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T (2002) Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 51:536–540
- Stumvoll M, Tschrötter O, Fritsche A, Staiger H, Renn W, Weisser M, Machicao F, Haring H (2002) Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. *Diabetes* 51:37–41
- Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, Ouchi N, Kihara S, Kawamoto T, Sumitsuji S, Funahashi T, Matsuzawa Y (2002) Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes* 51:2325–2328
- Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warram JH, Scherer PE, Trischitta V, Doria A (2002) A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes* 51:2306–2312
- Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, Boutin P, Vaxillaire M, Lepretre F, Dupont S, Hara K, Clement K, Bihain B, Kadowaki T, Froguel P (2002) Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet* 11:2607–2614

25. Zietz B, Barth N, Scholmerich J, Schmitz G, Schaffler A (2001) Gly15Gly polymorphism within the human adipocyte-specific apM-1 gene but not Tyr111His polymorphism is associated with higher levels of cholesterol and LDL-cholesterol in caucasian patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 109:320–325
26. Takahashi M, Arita Y, Yamagata K, Matsukawa Y, Okutomi K, Horie M, Shimomura I, Hotta K, Kuriyama H, Kihara S, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y (2000) Genomic structure and mutations in adipose-specific gene, adiponectin. *Int J Obes Relat Metab Disord* 24:861–868
27. Schaffler A, Barth N, Palitzsch KD, Drobnik W, Scholmerich J, Schmitz G (2000) Mutation analysis of the human adipocyte-specific apM-1 gene. *Eur J Clin Invest* 30:879–887
28. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM (2001) Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 86:3815–3819
29. Fingar DC, Birnbaum MJ (1994) Characterization of the mitogen-activated protein kinase/90-kilodalton ribosomal protein S6 kinase signaling pathway in 3T3–L1 adipocytes and its role in insulin-stimulated glucose transport. *Endocrinology* 134:728–735
30. Yan H, Yuan W, Velculescu VE, Vogelstein B, Kinzler KW (2002) Allelic variation in human gene expression. *Science* 297:1143
31. Mendell JT, Dietz HC (2001) When the message goes awry: disease-producing mutations that influence mRNA content and performance. *Cell* 107:411–414